Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 105 (2006) 286–293
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa

M.C. Mathabe [a], R.V. Nikolova [a], N. Lall [b,*], N.Z. Nyazema [c]

[a] *Biodiversity Discipline, School of Molecular and Life Sciences, University of Limpopo, Private Bag X 1106, Sovenga 0727, South Africa*
[b] *Department of Botany, University of Pretoria, Pretoria 0002, South Africa*
[c] *Department of Pharmacology, Pharmacy programme, University of Limpopo, Private Bag X 1106, Sovenga 0727, South Africa*

Received 30 May 2005; received in revised form 6 January 2006; accepted 25 January 2006

Abstract

The ethnobotanical survey conducted in this study showed that 21 plant species belonging to 14 families are used in traditional medical practice in Limpopo Province, South Africa, for the treatment of diarrhoea. Methanol, ethanol, acetone and hot water extract from different plant parts (leaves, roots, bark and stem rhizome), of several of these plants (*Indigofera daleoides*, *Punica granatum*, *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra*, *Schotia brachypetala* and *Spirostachys africana*), were screened for antibacterial activity against *Vibro cholera*, *Escherichia coli* and *Staphylococcus aureus*, *Shigella* spp., *Salmonella typhi*. The antibacterial activity was determined by agar-well diffusion method and expressed as the average diameter of the zone of inhibition of bacterial growth around the wells. The minimum inhibitory concentration (MIC) of active extracts was determined by using the micro-plate dilution assay. Most of the extracts showed relatively high antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 10 and 31 mm. Of the plants studied, the most active extracts were those obtained from *Punica granatum* and *Indigofera daleoides*. All extracts from two plants, namely, *Punica granatum* and *Ozoroa insignis*, were active against all bacterial strains while only organic extracts of *Indigofera daleoides* inhibited the growth of all tested microorganisms. Water extract of *Punica granatum* were equally active as organic extracts against bacteria such as *Staphylococcus aureus*, *Shigella sonnei* and *Shigella flexneri*. All extracts of *Elephantorrhiza elephantina*, *Elephantorrhiza burkei* and *Ximenia caffra* and *Schotia brachypetala* were not active against *Escherichia coli* and *Salmonella typhi*. The MIC values for active extracts ranged between 0.039 and 0.6 mg/ml. The results obtained appeared to confirm the antibacterial potential of the plants investigated, and their usefulness in the treatment of diarrhoea.

Keywords: Medicinal plants; Antibacterial activities; Diarrhoea

1. Introduction

Intestinal infection is the most common cause of diarrhoea worldwide and is estimated to be responsible for the deaths of 3–4 million individuals each year, particularly infants and young children (WHO, 1996). Acute watery diarrhoea accounts for approximately 80% of such episodes, persistent diarrhoea for 10%, and dysentery for up to 10% (Bhan, 2000). The major burden of infection is due to food-borne infections caused by *Salmonella*, *Campylobacter jejuni* and *Escherichia coli*, and water-borne infections particularly as a result of contamination of domestic water supplies with the cysts of *Giardia intestinals* and *Cryptosporidium parvum*.

The World Health Organization (WHO) recommends the use of oral rehydration therapy, breast and complimentary feeding in children as the cornerstone of managing diarrhoea. Antimicrobial chemotherapeutic agents are also used in the treatment of some bacterial infections which result in diarrhoea. The South African Essential Drugs Programme (1998) has adopted WHO recommendations for the drugs to be used to treat and manage diarrhoea. Be that as it may, people still seek help from traditional healers. They provide alternative health care services including use of medicines derived from plants because they are easily available and cheaper than modern medicine (Otshudi et al., 2000).

* Corresponding author. Tel.: +27 12 420 2524; fax: +27 12 369 5099.
E-mail address: namrita.lall@up.ac.za (N. Lall).

0378-8741/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2006.01.029

Ethnophamacological studies have been conducted to evaluate the effectiveness of traditional medicines used in treating diarrhoea (Ahsan et al., 1996; Menezes and Rao, 1998; Mukherjee et al., 1998; Offiah and Chikwendu, 1999; Chikwendu, 1999; Tona et al., 1999; Rahman et al., 2003; Tangpu and Yadav, 2004). In South Africa, similar studies have been carried out in KwaZulu Natal Province on plants used by Zulu speaking (Lin et al., 2002) traditional healers and in Limpopo Province on plants used by Vhenda speaking traditional healers (Ngobeli, 2002). These studies showed that traditional healers from different localities use different medicinal plants for the treatment of diarrhoea. No studies on plants used by North Sotho speaking traditional healers have been reported. The present study was carried out to document medicinal plants used by North Sotho traditional healers for the treatment of diarrhoea and to investigate their activity against selected diarrhoea causative microorganisms.

2. Materials and methods

2.1. Ethnobotanical survey

An ethnobotanical survey was conducted in three localities, Mentz, Botlokwa and Seshego of the Capricon District in Limpopo Province, South Africa (SA). The selection of this district was based on reports by the Limpopo Health Department in 2002, which indicated diarrhoea outbreaks in the district. The survey was conducted by interviewing four traditional healers (two men and two women) in each locality using the local language. Each interview followed a semi-structured questionnaire designed to obtain the following information: plants used for the treatment of diarrhoea; vernacular plant names; plant parts used; collection of plant material; mode of preparation and administration.

2.2. Plant material

Different plant parts (roots, leaves and bark) or whole plants from selected plants: *Indigofera daleoides* Benth. ex Harv. & Sond. (Fabaceae), *Punica granatum* (L.) (Punicaceae), *Syzygium cordatum* Hochst. ex C. Krauss. (Myrtaceae), *Gymnosporia senegalensis* Loes. (Celastraceae), *Ozoroa insignis* Delile. (Anacardiaceae), *Elephantorrhiza elephantine* (Burch.) Skeels (Fabaceae), *Elephantorrhiza burkei* Benth. (Fabaceae), *Ximenia caffra* Sond. var. caffra (Olacaceae), *Schotia brachypetala* Sond. (Caesalpinaceae) and *Spirostachys africana* Sond. (Euphorbiacea) were collected from the selected localities with the help of the traditional healers. The Herbarium at the University of Limpopo was used as reference point for identification of plants and deposition of voucher specimens (Table 1). Collected plant materials were air-dried under shade at room temperature and then ground with an electric grinder into fine powders which were stored into airtight containers at room temperature.

2.3. Plant extracts

Dried powdered plant materials were extracted with different solvent (methanol, acetone, ethanol and boiling water) for comparative analyses. Ten grams of each plant sample was mixed with 150 ml of each solvent. The mixtures were left overnight on a mechanical shaker at 150 rpm for 24 h at room temperature and then filtered through Whatman No. 1 filter using Büchner funnel. The extracts were further concentrated to dryness under reduced pressure at 37 °C, using a Büchi rotary evaporator. The yields from the different extracts were weighed, recorded and

Table 1
Ethnomedicinal plants used for the treatment of diarrhoea

Species, family, voucher number	Vernacular name	Parts[a]	Preparation/oral administration
Aloe greatheadii Schönl. (Selmar Schönlud) (Liliaceae) MATHABE 12 UNIN	Sekgopha	L	Decoction drunk
Asparagus cooperi Bak. (Asparagaceae) MATHABE 16 UNIN	Lefatšhana	WP	Decoction drunk
Bidens pilosa L. (Asteraceae) MATHABE 5 UNIN	Moshitji	WP	Decoction drunk
Bulbine natalensis Bak. cf. roowortel (Asphodelaceae) MATHABE 14 UNIN		L	Decoction drunk
Carpobrotus edulis (L.) N. E. Br (Aizoaceae) MATHABE 13 UNIN		L	Decoction drunk
Combretum imberbe Wawra. (Combretaceae) MATHABE 10 UNIN		RT	Decoction used to prepare soft porridge
Elephantorrhiza burkei Benth. (Fabaceae) MATHABE 11 UNIN	Lešhitšana	SR	Decoction used to prepare soft porridge
Elephantorrhiza elephantina (Burch.) Skeels (Fabaceae) MATHABE 15 UNIN	Lešhitšana	SR	Decoction used to prepare soft porridge
Guilleminea densa Moq. (Amaranthaceae) MATHABE 8 UNIN		WP	Decoction drunk
Gymnosporia senegalensis Loes. (Celastraceae) MATHABE 3 UNIN	Sephatho	RT	Decoction drunk
Ilex mitis L. Radlk. (Aquifoliaceae) MATHABE 1 UNIN	Monamane	RBK	Decoction drunk
Indigofera daleoides Benth. ex Harv. & Sond. (Fabaceae) MATHABE 7 UNIN		WP	Decoction drunk
Ozoroa insignis Delile. (Anacardiaceae) MATHABE 20 UNIN	Monoko	SBK	Decoction drunk
Punica granatum L. (Punicaceae) MATHABE 19 UNIN	Mokgaranata	RT	Decoction used to prepare soft porridge
Schotia brachypetala Sond. (Caesalpinaceae) MATHABE 2 UNIN	Molope	SBK	Decoction drunk
Sclerocarya birrea (A. Rich) (Anacardardiaceae) MATHABE 4 UNIN	Morula	SBK	Decoction drunk
Solanum supinum Dun. (Solanaceae) MATHABE 21 UNIN	Thola	RT	Decoction drunk
Spirostachys africana Sond. (Euphorbiacea) MATHABE 18 UNIN	Morekhure	SBK	Decoction drunk
Syzygium cordatum Hochst. ex C. Krauss. (Myrtaceae) MATHABE 17 UNIN	Montlho	SBK	Decoction drunk
Waltheria indica L. (Sterculiaceae) MATHABE 6 UNIN		WP	Decoction drunk
Ximenia caffra Sond. Var. caffra (Olacaceae) MATHABE 9 UNIN	Motšhidi	SBK	Decoction used to prepare soft porridge

[a] Parts: WP, whole plant; SBK, stem bark; RT, roots; RBK, root bark; L, leaves; SR, stem rhizome; BLB, bulb.

dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 100 mg/ml. The samples were then stored at 4 °C and further used for antibacterial tests. The yields from water extract of *Indigofera daleoides* obtained by using both, rotary evaporator and freeze drier, were insoluble in DMSO and in water, and therefore, it was not possible to test them for antibacterial activity.

2.4. Antibacterial assays

2.4.1. Bacterial strains

Microorganisms used in the determination of antibacterial activities of different plant extracts were as follows—Gram-positive: *Staphylococcus aureus* ATCC 25923; Gram-negative: *Salmonella typhy* ATCC 0232, *Vibro cholera*, *Escherichia coli* ATCC 35218 and *Shigella* spp. batch 0.57 (*Shigella dysentery*, *Shigella flexneri*, *Shigella sonnei*, *Shihella boydii*). All bacterial strains were obtained from the National Health Laboratory Services (NHLS) Polokwane Provincial Hospital. Different bacterial strains were maintained on nutrient agar (Collee and Marr, 1989) and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37 °C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 Mc Farland standard.

2.4.2. Antibacterial screening

2.4.2.1. Agar-well diffusion assay. The antibacterial tests were performed using agar-well diffusion assay (Perez et al., 1990). Agar plates were prepared using sterile Mueller–Hinton (MH) agar (Biolab). Bacterial strains of standardised cultures were evenly spread onto the surface of the agar plates using sterile swab sticks. Four wells (5 mm diameter) were made in each plate with sterile Pasteur pipettes. Ten microliters of methanol, ethanol, acetone and water extract (100 mg/ml) were added in each well. Ten microliters of DMSO per well was used as a negative control. Discs (5 mm diameter) of nalidixic acid (30 μg), erythromycin (15 μg) and cotrimoxazole (25 μg) were used as positive controls. Diffusion of the extracts and DMSO was allowed at room temperature for 1 h in a laminar flow cabinet. The agar plates were then covered with lids and incubated at 37 °C for 24 h.

The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of the zone inhibition in millimeters. The absence of a zone inhibition was interpreted as the absence of activity. Each extract was tested in triplicate and each experiment was repeated twice.

2.4.2.2. Serial dilution assay for determination of the minimal inhibitory concentration (MIC). A micro-dilution technique using 96 well micro-plates, as described by Eloff (1998) was used to obtain MIC values of the crude extracts against the following bacteria: *Staphylococcus aureus*, *Salmonella typhy*, *Escherichia coli*, *Shigella* spp. and *Vibro cholera*. Each plant extract acetone, methanol, ethanol and water (10 mg/ml), was serially diluted to obtain 2.5 mg/ml starting concentration in the first well. Similar serial dilution was performed for nalidixic acid (1 mg/l), a positive control obtained from Sigma. The starting concentration in the first well after the dilution was 0.25 mg/ml. An equal volume of 100 μl fresh bacterial cultures were added to the wells. Micro-plates were covered with lids and incubated at 37 °C overnight. *P*-Iodonitrotetrazolium violet (Sigma) reagent (0.2 mg/ml) was used to indicate the presence of uninhibited bacterial growth (a pink/purple colour) or inhibition (colourless) of bacterial growth in each well. The lowest concentration of the extract that inhibited the bacterial growth after incubation was taken as the MIC of a crude extract. Only extracts that showed antibacterial activity from agar-well diffusion assay were tested for MIC.

2.5. Statistical analysis

For data on agar diffusion assays, ANOVA was used to test the effects of bacteria within extracts on zone of inhibition using general linear model (GLM) procedure of Statistical Analysis System (SAS, 1998) for randomised complete block design. If significant *P*-values occurred, then comparison of means was done using probability of difference (*P*-diff) facility of SAS.

3. Results

3.1. Ethnobotanical survey

According to traditional healers who took part in the study, the Northern Sotho name for diarrhoea was reported to be letšhologo (acute and watery diarrhoea) or Tenghwibidu (dysentery). Contaminated water, food, utensils, breast feeding and/or teething is considered by traditional healers to be the reasons for diarrhoea outbreaks in the studied localities.

The ethnobotanical survey revealed that 21 plant species belonging to 14 families were used as traditional remedies for the treatment of diarrhoea by different communities in a district of the Limpopo Province (Table 1). From these plants, 13 species are trees and shrubs, 6 species are herbs and 1 species is a climber. It appears that not all plants used by traditional healers have local vernacular names, for example, *Waltheria indica* (Table 1). *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Sclerocarya birrea* and *Schotia brachypetala* were found to be commonly used by traditional healers in three localities while *Indigofera daleoides*, *Punica granatum* and *Asparagus cooperi* were only used in Mentz, Botlakwa and Seshego, respectively. Traditional healers use whole plant or different plant parts (leaves, bark, roots, stem bark and bulbs) for the preparation of remedies for the treatment of diarrhoea with stem bark being the most commonly used plant material. Plants are collected at anytime of the year, depending on their seasonal availability and preferably in the morning.

It appears that plant remedies were prepared using a single plant or occasionally a mixture of plants, for example, *Indigofera daleoides* and *Gymnosporia senegalensis* or *Indigofera daleoides* and *Waltheria indica* as mentioned by one of the traditional healer from Mentz. Hot water is usually used by traditional

Table 2
Antibacterial activities of methanol, ethanol, acetone and water extracts (100 mg/ml) from medicinal plants used traditionally in Limpopo Province

Sample	Bacteria[a] tested zone of inhibition (mm)							
	Sa	*Ec*	*St*	*Vc*	*Sd*	*Ss*	*Sf*	*Sb*
Elephantorrhiza burkei								
Methanol extract	22.0^{e}	R	R	14.6^{d}	16.3^{e}	R	15.0^{d}	10.0^{g}
Ethanol extract	21.3^{f}	R	R	13.3^{f}	15.3^{f}	R	22.3^{b}	10.3^{f}
Acetone extract	21.3^{g}	R	R	14.0^{f}	14.7^{d}	R	23.3^{b}	10.3^{f}
Water extract	22.0^{e}	R	R	19.3^{b}	19.3^{c}	14.3^{b}	17.3^{e}	11.0^{e}
Elephantorrhiza elephantine								
Methanol extract	23.3^{d}	R	R	17.7^{c}	18.3^{d}	15.7^{a}	20.0^{c}	14.0^{f}
Ethanol extract	23.7^{d}	R	R	15.3^{d}	17.7^{e}	R	20.0^{c}	14.0^{e}
Acetone extract	24.0^{d}	R	R	18.0^{c}	19.3^{c}	R	21.7^{c}	14.0^{e}
Water extract	25.0^{c}	R	R	15.7^{d}	18.3^{d}	14.7^{b}	19.7^{c}	15.7^{d}
Gymnosporia senegalensis								
Methanol extract	23.7^{d}	13.3^{b}	12.3^{b}	14.7^{d}	20.3^{c}	14.0^{b}	15.3^{d}	17.0^{d}
Ethanol extract	24.3^{c}	14.0^{b}	12.3^{b}	17.3^{c}	21.3^{b}	14.3^{b}	15.0^{f}	15.7^{d}
Acetone extract	24.3^{c}	12.3^{b}	12.3^{b}	14.3^{e}	21.7^{b}	14.3^{b}	14.7^{h}	18.0^{c}
Water extract	19.3^{f}	R	R	13.5^{f}	13.0^{f}	R	R	R
Indigofera daleoides								
Methanol extract	28.7^{b}	17.4^{a}	17.7^{a}	18.3^{b}	24.3^{a}	14.7^{b}	23.7^{b}	19.3^{b}
Ethanol extract	29.0^{a}	17.0^{a}	17.7^{a}	20.2^{a}	27.0^{a}	16.0^{a}	17.3^{e}	19.7^{a}
Acetone extract	28.0^{b}	16.0^{a}	15.7^{a}	19.6^{b}	25.0^{a}	14.3^{b}	20.0^{d}	19.7^{b}
Ozoroa insignis								
Methanol extract	21.7^{e}	12.0^{b}	11.3^{b}	14.3^{d}	14.7^{f}	13.0^{c}	13.0^{f}	15.0^{e}
Ethanol extract	25.3^{b}	11.0^{d}	11.3^{b}	14.2^{e}	20.3^{c}	12.7^{c}	15.3^{f}	17.7^{b}
Acetone extract	22.0^{f}	12.0^{b}	12.3^{b}	17.3^{d}	21.3^{b}	15.7^{a}	16.7^{g}	18.3^{c}
Water extract	25.3^{b}	15.0^{b}	16.3^{a}	18.7^{c}	20.3^{b}	15.3^{a}	18.3^{d}	19.7^{b}
Punica granatum								
Methanol extract	29.3^{a}	13.0^{b}	13.3^{b}	20.0^{a}	21.7^{b}	13.3^{c}	25.3^{a}	18.7^{c}
Ethanol extract	29.3^{a}	13.0^{c}	13.3^{b}	18.9^{b}	19.7^{d}	13.0^{c}	30.0^{a}	16.3^{c}
Acetone extract	29.3^{a}	11.7^{b}	16.0^{a}	21.3^{a}	21.0^{b}	13.0^{c}	30.7^{a}	21.0^{a}
Water extract	27.0^{a}	18.0^{a}	16.6^{a}	21.3^{a}	22.3^{a}	12.7^{c}	30.7^{a}	21.3^{a}
Spirostachys africana								
Methanol extract	23.3^{e}	11.3^{c}	10.0^{c}	14.3^{f}	21.0^{b}	R	18.3^{e}	14.3^{f}
Ethanol extract	23.0^{d}	R	R	18.7^{c}	18.7^{d}	R	15.3^{f}	15.3^{d}
Acetone extract	22.3^{e}	11.3^{c}	10.0^{c}	14.3^{f}	21.0^{b}	R	18.3^{f}	14.7^{d}
Water extract	23.0^{d}	R	R	18.7^{c}	18.7^{d}	R	15.7^{f}	15.3^{d}
Schotia brachypetala								
Methanol extract	23.3^{e}	R	R	14.0^{f}	13.3^{g}	R	13.3^{f}	R
Ethanol extract	19.7^{e}	R	R	14.7^{e}	14.7^{g}	R	R	R
Acetone extract	23.3^{e}	R	R	14.7^{e}	14.3^{d}	R	13.3^{i}	R
Water extract	19.7^{e}	R	R	14.3^{f}	14.0^{e}	R	R	R
Syzygium cordatum								
Methanol extract	19.3^{h}	R	12.0^{b}	15.0^{c}	20.7^{c}	R	18.3^{e}	20.7^{a}
Ethanol extract	25.0^{b}	13.0^{b}	11.7^{b}	19.3^{b}	18.7^{d}	13.0^{d}	19.3^{d}	18.3^{b}
Acetone extract	19.3^{h}	R	12.0^{b}	14.7^{e}	14.7^{d}	R	18.0^{f}	17.7^{c}
Water extract	25.0^{b}	12.9^{c}	11.7^{b}	19.3^{b}	18.7^{d}	12.7^{c}	20.3^{b}	18.3^{c}
Ximenia caffra								
Methanol extract	25.0^{c}	R	R	14.0^{e}	12.0^{h}	R	15.0^{d}	14.3^{f}
Ethanol extract	25.3^{b}	R	R	13.3^{f}	12.7^{h}	R	15.0^{f}	R
Acetone extract	23.0^{e}	R	R	13.0^{g}	13.0^{e}	R	19.0^{e}	R
Water extract	20.7^{e}	R	R	13.0^{g}	12.0^{g}	R	12.0^{g}	R
Nalidixic acid (10 μg)	35.0	21.3	21.7	22.3	30.0	23.3	22.3	29.0
Erythromycin (15 μg)	23.7	R	R	19.7	14.7	R	14.3	14.0
Contrimoxazole (25 μg)	17.7	11.3	13.0	R	R	27.0	R	17.3

Column means with same superscripts (a–i) of same plants do not differ significantly ($P < 0.05$).

[a] Bacteria: *Sa*, *Staphylococcus aureus*; *Ec*, *Escherichia coli*; *St*, *Salmonella typhi*; *Vc*, *Vibro cholera*; *Sd*, *Shigella dysentery*; *Ss*, *Shigella sonnei*; *Sf*, *Shigella flexneri*; *Sb*, *Shihella boydii*; R, resistant.

Table 3
Minimum inhibitory concentrations from selected medicinal plants used for the treatment of diarrhoea in Limpopo Province

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)															
	Sa	*Ec*	*St*	*Vc*	*Sd*	*Ss*	*Sf*	*Sb*	*Sa*	*Ec*	*St*	*Vc*	*Sd*	*Ss*	*Sf*	*Sb*
	Methanol								Ethanol							
Elephantorrhiza burkei	0.156	–	–	0.312	0.156	–	0.156	0.625	0.156	–	–	0.312	0.312	–	0.078	0.625
Elephantorrhiza elephantina	0.156	–	–	0.312	0.156	0.625	0.156	0.625	0.156	–	–	0.312	0.312	–	0.078	0.625
Gymnosporia senegalensis	0.156	0.312	0.312	0.156	0.078	–	0.156	0.312	0.156	0.156	0.156	0.156	0.156	–	0.156	0.312
Indigofera daleoides	0.078	0.078	0.156	0.078	0.078	0.312	0.078	0.156	0.039	0.078	0.078	0.039	0.039	0.312	0.156	0.156
Ozoroa insignis	0.156	0.078	0.312	0.156	0.078	0.625	0.156	0.312	0.156	0.156	0.156	0.156	0.156	0.312	0.156	0.312
Punica granatum	0.078	0.078	0.156	0.078	0.078	0.312	0.078	0.156	0.078	0.078	0.156	0.078	0.039	0.312	0.039	0.156
Spirostachys africana	0.156	–	0.625	0.156	0.156	–	0.312	–	0.156	0.156	–	0.156	0.312	–	0.312	0.625
Schotia brachypetala	0.156	–	–	0.312	0.156	–	0.312	–	0.156	–	–	0.312	0.312	–	0.312	–
Syzygium cordatum	0.312	0.312	0.312	0.156	0.156	–	0.156	0.156	0.156	0.156	0.156	0.156	0.156	–	0.156	0.156
Ximenia caffra	0.156	–	–	0.312	0.156	0.312	–	–	0.312	–	–	0.156	0.156	–	0.156	–
	Acetone								Water							
Elephantorrhiza burkei	0.312	–	–	0.156	0.156	–	0.078	0.312	0.156	–	–	0.156	0.625	–	0.156	0.312
Elephantorrhiza elephantina	0.312	–	–	0.156	0.156	–	0.078	0.312	0.156	–	–	0.156	0.625	0.625	0.156	0.312
Gymnosporia senegalensis	0.312	0.156	0.156	0.312	0.156	–	0.156	0.156	0.312	0.156	–	0.312	0.312	–	–	–
Indigofera daleoides	0.078	0.156	0.156	0.078	0.078	0.312	0.156	0.156	–	–	–	–	–	–	–	–
Ozoroa insignis	0.156	0.156	0.156	0.078	0.078	0.312	0.156	0.156	0.156	0.625	0.625	0.156	0.312	0.312	0.156	0.625
Punica granatum	0.078	0.156	0.156	0.039	0.078	0.312	0.039	0.078	0.078	0.156	0.156	0.156	0.312	0.156	0.156	0.156
Spirostachys africana	0.312	–	0.156	0.156	0.312	–	0.156	0.156	0.156	0.625	–	0.156	0.312	–	0.312	0.312
Schotia brachypetala	0.312	–	–	0.156	0.156	–	0.156		0.312	–	–	0.156	0.625	–	0.312	–
Syzygium cordatum	0.312	–	–	0.156	0.156	–	0.156	0.156	0.078	0.625	0.156	0.156	0.312	–	0.312	0.625
Ximenia caffra	0.312	–	–	0.156	0.625	–	0.156	–	0.312	–	–	0.156	0.625	–	0.312	–
+Control (nalidixic acid)	0.001	0.007	0.007	0.007	0.003	0.007	0.007	0.001	–	–	–	–	–	–	–	–

[a] Bacteria: *Sa*, *Staphylococcus aureus*; *Ec*, *Escherichia coli*; *St*, *Salmonella typhi*; *Vc*, *Vibro cholera*; *Sd*, *Shigella dysentery*; *Ss*, *Shigella sonnei*; *Sf*, *Shigella flexneri*; *Sb*, *Shihella boydii*; R, resistant; positive; (–), not performed.

healers to prepare their remedies. The dose given to the patient depends on age, and it ranged from three teaspoons of decoction per day for children to one cup (three times) per day for adults. In some cases, the decoction was mixed with maize meal to prepare soft porridge and is given to patients once a day.

3.2. Antibacterial screening

3.2.1. Agar-well diffusion assay

As shown in Table 2, the extracts from different plant species studied showed some antibacterial activity against all/or some of the tested diarrhoea causative microorganisms, with the diameters of zone of inhibition ranging between 10 and 31 mm. There were significant differences ($P < 0.05$) in the antibacterial activity of methanol, ethanol, acetone and water extract from *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra*, *Schotia brachypetala* and *Spirostachys africana*. Of the plants studied, the most active extracts were those obtained from *Punica granatum* and *Indigofera daleoides*. All organic solvent (methanol, ethanol, acetone) and water extract from two plants, namely, *Punica granatum* and *Ozoroa insignis* showed positive antibacterial activity against all bacterial strains. There were no significant differences ($P < 0.05$) in activity between organic solvents and water extract of *Punica granatum* against *Staphylococcus aureus*, *Shigella sonnei* and *Shihella boydii*. Methanol, ethanol and acetone extracts of *Indigofera daleoides* inhibited the growth of all tested microorganisms. There were no significant differences ($P < 0.05$) between the antibacterial activity of methanol, ethanol and acetone extracts of *Indigofera daleoides* against *Escherichia coli*, *Salmonella typhy* and *Shigella dysentery*. All extracts of *Elephantorrhiza elephantina*, *Elephantorrhiza burkei* and *Ximenia caffra* and *Schotia brachypetala* were not active against *Escherichia coli* and *Salmonella typhy*.

All the bacteria in the study were sensitive to nalidixic acid with *Staphylococcus aureus*, *Shigella dysentery* and *Shihella boydii* being the most sensitive (inhibition zone values of 35, 30 and 29 mm, respectively). *Escherichia coli*, *Salmonella typhy* and *Shigella sonnei* were resistant to erythromycin. *Vibro cholera*, *Shigella dysentery* and *Shigella flexneri* were found to be resistant to contrimoxazole.

3.2.2. Minimum inhibitory concentration

Table 3 shows MIC values of the active extracts on all tested microorganisms. The MIC values obtained in this study from all plant extracts tested ranged from 0.625 to 0.039 mg/ml. The highest MIC value of 0.625 was observed for methanol, ethanol and water extract from *Elephantorrhiza elephantina* and *Elephantorrhiza burkei*, depending on the bacterial strains. Ethanol and acetone crude extracts of *Indigofera daleoides* and *Punica granatum* had the lowest MIC value of 0.039 mg/ml against most tested diarrhoea causative microorganisms. MIC values of *Indigofera daleoides* ethanol extracts reflect high sensitivity of *Staphylococcus aureus*, *Vibro cholera* and *Shigella dysentery* to this extract. *Vibro cholera*, *Shigella dysentery* and *Shigella flexneri* were found to be susceptible to ethanol and acetone extracts of *Punica granatum*. Nalidixic acid, which was used as a positive control, had MIC values ranging from 0.001 to 0.007 mg/ml. *Staphylococcus aureus*, *Vibro cholera*, *Shigella dysentery* and *Shigella flexneri* with high sensitivity to some extracts, were also sensitive to nalidixic acid at a concentration three times lower than crude extracts.

4. Discussion and conclusions

As seen from the results obtained from the ethnobotanical survey part of the present study, there were 21 plant species used by North Sotho traditional healers in selected areas in the Capricon District Limpopo Province. The high diversity of medicinal plants used in the treatment of diarrhoea in the Capricon District could be attributed to different interpretations on what caused diarrhoea and the abundance of a particular plant in that specific locality. Different plants from those found in this study, were reported to be used for diarrhoeal treatment by Vhenda (Ngobeli, 2002) and Zulu (Lin et al., 2002) speaking traditional healers. *Punica granatum*, *Syzygium cordatum* and *Spirostachys africana* have also been reported to be used for diarrhoeal treatment in other parts of the world (Chinemana et al., 1985; Bandeira et al., 2000; Tabuti et al., 2003; Vidal et al., 2003). The fact that these plants are used in other parts of the world may suggest their pharmacological potential in diarrhoeal treatment.

Most of the plants used by traditional healers in the Capricon District were trees and the widely sought after plant part in the preparation of remedies was the bark. The bark was harvested at any time of the year. This removal of the bark from a tree has been reported to accelerate the death of a tree (Grace et al., 2002). It was not surprising, therefore, that during the study it was difficult to obtain bark from some trees such as *Ximenia caffra* and *Spirostachys africana* since some of the trees were dead due to debarking. This may be a good reason to encourage conservation among the communities and traditional healers by domesticating some of these plants, an idea being promoted by a lot of other researchers (Kala, 2000; Heywood and Iriondo, 2003; Shinwari and Gilani, 2003).

Of the plants studied, the most active extracts were those obtained from *Punica granatum* and *Indigofera daleoides*. Nothing has been reported on *Indigofera daleoides* with regard to its antimicrobial activity. However, other species such as *Indigofera dendroides*, *Indigofera longeraceae* and *Indigofera oblingifolia* were found to have antimicrobial activity (Dahot, 1999; Esimone et al., 1999; Thangadurai et al., 2002). Results obtained in this study on antibacterial activity of *Punica granatum*, seem to agree with those obtained by Ahmad and Beg (2001) who reported that alcohol extracts of *Punica granatum* fruits showed antibacterial activity when tested against *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysentery*. Prashanth et al. (2001) also reported the methanolic extracts of *Punica granatum* fruit rind to be active against all tested microorganisms. The present study showed that *Punica granatum* organic extracts from roots have similar activity like that of fruits as reported by the two aforesaid authors.

The antibacterial activity shown by the water extract of *Punica granatum* in this study could be of interest since traditional healers use water generally as a way of preparing remedies

from medicinal plants. This study showed that *Punica granatum* water extract were equally active as organic extracts against Gram-negative bacteria such as *Shigella sonnei* and *Shihella boydii*. This is in contrast to the work done by other researchers who have reported water extract to show low activities especially towards Gram-negative bacteria, as compared to organic extracts (Shale et al., 1999; Lall and Meyer, 2000; Matu and Van Staden, 2003). This seemed to confirm the antibacterial potential of *Punica granatum* and its use in traditional medical practice. Some researchers have attributed this type of activity to the presence of water soluble tannins which are well known to possess antimicrobial properties (Djipa et al., 2000; Otshudi et al., 2000). New gallotannins and ellagitannins isolated from *Punica granatum* fruit rind have been reported to be the principal components responsible for the antimicrobial action (Hussein et al., 1997; Machado et al., 2003; Vidal et al., 2003).

In this study, *Staphylococcus aureus*, *Vibro cholera* and *Shigella dysentery*, some of which showed resistance to contrimoxazole, were found to be the most susceptible bacteria to all tested extracts obtained from different plants. Our results agree with those that have reported the susceptibility of *Staphylococcus aureus* and *Shigella dysentery* to some plant extracts (Ilori et al., 1996; Machado et al., 2003; Omer and Elnima, 2003). In the present study, it was found that *Escherichia coli* and *Salmonella typhy*, Gram-negative bacteria resistant to erythromycin, were also resistant to all extracts of *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra* and *Schotia brachypetala* except for water extract of *Elephantorrhiza elephantina* and *Elephantorrhiza burkei*. This was in agreement with what has been reported that Gram-negative bacteria are resistant to most plant extracts (Lall and Meyer, 2000). From the MIC results obtained in the present, it can be concluded that plants that showed low MIC (0.039 mg/ml), in particular ethanol and acetone extracts of *Indigofera daleoides* and *Punica granatum*, could be a good source of bioactive components with antimicrobial potency. According to Rios et al. (1988), plant extracts that are active at concentration 100 μg/ml when using the micro-plate dilution method could be considered to have good an antimicrobial potency level.

The results obtained in this study appear to confirm the antibacterial potential of the plants investigated, and their usefulness in the treatment of diarrhoea that may be as a result of infection.

Acknowledgements

National Research Foundation, Pretoria is thanked for financial assistance. We are grateful to traditional healers who were consulted during the study for sharing their knowledge and information.

References

Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74, 113–123.

Ahsan, M., Chowdhury, A.K.A., Islam, S.N., Ahmed, Z.N., 1996. Garlic extract and allicin: broad spectrum antibacterial agents effective against multiple drug resistant strain of *Shigella dysentery* type 1 and *Shigella flexneri*, *enterotoxigenic Escherichia coli* and *Vibro cholerae*. Phytotherapy Research 10, 329–331.

Bandeira, S., Gaspar, F., Pagula, F.P., 2000. Ethnobotany and Healthcare in Mozambique. http://www.aaas.org/international/africa/moz/bandeira.html.

Bhan, M.K., 2000. Current and future management of childhood diarrhoea. International Journal of Antimicrobial Agents 14, 71–73.

Chikwendu, U.A., 1999. Antidiarrhoeal effects of *Ocium gartissium* leaf extract in experimental animals. Journal of Ethnopharmacology 68, 327–330.

Chinemana, F., Drummond, R.B., Mavi, S., De Zoysa, I., 1985. Indigenous plant remedies in Zimbabwe. Journal of Ethnopharmacology 14, 159–172.

Collee, J.G., Marr, W., 1989. Cultivation of bacteria. In: Mackie and Mc Cartney Practical Medical Microbiology, 30 ed. Churchhill Livingstone, Edinburg, Scotland, pp. 432–454.

Dahot, M.U., 1999. Antibacterial and antifungal activity of small protein of *Indigofera oblingifolia* leaves. Journal of Ethnopharmacology 64, 277–283.

Djipa, C.D., Delmée, M., Quetin-Leclercq, J., 2000. Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). Journal of Ethnopharmacology 71, 307–313.

Eloff, N.J., 1998. A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.

Esimone, C.O., Adikwu, K.N., Muko, K.N., 1999. Antimicrobial properties if *Indigofera dendroides* leaves. Fitoterapia, 517–520.

Grace, O.M., Prendergast, H.D.V., Van Staden, J., Jager, A.K., 2002. The status of bark in African traditional health care. South African Journal of Botany 68, 21–30.

Heywood, V.H., Iriondo, J.M., 2003. Plant conservation: old problems, new perspectives. Biological Conservation 113, 312–335.

Hussein, S.A.M., Barakat, H.H., Merfort, I., Nawwar, M.A., 1997. Tannins from the leaves of *Punica granatum*. Phytochemistry 45, 819–823.

Ilori, M.O., Sheteolu, A.O., Omonigbehin, E.A., Adeneye, A.A., 1996. Antidiarrhoeal activities of *Ocium gratissimum* (Lamiaceae). Journal of Diarrhoeal Disease Research 14, 283–285.

Kala, C.P., 2000. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalya. Biological Conservation 93, 371–379.

Lall, N., Meyer, J.J.M., 2000. Antibacterial activity of water and acetone extracts of roots of *Euclea natalensis*. Journal of Ethnopharmacology 72, 313–316.

Lin, J., Puckree, T., Mvelase, T.P., 2002. Antidiarrhoeal evaluation of some medicinal plants used by Zulu traditional healer. Journal of Ethnopharmacology 79, 53–56.

Machado, T.B., Pinto, A.V., Pinto, M.C.F.R., Leal, I.C.R., Silva, M.G., Amaral, A.C.F., Kuster, R.M., Netto-dosSantos, K.R., 2003. In vitro activity of Brazilian medicinal plants naturally occurring naphthoquinones and their analogues, against methicillin resistant *Staphylococcus aureus*. International Journal of Antimicrobial Agents 21, 279–284.

Matu, E.N., Van Staden, J., 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology 87, 35–41.

Menezes, A.M., Rao, V.S., 1998. Effect of *Astronium urundeuva* (Aroeira) on gastrointestinal transit in mice. Brazilian Journal of Medical and Biological Research 213, 531–533.

Mukherjee, P.K., Saha, K., Murugesa, T., Mandal, S.C., Pal, M., 1998. Screening of antidiarrhoeal profile of some plant extracts of a specific region of West Bengal, India. Journal of Ethnopharmacology 60, 85–89.

Ngobeli, A.M., 2002. Biological and chemical screening of plants used traditionally to treat diarrhoea in children. Department of Botany, University of Venda for Science and Technology, Masters Thesis.

Offiah, V.N., Chikwendu, U.A., 1999. Antidiarrhoeal effects of *Ocium gartissium* leaf extract in experimental animals. Journal of Ethnopharmacology 68, 327–330.

Omer, M.E.F.A., Elnima, E.I., 2003. Antimicrobial activity of *Ximenia americana*. Fitoterapia 74, 122–126.

Otshudi, A.L., Vercruysee, A., Foriers, A., 2000. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). Journal of Ethnophamarcology 71, 411–423.

Perez, C., Paul, M., Bazerque, P., 1990. Antibiotic assay by agar-well diffusion method. Acta Biologiae et Medicine Experimentalist 15, 13–115.

Prashanth, D.I., Asha, M.K., Amit, A., 2001. Antibacterial activity of *Punica granatum*. Fitoterapia 72, 171–173.

Rahman, M.T., Khan, O.F., Saha, S., Alimuzzaman, M., 2003. Antidiarrhoeal activity of the bark extract of *Careya arborea* Roxb. Fitoterapia 74, 116–118.

Rios, J.L., Recio, M.C., Villar, A., 1988. Screening methods for natural products with antimicrobial activity: a review of literature. Journal of Ethnopharmacology 23, 127–149.

SAS institute, 1998. Statistical Analysis System user's guide. SAS institute Inc., Cary, M.C.

Shale, T.L., Stirk, W.A., Van Staden, J., 1999. Screening of medicinal plants used in Lesotho for antibacterial and anti-inflammatory activity. Journal of Ethnopharmacology 67, 347–354.

Shinwari, Z.K., Gilani, S.S., 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). Journal of Ethnopharmacology 84, 289–298.

Tabuti, J., Dhillion, S.S., Lye, K., 2003. Traditional medicine in Bulamogi Country, Uganda: its practitioners, uses and viability. Journal of Ethnophamarcology 85, 119–129.

Tangpu, V., Yadav, A.K., 2004. Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in albino mice. Fitoterapia 75, 39–44.

Thangadurai, D., Viswanathan, M.B., Ramesh, N., 2002. *Indigofera bietone*. A novel abietane diterpenoid from *Indigofera longeracemosa* with potential antituberculous and antibacter activity. Pharmazie 59, 336.

Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Piters, L., Totte, J., Vlientinck, A.J., 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. Phytomedicine 6, 59–66.

Vidal, A., Fallarero, A., Peña, B.R., Medica, M.E., Gra, B., Rivera, F., Gutierrez, Y., Vuorela, P.M., 2003. Studies on the toxicity of *P. granatum* L. (Punicaceae) whole fruit extracts. Journal of Ethnopharmacology 89, 295–300.

World Health Organisation, 1996. Water and Sanitation: Fact sheet, vol. 112. Geneva, World Health Organisation.