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In-vitro cytotoxicity and antimicrobial activities, against clinical isolates of *Campylobacter* species and *Entamoeba histolytica*, of local medicinal plants from the Venda region, in South Africa

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In the quest for alternative treatments against *Campylobacter jejuni* and *Entamoeba histolytica*, which are both aetiological agents of diarrhoea world-wide, the in-vitro activities against the two pathogens of extracts of 18 South African medicinal plants have recently been assessed.

Forty extracts from the 18 plant species were prepared and tested against 110 clinical isolates of *Campylobacter* spp. In addition, extracts from eight of the plant species were tested against a standard strain (HM-1:IMSS) of *E. histolytica*, and the cytotoxicity of each of 19 extracts from 15 of the plant species was explored using Vero cell cultures and microdilution assays.

At least one extract of each plant species investigated was found to be active against some of the *Campylobacter* isolates. Extracts of *Lippia javanica* and *Pterocarpus angolensis* had the highest antibacterial activity, each giving a minimum inhibitory concentration (MIC) of 90 μg/ml. Of the extracts tested against *E. histolytica*, however, only those of *P. angolensis* and *Syzigium cordatum* were found to have anti-amoebic activity, with MIC of 1.2 and 7.5 mg/ml, respectively. Although most of the extracts showed little toxicity against Vero cells, with most of the median inhibitory concentrations (IC_{50}) recorded exceeding 400 μg/ml, an extract of *Bauhinia galpini* was quite toxic, with an IC_{50} of just 2.7 μg/ml. Acetone and methanol extracts of several of the plants show promise as templates for the design of new anti-diarrhoeal therapies.

Entamoeba histolytica and *Campylobacter* species are aetiological agents of diarrhoea, particularly in developing countries, where diarrhoeal diseases constitute a very important cause of morbidity and mortality among children and young adults (WHO, 2002). Recent estimates indicate that, each year, about 1.9 million children die because of infectious diarrhoea (Bryce *et al.*, 2005). Also of concern are the under-recognised long-term effects of frequent early childhood diarrhoeal episodes, which include permanent shortfalls in physical and cognitive development. In some settings, decrements of up to 8 cm in height, 10 intelligence-quotient points, and 12 months of schooling appear attributable to early childhood diarrhoea and enteric parasitic infection (Steiner *et al.*, 2006; Guerrant *et al.*, 2008). Diarrhoea is also a hallmark of HIV infections, with about 90% of HIV-infected individuals in Africa suffering from

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persistent diarrhoea. The control of *En. histolytica*, *Campylobacter* and other diarrhoeagenic infections could thus greatly improve the lives of millions of individuals, especially those resident in developing areas and/or living with HIV/AIDS.

Although erythromycin is currently the drug of choice for the treatment of campylobacteriosis, Samie *et al.* (2007) recently found 35% of the *Campylobacter* isolates they investigated, which were collected from diarrhoeal stools in the Vhembe district of the South African province of Limpopo, to be resistant to this antibiotic. Limpopo is predominantly rural and, as the result of poor water quality and limited or even absent sewage disposal, its human populations are very prone to infections by diarrhoeagenic microorganisms.

There have also been several cases of treatment failure (Hanna *et al.*, 2000), as well as undesirable side-effects (Kim *et al.*, 2005), following the use of metronidazole against amoebiasis. A search for novel and more effective compounds for the treatment of campylobacteriosis and amoebiasis appears justified.

Medicinal plants have been used as source of many compounds used in the treatment of infectious organisms, and about 70% of the African population still relies on medicinal plants for basic healthcare (Kamanzi *et al.*, 2002). Samie *et al.* (2005) tested some 14 medicinal plants used in the Venda region of South Africa against 15 different bacterial species (although activity against *Campylobacter* spp. was not explored). In the present study, the in-vitro antibacterial activities of extracts or essential oil from 18 species of medicinal plant from the Venda region — including 12 of the 14 species investigated by Samie *et al.* (2005) — were tested against 110 *Campylobacter* isolates collected from diarrhoeal patients in the Vhembe district. Eight of the extracts were also tested for their anti-*En. histolytica* activity *in vitro*, and the cytotoxicity of 15 of the extracts, against Vero cell cultures, was also evaluated.

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Extracts and Essential Oil

The plant materials used in this study (Table 1) were collected from various sites in the Vhembe district of South Africa. Voucher specimens were identified and deposited at the Thohoyandou Botanical Garden Herbarium, Thohoyandou, South Africa. Each sample was cleaned and dried at room temperature for 2 weeks and ground to fine particles before 100-g subsamples were extracted with solvent (most frequently methanol, acetone or hexane; see Table 2) for 3 days. [Polar solvents were often used because most of the plants are traditionally used as aqueous decoctions (and water is a polar solvent) and because acetone or methanol extracts of most of the plants have previously been found to have the highest antibacterial activities (Samie *et al.*, 2005)]. Each extract was dried in a rotary evaporator and then dissolved in 10% dimethyl sulfoxide (DMSO) for testing. An essential oil was also prepared from the fresh leaves of *Lippia javanica*, by hydrodistillation, for 3 h, in a Clevenger-type apparatus. All the extracts and the essential oil were kept at 4°C in the dark until further use.

Antibacterial Activities *in Vitro*

Overall, 110 strains of *Campylobacter* were isolated from diarrhoeal stool samples and identified as previously described (Samie *et al.*, 2007). Briefly, a suspension of faecal material was prepared in sterile saline. This suspension was used to flood the top surface of a sterile, 47-mm-diameter membrane filter (with 0.6-μm pores) that had been placed on a plate of Columbia blood agar (Oxoid, Basingstoke, U.K.) supplemented with tryptose (Oxoid). After 15 min, the filter was carefully removed so that none of the suspension spilt directly onto the agar. Suspected *Campylobacter* colonies that grew on the plates were confirmed by Gram

TABLE 1. *Ethnobotanical information on the 18 medicinal plants used by the Venda and in the present study*

Family and species	Common name(s)	Plant part(s) used	Traditional use (Mabogo, 1990; unpubl. obs.)	Voucher specimen
ANNONACEAE				
Annona sp.	–	Fruit	Abscesses	AS19
APOCYNACEASE				
Carissa edulis	*Thungulu* (V)	Leaf	Stomach ache, cough, cataracts	JF07
CAESALPINIACEAE				
Peltophorum africanum	*Musese* (V), African wattle (E)	Bark, root	Tuberculosis, stomach complaints, intestinal parasites	BP01
CELASTRACEAE				
Elaeodendron transvaalensis (Burtt)	*Mulumanama* (V), bushveld saffron (E)	Root	Fungal infections, stomach disorders and ulcers, venereal diseases.	BP05
CUCURBITACEAE				
Mormodica balsamina	*Lubavhe* (V)	Whole plant	Diabetes, childhood diarrhoea	JF03
EUPHORBIACEAE				
Bridelia micrantha	*Munzere* (V), mitzeerie (E)	Root, bark, seed	Stomach ache, tapeworm, diarrhoea, headache, sore joints, sore eyes, venereal diseases, fevers	BP03
FABACEAE				
Bauhinia galpinii	*Mutzwiriri* (V), Pride of the Cape (E)	Root	Diarrhoea	AS04
Pterocarpus angolensis	*Mutondo* (V), iron wood (E)	Bark	Wounds	AS05
MALVACEA				
Sida alba	–	Leaf	Diarrhoea, dysentery	AS12
MENISPERMACEAE				
Cissampelos torulosa	*Lukandululo* (V)	Leaf	Diarrhoea, dysentery, sore throat	AS13
MORACEAE				
Ficus sycomorus	*Muhuyu* (V)	Bark	Chest conditions, coughing, scrofula	AS06
MYRTACEAE				
Syzygium cordatum	*Mutu* (V), water berry (E)	Bark, leaf	Stomach troubles, cold, fever, diarrhoea, wounds	AS14
OLACACEAE				
Ximenia caffra	*Mutshili* (V), sourplum (E)	Leaf, root	Diarrhoea, dysentery, fever, cough, venereal diseases	AS15
PAPILIONACEAE				
Mucuna coriacea	*Mulada* (V)	Root	Toothache, diarrhoea	BP02
Zornia milneana	*Lukandululo* (V)	Whole plant	Dysentery, diarrhoea	AS16
URTICACEAE				
Pouzolzia mixta	*Muthanzwa* (V), soap nettle (E)	Root, stem, leaf	Diarrhoea, dysentery, general body health	AS17
VERBENACEAE				
Lippia javanica	*Musudzungwane* (V), fever tea (E)	Leaf	Asthma, malaria, diarrhoea	AS19
VITACEAE				
Rhoicissus tridentata	*Murumbulashedo*(V), bitter grape (E)	Root, tuber, fruit	Prevention of miscarriages, diarrhoea	AS18

V, Tshivenda; E, English.

TABLE 2. *Antibacterial profiles of the 40 tested extracts, produced from 18 species of medicinal plant, against 110 clinical strains of Campylobacter*

		No. and (%) of *Campylobacter* isolates suppressed with extract at a concentration (mg/ml) of:							
	Part and solvent	>6	6	3	1.5	0.75	0.35	0.18	0.09
PLANT									
Annona sp.	Fruit, acetone	18 (16)	62 (56)	13 (12)	4 (3.6)	13 (12)			
	Fruit, ethyl acetate	24 (22)	86 (78)						
	Fruit, hexane			6 (5.5)	22 (20)	77 (70)	5 (4.5)		
Bauhinia galpinii	Bark, acetone		6 (5.4)	23 (21)	78 (71)	3 (2.7)			
Bridelia micrantha	Bark, methanol	18 (16)	57 (52)	35 (32)					
	Roots, methanol	5 (4.5)	86 (78)	19 (17)					
	Seed, acetone	5 (4.5)	29 (26)	5 (4.5)	67 (61)	5 (4.5)			
Carissa edulis	Leaves, acetone	9 (8.2)	9 (8.2)	79 (72)	13 (12)				
Cissampelos torulosa	Leaves, methanol	3 (2.7)	42 (38)	36 (33)	26 (24)	3 (2.7)			
Elaeodendron transvaalensis	Root, methanol		12 (11)	31 (28)	43 (39)	24 (22)			
Ficus sycomorus	Bark, acetone			16 (15)	65 (59)	26 (24)	3 (2.7)		
Lippia javanica	Leaves, methanol						47 (43)	55 (50)	8 (7.3)
	Leaves, acetone					11 (10)	44 (40)	50 (45)	5 (4.6)
	Leaves (essential oil)	18 (16)	43 (39)	37 (34)	12 (11)				
Mormodica balsamina	Leaves, acetone				22 (20)	31 (28)	57 (52)		
	Leaves, hexane	24 (22)	33 (30)	53 (48)					
Mucuna coriacea	Root, methanol	24 (22)	61 (55)	25 (23)					
Peltophorum africanum	Bark, methanol		9 (8.2)	79 (72)	22 (20)				
Pouzolzia mixta	Roots, methanol				39 (35)	71 (65)			
	Stem, methanol	10 (9)	19 (17)	24 (22)	28 (25)	29 (26)			
	Leaves, methanol		102 (93)	8 (7)					
	Roots, water	103 (94)	7 (6.4)						
Pterocarpus angolensis	Bark, methanol		8 (7.3)	63 (57)	39 (36)				
	Bark, acetone		2 (1.8)	33 (30)	61 (56)	14 (13)			
	Bark, dichloromethane			13 (12)	53 (48)	44 (40)			
	Bark, ethyl acetate				43 (39)	12 (11)	37 (34)	6 (5.5)	12 (11)
	Bark, hexane	24 (22)	43 (39)	37 (33)	6 (5.5)				
	Bark, chloroform	24 (22)	19 (17)	14 (13)	48 (44)	5 (4.6)			
Rhoicissus tridentata	Tuber, acetone			60 (55)	39 (35)	11 (10)			
	Fruit, methanol				24 (22)	67 (61)	19 (17)		
	Fruit, acetone			14 (13)	14 (13)	29 (26)	43 (39)	10 (9.1)	
Sida alba	Leaves, acetone			47 (43)	55 (50)	8 (7.3)			

TABLE 2. Continued.

		No. and (%) of *Campylobacter* isolates suppressed with extract at a concentration (mg/ml) of:							
	Part and solvent	>6	6	3	1.5	0.75	0.35	0.18	0.09
Syzygium cordatum	Bark, methanol			10 (9.1)	65 (59)	24 (22)	11 (10)		
	Bark, acetone			3 (2.7)	44 (40)	38 (35)	17 (16)	8 (7.3)	
	Leaves, methanol		91 (83)	19 (17)					
	Leaves, acetone			33 (30)	68 (62)	9 (8.2)			
Ximenia caffra	Bark, acetone				8 (7.3)	39 (37)	63 (57)		
	Leaves, acetone		5 (4.6)	48 (44)	48 (44)	9 (8.2)			
Zornea milneana	Whole, methanol					18 (16)	84 (76)	9 (8.2)	
	Whole, acetone		3 (2.6)		6 (5.4)	23 (21)	68 (62)	10 (9.1)	
Gentamicin (control)									115 (100)

staining, oxidase, catalase and motility tests, and a commercial, *Campylobacter*-specific haemagglutination assay (Dryspot Campylobacter test; Oxoid) used as recommended by the manufacturer.

A microdilution method (Samie *et al.*, 2005) was used to test the activity of dilutions (0.09–6 mg/ml) of each of the 40 extracts from the 18 plant species against each of the 110 *Campylobacter* isolates, with the results expressed as minimum inhibitory concentrations (MIC). Gentamicin was used as a positive control, at concentrations varying between 0.25 and 32 μg/ml.

Anti-amoebic Activities *in Vitro*

After the antibacterial assays, there was sufficient of each of 16 extracts (representing eight plant species) left for the 16 to be used in tests against axenic cultures of *En. histolytica* strain HM-1:IMSS. The amoebic trophozoites were maintained, at 37°C and under anaerobic conditions, in TYI-S-33 medium supplemented with 10% bovine serum, and exposed to the extracts when they were in the log phase of their growth. Another microdilution method (Upcroft and Upcroft, 2001) and 96-well flat-bottomed plates (Costar, Cambridge, MA) were used to evaluate activity against the *En. histolytica* cultures. In brief, *En. histolytica* trophozoites (6×10^4/well) were incubated, for 48 h at 37°C and under anaerobic conditions, in the presence of different concentrations (0.8–10 mg/ml) of a crude extract in 10% DMSO. Each test included metronidazole, at concentrations varying between 0.01 and 2 μg/ml, as a positive control, and diluent (i.e. extract-free culture medium with an appropriate concentration of DMSO) as a negative control. After the incubation, an inverted microscope was used to check the appearance and numbers of trophozoites in the wells, each well being scored from 1 (indicating total inhibition of growth) to 4 (indicating no inhibition). Although these scores allowed the inhibitory concentrations to be roughly determined, Trypan-Blue staining and haemocytometer counts were used to get more accurate estimates. For this, the plates were chilled, to release the trophozoites from the plate surface, so that the trophozoites from each well could be stained with Trypan Blue and the non-viable (blue-staining) and viable trophozoites separately counted in a haemacytometer. A median inhibitory concentration (IC_{50}) and an IC_{90} were determined for each tested extract, as the lowest concentrations that inhibited (as indicated by the rounding up of the cells before they were chilled and/or staining with Trypan Blue) at least 50% and 90%, respectively, of the number of non-rounded/viable cells in the negative-control well. These experiments were performed in duplicate and repeated at least three times.

Cytotoxicity *in Vitro*

Nineteen extracts, from a total of 15 plant species, were also tested for their cytotoxicity against cultures of Vero cells. Monolayers of the cells were prepared in 96-well microtitre trays by seeding each well with 200 μl of a suspension (containing 10^5 cells/ml medium) of cells in Eagle's minimum essential medium supplemented with 10% heat-inactivated foetal calf serum. After allowing the cells to settle for 24 h at 37°C, doubling dilutions of an extract, from 8–400 μg/ml, were prepared in medium and added to the cell cultures (at 200 μl/well). Only extract-free medium was added to some of the wells, as a negative control. The cells were then incubated for another 7 days at 37°C and monitored daily. Any microscopically detectable alteration in the cells (i.e. loss of the monolayer, rounding or shrinking of cells, and granulation or vacuolization of the cytoplasm) was recorded. Results were expressed as IC_{50}, defined, for this part of the study, as the lowest extract concentration that inhibited at least 50% of cell growth (compared with that in the extract-free controls) after the 7 days of incubation.

Potential Usefulness of the Extracts in Therapy or Drug Development

To evaluate the potential usefulness of each of the 19 extracts tested for cytotoxicity, the IC_{50} against Vero cells of each such extract was divided by the lowest MIC recorded, for the same extract, against any *Campylobacter* isolate, to give a therapeutic index (TI). To be considered of potential use in the development of new therapies, an extract had to give a TI of at least 1.0.

RESULTS

Anti-*Campylobacter* Activities

At least one extract of each of the 18 plant species investigated had activity against some of the *Campylobacter* isolates (Table 2). The gentamicin used as a positive control was, however, active against every isolate.

Anti-amoebic Activities

Of the 16 extracts tested against *En. histolytica*, six, from a total of four plant species, showed significant activity, with IC_{50} varying between 1.25 and 10 mg/ml and IC_{90} varying between 5 and 10 mg/ml (Table 3). The active extracts, like the metronidazole control, caused rounding and lysis of the trophozoites (Fig. 1).

Cytotoxicity

The IC_{50} recorded in the cytotoxicity assays varied from 2.725 μg/ml for the acetone extract of the bark of *Bauhinia galpinii* to >400 μg/ml for six other extracts (Table 4).

Usefulness of the Plant Extracts

Of the 19 extracts tested for cytoxicity, only five gave TI of ⩾1.0 (Fig. 2).

DISCUSSION

In spite of the high prevalence of *Campylobacter* and *En. histolytica* infection among diarrhoeal patients in the Venda region of South Africa (Samie *et al.*, 2006*a*, *b*), as well as the increasing antibiotic

TABLE 3. *The in-vitro anti-amoebic activities of 16 extracts produced from eight species of medicinal plant*

		Inhibitory concentrations	
	Part and solvent	IC_{50}	IC_{90}
PLANT			
Annona sp.	Fruit, methanol	>10 mg/ml	>10 mg/ml
	Fruit, hexane	5 mg/ml	>10 mg/ml
Cissampelos torulosa	Whole plant, methanol	>10 mg/ml	>10 mg/ml
Lippia javanica	Leaves, acetone	>10 mg/ml	>10 mg/ml
	Leaves (essential oil)	1.25 mg/ml	5 mg/ml
Pouzolzia mixta	Roots, hexane	>10 mg/ml	>10 mg/ml
	Stem, hexane	>10 mg/ml	>10 mg/ml
	Root, methanol	>10 mg/ml	>10 mg/ml
	Leaves, acetone	>10 mg/ml	>10 mg/ml
Pterocarpus angolensis	Bark, methanol	1.25 mg/ml	5 mg/ml
	Bark, hexane	>10 mg/ml	>10 mg/ml
Syzygium cordatum	Bark, acetone	2.5 mg/ml	7.5 mg/ml
	Bark, methanol	5 mg/ml	>10 mg/ml
	Leaves, methanol	2.5 mg/ml	10 mg/ml
Ximenia caffra	Leaves, acetone	>10 mg/ml	>10 mg/ml
Zornia milneana	Whole plant, acetone	>10 mg/ml	>10 mg/ml
Diluent (negative control)		>10 mg/ml	>10 mg/ml
Metronidazole (positive control)		0.05 μg/ml	0.125 μg/ml

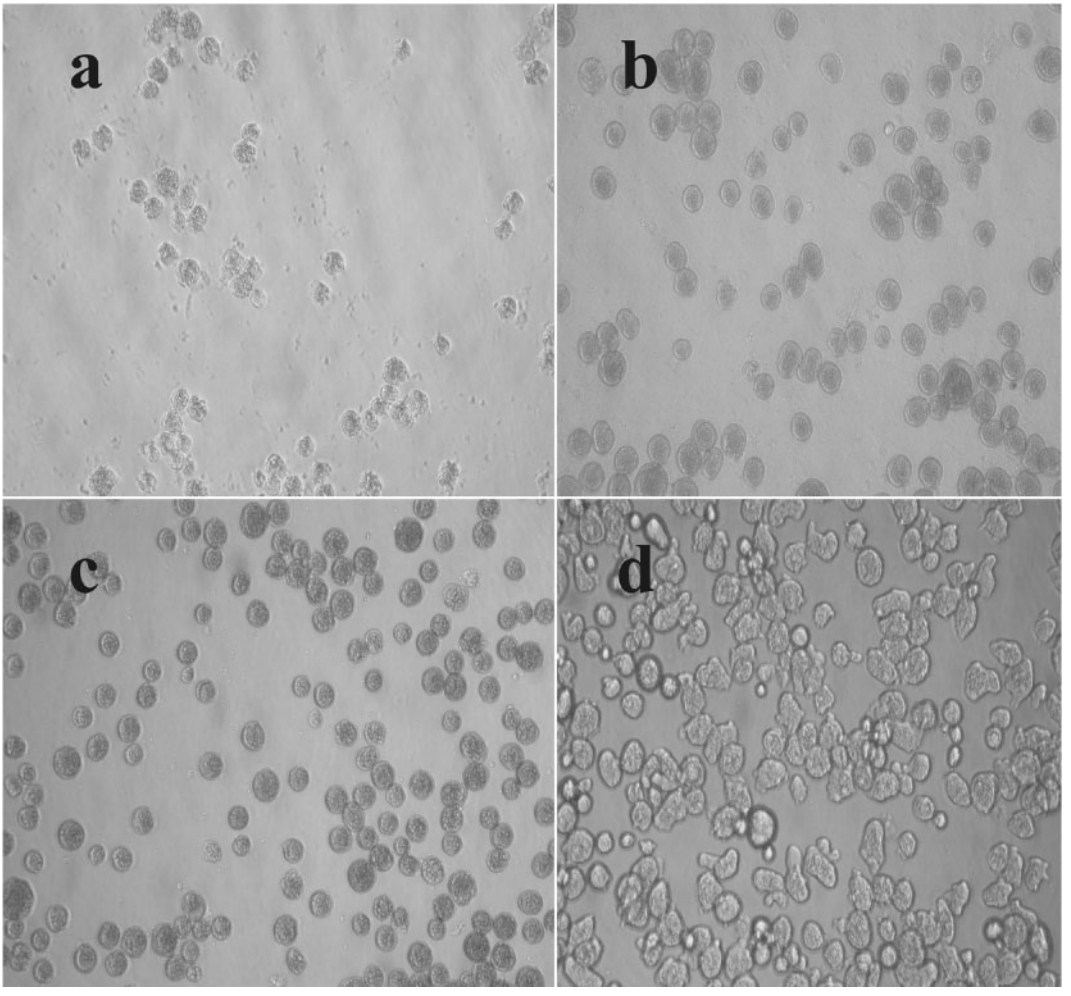

FIG. 1. Cytopathological effects of the metronidazole used as the positive control (a), an essential oil prepared from the leaves of *Lippia javanica* (b), and a bark extract of *Pterocarpus angolensis* (c), on cultures of *Entamoeba histolytica*. Only in the cultures in which the extract diluent was used as a negative control (d) were pseudopodia visible on the amoebae.

resistance of these pathogens in developing countries in general, few studies have examined the activities of the medicinal plants used in Africa against these organisms. In one recent investigation, some compounds from medicinal plants used in Thailand have, however, been found to have good activity against *Campylobacter* spp. (Wannissorn *et al.*, 2005). In the present, South African study, every medicinal plant investigated had some activity against some of the local *Campylobacter* isolates. Although extracts of *Lippia javanica* leaves and *Pterocarpus angolensis* bark appeared to have the highest antibacterial activities, the activities of these two plants against *Campylobacter* spp. (and, in fact, the activity of *Pt. angolensis* against any bacteria) do not appear to have been evaluated before. Although Steenkamp *et al.* (2004) attempted to prepare water and methanol extracts of *Pt. angolensis*, the yields were too small for any antimicrobial tests. Samie *et al.* (2005) found that an acetone extract of *L. javanica* leaves showed consistent activity against all of their (Gram-negative and Gram-positive) test

TABLE 4. *The in-vitro cytotoxic activities of 19 extracts, produced from 15 species of medicinal plant, against Vero cells*

Plant	Part and solvent	Mean value and (S.D.) for the median inhibitory concentration (μg/ml)
Annona sp.	Fruit, hexane	>400
Bauhinia galpini	Bark, acetone	2.7(2.52)
Bridelia micrantha	Bark, methanol	60.01(3.80)
Cissampelos torulosa	Leaves, methanol	206.4(2.32)
Ficus sycomorus	Bark, acetone	261.0(2.50)
Lippia javanica	Leaves, acetone	105.6(6.11)
Mormodica balsamina	Leaves, acetone	>400
Peltophorum africanum	Root, methanol	137.2(3.15)
Pterocarpus angolensis	Bark, ethanol	322.2(0.79)
Pouzolzia mixta	Leaves, acetone	
	Root, methanol	
Rhoicissus tridentata	Tuber, acetone	205.8(3.29)
	Fruit, methanol	111.6(3.63)
Sida alba	Leaves, acetone	352.9(1.05)
Syzygium cordatum	Bark, acetone	37.34(1.93)
	Leaves, methanol	>400
Ximenia caffra	Leaves, acetone	102.6(4.47)
	Bark, acetone	130.8(2.86)
Zornia milneana	Whole plant, methanol	>400

bacteria (which included no *Campylobacter*). Previously, McGaw *et al.* (2000) had reported that extracts of *L. javanica* leaves showed no antibacterial or anti-amoebic activity. In the present study, an acetone extract of *L. javanica* leaves also had no significant anti-amoebic activity (even when tested at 10 mg/ml), although the essential oil from the leaves of this plant gave an IC_{50} against *En. histolytica* of 1.25 mg/ml. The activities of some other plants against *Campylobacter* have been recorded. Paulo *et al.* (1994), for example, found that the activity of an ethanol extract of cryptolepine (the main alkaloid of *Cryptolepis sanguinolenta* — a plant used in traditional medicine in West Africa) against *Campylobacter* was higher than that of co-trimoxazole and sulfamethoxazole, with the *Campylobacter* strains investigated just as susceptible to cryptolepine as to ampicillin. Silva *et al.* (1997) found that the activity, against *Campylobacter* strains, of an ethanol extract of the decorticated roots of *Terminalia macroptera* was similar to that of co-trimoxazole and even higher than that of sulfamethoxazole, although lower than that of tetracycline, erythromycin, ampicillin or streptomycin; ellagitannins appeared to be the major compounds in the extract and the most active fractions of the plant.

Although the activities of many plants against *En. histolytica* have been investigated in the past, the present study appears to be the first to explore the anti-amoebic properties of medicinal plants collected in the Venda region.

Unfortunately, the compounds found in some plant species are highly poisonous to humans (Steenkamp *et al.*, 2004). Many plants hold compounds with good antibacterial or antibiotic activity but, to be useful in clinical medicine, such compounds should either not be toxic to humans or offer such clinical benefits that they may still be useful despite having some toxicity. In the treatment of cancers, some cytotoxicity is, in fact, often beneficial. In the present study, against Vero cells, an extract of the bark of *Ba. galpinii* had the highest level of cytotoxicity recorded. Using a *Salmonella* model, Reid *et al.* (2006) recently found that a leaf extract of *Ba. galpinii* had

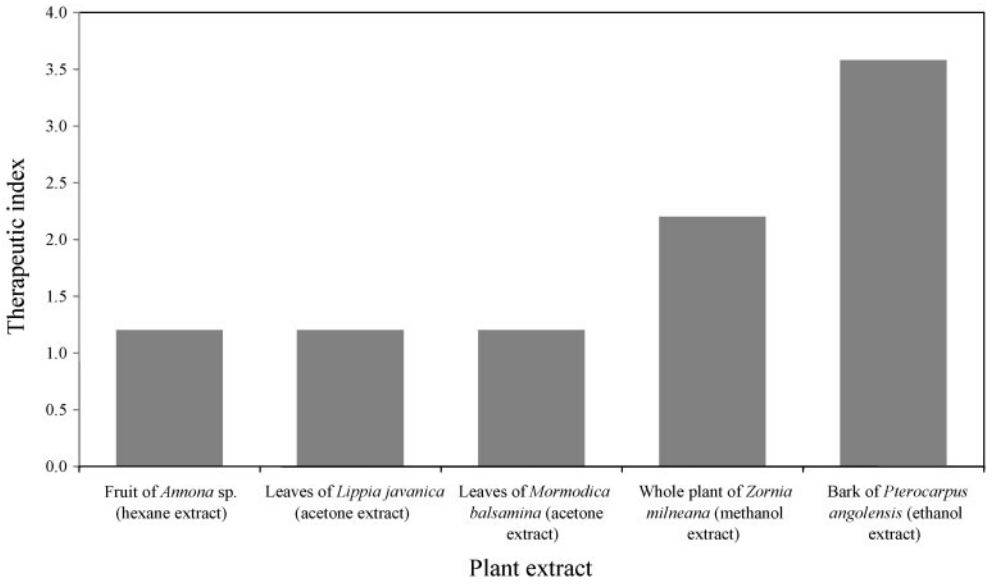

FIG. 2. The therapeutic indices for the most promising extracts (i.e. those showing relatively low cytotoxicity against Vero cells when used at concentrations found to inhibit many of the *Campylobacter* isolates).

antimutagenic activities. Investigation of the potential usefulness of *Ba. galpinii* extracts in cancer therapy appears justified.

Although most of the plant species investigated in the present study have not been tested previously against any *Campylobacter* strains, most were already known to be active against a wide variety of other bacteria, including *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Samie *et al.*, 2005). One of the plant species known to have antibacterial activity, *Syzygium cordatum*, is commonly found in southern Africa. In the present study, a methanol extract of the bark of this tree was very active against *Campylobacter* isolates but had high toxicity, and consequently gave a very low therapeutic index. Although a methanol extract of the leaves was less toxic against the Vero cells, it was also much less active against the *Campylobacter* isolates. Verschaeve *et al.* (2004) found that *Sy. cordatum* was itself slightly genotoxic at 500 μg/ml (P=0.05) but could also protect against mitomycin-C-induced genotoxicity, depending on the concentration used. Elgorashi *et al.* (2003) reported, however, that neither *Sy. cordatum* nor *Pouzolzia mixta* appeared genotoxic when investigated using the Ames test. Any plant used in traditional medicine, or compound from it, should be thoroughly screened for genotoxicity before its long-term usage is recommended.

Kloos *et al.* (1987) reported that *Zornia setosa obvata* gave 100% kill against the schistosome host, *Biomphalaria pfeifferi*, in the Machakos district of Kenya. Confusingly, *Zornia milneana* and *Cissampelos torulosa* are both called *lukandululo* in Venda, although traditional healers in the area of the present study indicated that *Z. milneana* was more active than *Ci. torulosa* in the treatment of childhood diarrhoea (unpubl. obs.), and the present results seem to support this belief. When Benoit-Vical *et al.* (2006) investigated *Mormodica balsamina*, a plant traditionally used against malaria in Niger, they found that the plant did have significant antimalarial activity, both *in vitro* and *in vivo*, and showed no signs of toxicity in healthy mice. Aqueous and ethanol extracts of the same plant species were effective against multiple-drug-resistant strains of *Salmonella*

typhi, with MIC of 9.60–14 μg/ml and minimum bactericidal concentrations of 24–33 μg/ml (Akinyemi *et al.*, 2005). Iwalokun *et al.* (2001) also found low shigellocidal activity in extracts of *Mo. balsamina*.

Campylobacter spp. and *En. histolytica* are both very common among patients attending the hospitals in the Venda region and appear to be significantly associated with diarrhoea and inflammation, especially in children (Samie *et al.*, 2006*a*, *b*). The present results indicate the potential usefulness of some of the plants used by the traditional healers in the region, in the treatment of such diarrhoeagenic infections. Further isolation and characterisation of the active compounds, especially those in the plants giving the higher therapeutic indices, should be a priority of future studies.

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