UNIVERISTY OF ZULULAND

AN ETHNOBOTANICAL AND ANTIDIARRHOEAL INVESTIGATION OF PLANTS USED TRADITIONALLY IN THE MAPUTALAND AREA HOMESTEADS

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AN ETHNOBOTANICAL AND ANTIDIARRHOEAL INVESTIGATION OF PLANTS USED TRADITIONALLY IN THE MAPTUALAND AREA HOMESTEADS

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Declaration

I, Mduduzi Nkosinathi Nkwanyana hereby declare that this research report is my own work. It is being submitted for the degree of Master of Science in Botany at the University of Zululand. It has not been submitted before for any degree or examination at this or any university.

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Supervisor:

Date:

Abstract

Zulu people living in the rural area of Maputaland (KwaZulu-Natal, South Africa) rely heavily on medicinal plants, particularly for the treatment of diarrhoea. The main aim of this study was to determine which plants are used in this area to treat diarrhoea and to validate their antidiarrhoeal efficacy against diarrhoeal-related pathogens.

An ethnobotanical survey was conducted in four different localities in northern Maputaland (Mabibi, Mseleni, Mbazwana/ Olakeni and Tshongwe) on plants that are used to treat diarrhoeal infections. Twenty three plant species were documented and collected in the survey. A muthi market survey was also performed where three more plant species namely *Sarcophyte sanguinea*, *Ximenia caffra* and *Strychnos henningsii* were identified to treat diarrhoea. *Acacia burkei, Brachylaena transvaalensis, Cissampelos hirta* and *Sarcostemma viminale* were recorded for the first time globally as antidiarrhoeal remedies. *Psidium guajava* was the most mentioned (43%) plant to be used traditionally to treat diarrhoea followed by *Catharanthus roseus* and *Melia azedarach*. Most of the interviewees mentioned the use of plants individually but some mentioned the use of plants in combinations. The following plants were said to be used in combination; *Brachylaena transvaalensis* with *Psidium guajava*; *Sclerocarya birrea*, *Acanthospermum glabratum* in combination with *Krauseola mosambicina*; *Psidium guajava* and *Mangifera indica* in combination with *Sarcophyte sanguinea*. Most plants were used as leaf decoctions. The study also revealed that the choice of plants used was based on the availability of the plant in and around the interviewees' homestead. One new vernacular name was recorded which demonstrates the importance of recording this information.

Antidiarrhoeal studies were performed with the crude extracts against diarrhoeal pathogens. Organic extracts of *Terminalia sericea* showed noteworthy antibacterial activity (mean MIC value of 0.04 mg/ml against *Shigella flexneri*). Many other plant species showed noteworthy activities against different pathogens. More than 80% of plant species screened were active against at least one out of seven of the diarrhoeal pathogens. *Proteus vulgaris* showed overall the least susceptibility, while *Shigella flexneri* proved to be the most susceptible pathogen. Aqueous extracts generally showed poorer antimicrobial activity with some exceptions i.e. *Acacia*

burkei, Garcinia livingstonei, Sclerocarya birrea and *Terminalia sericea*. The antibacterial activity of plant species collected from the homesteads demonstrated better activity compared to those collected from the muthi markets. In the combination studies, synergy (ΣFIC < 0.50) was observed against at least four or more pathogens. Combinations of *Acanthospermum glabratum* with *Psidium guajava* and *Brachylaena transvaalensis* with *Psidium guajava*, proved to be the most favourable combinations. Some aqueous extracts in combination showed synergistic interactions. The combination of *Acanthospermum glabratum* with *Krauseola mosambicina* showed (76%) synergistic interactions when investigated in various ratios. The results from this study correlates to a certain extent with the use of particular plant species to treat diarrhoea infections. Furthermore there is some correlation between the best antimicrobial activities and the most frequently used plant species and plant combinations used by the lay people in the Maputaland area.

Publication

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Dedication

To my late grandmother Bongiwe Ndwandwe. I hope that this report will serve to demonstrate that hard work, perseverance, dedication and most importantly discipline pays off.

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First and foremost, I must give thanks to my creator God almighty for guiding and giving me strength throughout this lengthy journey. If it wasn't for Him I wouldn't be where I am today.

Secondly for myself for putting in an effort even if it seemed endless and proving to the world what I am capable of. If it wasn't for this piece of work I wouldn't have realised the strength and knowledge I have or lack for that matter.

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Abbreviations

Aids - Acquired immunodeficiency syndrome

ATCC - American Type Culture Collection

CFU - Colony Forming Units

DMSO - Dimethylsulfoxide

ETEC - Enterotoxigenic *Escherichia coli*

FIC - Fractional inhibition concentration

g - Gram

HIV - Human immune virus

hrs - Hours

INT - *p*-Iodonitrotetrazolium Violet

Mg - milligram

mg/ml - milligram per millilitre

MIC - Minimum inhibition concentration

MRSA - Methicillin Resistant *Staphylococcus aureus*

ND - Not Determined

NS - Not susceptible

Ors - orally

Spp. - Species

WHO - World Health Organisation

µl - Micro Litre

°C - Degrees Celsius

Chapter 1

Introduction

1.1 Plants as source of medicine

More than 50% of all drugs in clinical use in the world today are derived from natural products and their derivatives. As an example, more than two thirds of the anticancer drugs approved between the 1940s and 2006 are either natural products, or were developed on the knowledge gained from natural products (Newman and Cragg, 2007). Other well known examples of plant derived medicines include quinine, morphine, codeine, colchicine, atropine, reserpine, digocin, taxol and vincristine (Van Wyk and Wink, 2004). In this context, natural product research and herbal therapies have become thriving fields of research. Medicinal plants are thus something of the future and not of the past (Van Wyk *et al*., 2009). When conserving traditional medicinal knowledge, it is important that inventories of plants with therapeutic value are carried out and the knowledge related to their uses documented systematically. Other than conserving traditional knowledge these kinds of studies can have other values to the communities such as to help identify plants species with market value and thus helping communities generate incomes. When communities generate income it is perceived as an important motivation for the conservation of local plant species. Furthermore, studies related to herbal medicines can help to stimulate confidence in traditional medicine and enhance appreciation among local communities. As a result, local communities may have a higher appreciation of the value of their plant resources and take extra effort in conservation strategies (Tabuti *et al*., 2003).

1.2 Medicinal plants in South Africa

Ethnobotanical surveys in South Africa have shown that medicinal plants are the main life supporters for many rural people (Van Wyk *et al*., 2008; Dahlberg and Trygger, 2009; De Beer and Van Wyk, 2011). This was believed to be caused by the high cost of western medicine which makes it difficult for poorer communities, whereas it is easier to access traditional medicinal plants. Mander *et al.* (2006) demonstrated that for many South Africans, traditional medicine is part of a culture and tradition; and is not considered at all inferior to western medicine. It is rural

people's belief that traditional medicine treats a wide range of health problems that western medicine does not. Mander *et al*. (2006) reported that 84% of patients attending a Durban (RSA) based clinic used traditional medicines, with only 18% of the patients indicating that they may reduce their use of traditional medicines in the future. However, 97% of traditional healers' patients indicated that their use of traditional medicine was by choice and not as a result of access and cost issues associated with western medicines. Furthermore the study showed that traditional medicine was often more expensive (the communities pay more to traditional healers) compared to the clinic fees, and thus dispelling the myth that traditional medicine is a cheaper alternative (Mander *et al.*, 2006).

Unemployment, HIV/AIDS and rapid urbanization has led to high demand of traditional medicine thus over harvesting of wild medicinal plants is a major threat. Presently, 550 medicinal plant species are being sold at the muthi markets, of which at least 86% of these plant species when harvested will result in the death of the entire plant (Mander *et al.*, 2006). At least 13 300 rural households are said to survive with the money they get by selling medicinal plants. Campbell (2007) stated that South Africa's traditional medicine industry is estimated to be worth 2.9 billion rand. Thus the control of harvesting plants is needed so as not to cause extinction of some medicinal plants.

It is with no doubt that South Africa has a wide diversity of flora which boasts with a number of species being endemic to the country. In the editorial research done by Van Staden (2008), it is stated that about 70% of South Africans use plants for their cultural and health issues. The paper look at the reviews that have been published in a number of fields including commercially important South African plants and southern African plants that treat specific ailments and their potential to be used to develop novel drugs for anti-microbial, anti-malarial and anti-cancer treatment. Few reviews were also seen on plants used for mental illnesses and veterinary treatment. The genera of commercially useful medicinal plants were said to be mainly *Aloe, Hermannia, Helichrysum, Sceletium, Salvia, Commiphora* and *Vitex*. Commercially important herbal teas included *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides* which are distributed around the world now. *Agathosma betulina, Agathosma crenulata*, *Pelargonium sidoides, Hoodia gordonii*, *Hypoxis*

hemerocallidea and *Sutherlandia frutescens* are among the othernoteworthy species that were reviewed. Also considered, was the key issue on the efficacy and safety of medicinal plants. Such interest in medicinal plants has resulted in a board that has been implemented to provide a framework for the institutionalization of African Traditional Medicine in the South African health care system (Van Staden, 2008).

1.3 Diarrhoea in developing countries

Diarrhoea is a major problem in developing countries including South Africa as it is also an opportunistic disease for the HIV/AIDS patients (Agunu *et al*., 2005; Njoroge and Kibunga, 2007). It has been stipulated that about 6.9% of deaths in the low income countries are caused by diarrhoea making it the third leading cause of deaths in these countries (WHO, 2010). In 2004, it was documented that diarrhoea was the second leading cause of death in children under the age of five, after pneumonia and 80% of the 1.5 billion children mortality were under the age of two (WHO, 2010). In South Africa, more than 25 000 deaths of all premature mortality rates were said to be caused by diarrhoeal diseases in 2005 (Basson, 2009). Diarrhoea is normally noticed by passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual).There are three types of diarrhea observed normally, firstly it's the acute watery diarrhoea which lasts several hours or days and sometimes includes cholera. The second one is the acute bloody diarrhoea also called dysentery and lastly the persistent diarrhoea which lasts 14 days or longer.

Diarrhoea is mainly caused by a variety of bacterial, viral and parasitic organisms that lead to the infection of the intestinal tract. Contaminated food, contaminated drinking-water, poor hygiene or person-to-person transmissions are the main causes of diarrhoeal spread. Diarrhoea leads to severe dehydration which can be fatal if body fluids and electrolytes are not replenished, either through the use of oral rehydration salts (ORS) solution, or through an intravenous drip (WHO, 2010). Bacterial diarrhoea may be caused by Gram-positive bacteria such as *Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Enterococcus faecali*s and Gram-negative bacteria such as *Escherichia coli*, *Shigella flexneri, Proteus mirabilis, Salmonella typhimurium* and *Serratia marcescens* (Atlas, 1995; Giannella, 1996; Shen *et al*., 1996; Ryan and Ray, 2004; Yang and Yang, 2005). Diarrhoea is easily

preventable and treatable. The consumption of clean water, good sanitation and hygiene and vaccination against viruses that cause diarrhoea can reduce cases of infection. Rehydration with solutions that are rich in salts, consultation of health care facilities, eating nutrient rich food and zinc supplements helps treat diarrhoea (WHO, 2010). Medicinal plants such as *Acacia karoo, Elephantorrhiza elephantine, Psidium guajava, Strychnos henningsii, Ziziphus mucronata* and many more has been reported to treat diarrhoea (Lin *et al*., 2002; Njoroge and Kibunga, 2007; Appidi *et al*., 2008; Goncalves *et al*., 2008). The main concern is that very few of these plant species has been clinically proven to treat diarrhoea (Otshudi *et al*., 2000). Thus the present study is likely to reveal some novel plants species that are reported for the treatment of diarrhoea.

1.4 Study area

The study area (northern Maputaland) is one of the most poverty stricken areas in South Africa, where the availability of clean drinking water and sanitary ablutions are particularly problematic (Municipal Demarcation Board South Africa, 2009). Under these conditions diarrhoea is a major concern to resident rural communities. In a case study done in 2007 in the same area as the current study (Mbazwana) 12 plants were identified for treating diarrhoea (De Wet *et al*., 2008). These plants were tested against four ATCC strains for their antibacterial activity with promising results. Of the 12 plants tested, five were known for their antibacterial activity i.e. *Sclerocarya birrea, Psidium guajava, Syzigium cordatum, Melia azedarach* and *Vernonia oligocephala*, while the rest were investigated for the first time. Medicinal uses of two plants were recorded for the first time i.e. *Krauseola mosambicina* and *Secamone filiformis*, while two other plants were recorded for the first time to be used to treat diarrhoea i.e. *Krauseola mosambicina* and *Catharanthus roseus*. This case study emphasized that medicinal plants play an important role in the primary health care for these rural people and that there was a need to continue with these types of investigations as there is still a wealth of undiscovered ethnobotanical information to be documented in this remote area of KwaZulu-Natal.

The present study intends to broaden the investigation in this area on plants growing in the homesteads and plants sold at a couple of muthi markets for the treatment of diarrhoea. A comparison between these plants will give an indication of the efficacy

of the homestead plants. Plants collected from the homesteads are a sustainable way of harvesting and managing medicinal resources, especially in Maputaland, which is one of South Africa's HOTSPOTS "hot spots" (Scott-Shaw, 1999). The sustainable cultivation of these plants could then be encouraged in the communities, instead of buying plants at the muthi markets.

1.5 Aims and objectives of the project

The first objective of this project was to document the ethnobotanical knowledge on antidiarrhoeal plant use by the lay people of Mapulaland. The second objective was to validate whether the plants used to treat diarrhoea are antimicrobially effective when tested against diarrhoeal pathogens. These objectives can be subdivided into the following aims;

- To perform an ethnobotanical survey on plants used for diarrhoea treatment.
- To collect and identify the plants.
- To test for the antidiarrhoeal activity using the minimum inhibition concentration (MIC) assays.
- To perform interactive plant combination studies to determine the efficacy when plants are used together.
- To evaluate the antibacterial activity of plants which are sold on muthi markets for treatment of diarrhoea in the Maputaland region.

1.6 The significance of the study

This study is the first to be conducted in this specific region to investigate plants growing in the Homestead (Muzi's), which are used to treat diarrhoea. The study will also establish the antibacterial efficacies of these plant species against microorganisms that cause diarrhoeal infections. As the study is community based research, the findings from the research will help the researcher to motivate elders to pass the knowledge to the youngsters.

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Chapter 2

Materials and Methods

2.1 Ethnobotanical survey

An ethnobotanical survey of antidiarrhoeal plants was conducted during February-March 2008 in four different localities in the Maputaland area. Figure 2.1 shows the four localities namely: Mabibi, Mbazwana, Mseleni and Tshongwe. Ethical clearance was obtained from the ethics committee at the University of Zululand for the study (document S623/08). The survey was conducted by interviewing people from 72 homesteads (Muzis), (+/-20 homesteads per area). The interviews were conducted in Zulu with a structured questionnaire (Appendix 1). The questionnaire was designed to obtain the following information: sociodemographic data (age, gender and educational background), vernacular plant names, plant parts used, preparation and mode of administration. Each interviewee had to sign a form of consent which explains the projects aims before being interviewed (Appendix 2). A Zulu interpreter (Simon Khumalo) helped with the interviews. The interpreter used Zulu language to explain the whole process and those that could not sign their forms of consent used an X as the way of signing. As a token of appreciation each interviewee received a 2 kg bag of rice after being interviewed.

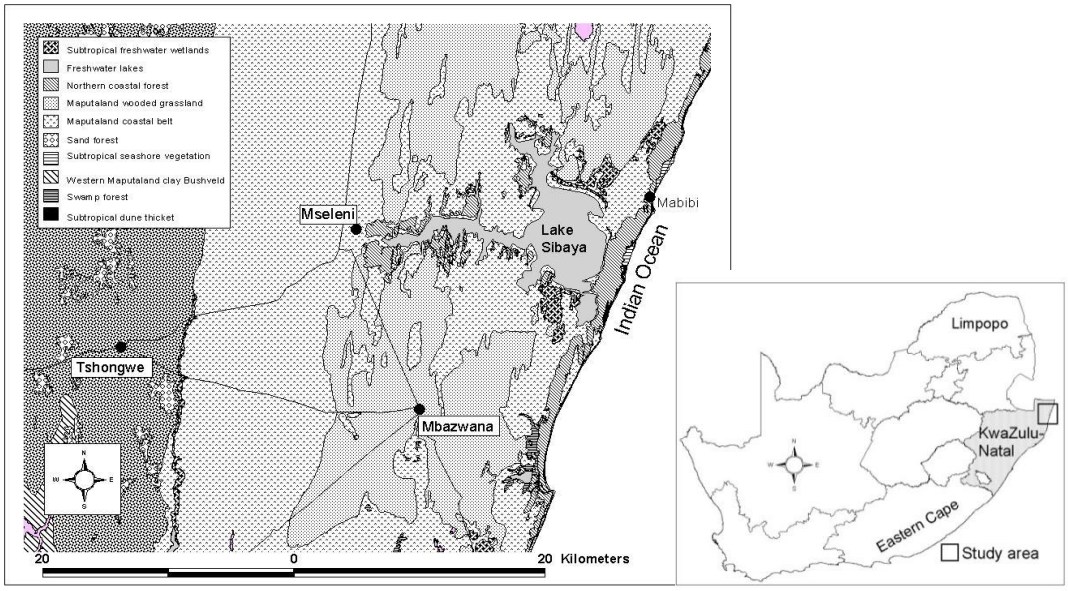

Figure 2.1 Study area – northern Maputaland located in KwaZulu-Natal province.

Plant species documented in the survey were collected, identified and voucher specimens were deposited in the herbarium at the Department of Botany, University of Zululand. Mr Ngwenya from the South African National Biodiversity Institute KwaZulu-Natal Herbarium identified the plant samples. The 23 plant species collected, with its voucher number and the locality of collection are shown in Table 2.1.

Table 2.1 Localities and voucher specimens of the plants collected from the Maputaland homesteads for antidiarrhoeal screening. ZULU = Herbarium, University of Zululand.

Plant species	**Part used**	**Voucher specimen**	**Localities**
Acacia burkei Benth	Bark	MN Nkwanyana 3 ZULU	Lake Sibaya, Mseleni, garden of Mary Khanyile, [2732 BC (Ubombo)]
Acanthospermum glabratum (DC) Wild	Whole plant	MN Nkwanyana 37 ZULU	Lake Sibaya, Mseleni, garden of Tholakele Ndlovu, [2732 BC (Ubombo)]
Brachylaena transvaalensis E.Phillips & Schweick	Leaves	MN Nkwanyana 30 ZULU	Mabibi, garden of Robert Zikhali, [2632 DD (Bela Vista)]
Catharanthus roseus (L.) G. Don.	Roots	MN Nkwanyana 7 ZULU	Lake Sibaya, Mseleni, garden of Duduzile Maphanga, [2732 BC (Ubombo)]
		MN Nkwanyana 32 ZULU	Mabibi, garden of Robert Zikhali, [2632 DD (Bela Vista)]
Chenopodium ambrosioides L.	Wholeplant	MN Nkwanyana 39 ZULU	Lake Sibaya, Mseleni, garden of Tholakele Ndlovu, [2732 BC (Ubombo)]
Cissampelos hirta Klotzch	Wholeplant	MN Nkwanyana 27 ZULU	Mabibi, garden of Thenjiwe Dlamini, [2632 DD (Bela Vista)]
Garcinia livingstonei T. Anderson	Bark	MN Nkwanyana 19 ZULU	Tshongwe, garden of Jabu Mabika, [2732 AD]
Gymnosporia senegalensis (Lam.) Loes.	Leaves	MN Nkwanyana 13 ZULU	Lake Sibaya, Mseleni, garden of Bonani Qwabe, [2732 BC (Ubombo)]

Table 2.1 Continued…

Plant species	Part used	Voucher specimen	Localities
Krauseola mosambicina (Moss.) Pax & K. Hoffm.	Whole plant	MN Nkwanyana 6 ZULU	Lake Sibaya, Mseleni, garden of Duduzile Maphanga, [2732 BC (Ubombo)]
		MN Nkwanyana 28 ZULU	Mabibi, garden of Nyameka Mehlomakhulu, [2632 DD (Bela Vista)]
Lippia javanica (Burm.f.) Spreng.	Leaves	MN Nkwanyana 20 ZULU	Lake Sibaya, Mseleni, garden of Emah Nxumalo, [2732 BC (Ubombo)]
Mangifera indica L.	Leaves	MN Nkwanyana 29 ZULU	Mabibi, garden of Lindokuhle Ngubane, [2632 DD (Bela Vista)]
Melia azedarach L.	Leaves	MN Nkwanyana 4 ZULU	Lake Sibaya, Mseleni, garden of Bonani Qwabe, [2732 BC (Ubombo)]
		M.N. Nkwanyana 10 ZULU	Lake Sibaya, Mseleni, garden of Mary Khanyile, [2732 BC (Ubombo)]
		MN Nkwanyana 22 ZULU	Lake Sibaya, Mseleni, garden of Zibangile Skhakhane, [2732 BC (Ubombo)]
		MN Nkwanyana 31 ZULU	Mabibi, garden of Robert Zikhali [2632 DD (Bela Vista)]
Psidium guajava L.	Leaves	MN Nkwanyana 5 ZULU	Lake Sibaya, Mseleni, garden of Mary Khanyile, [2732 BC (Ubombo)]
		MN Nkwanyana 14 ZULU	Lake Sibaya, Mseleni Garden of Zibangile Skhakhane, [2732 BC (Ubombo)]
		MN Nkwanyana 17 ZULU	Lake Sibaya, Mseleni, garden of Lindiwe Zikhali, [2732 BC (Ubombo)]
		MN Nkwanyana 21 ZULU	Lake Sibaya, Mseleni, garden of Marita Mnguni, [2732 BC (Ubombo)]
		MN Nkwanyana 24 ZULU	Tshongwe, garden of Thembi Mhlongo, [2732 AD]
		MN Nkwanyana 26 ZULU	Mabibi, garden of Thenjiwe Dlamini, [2632 DD (Bela Vista)]

Table 2.1 Continued…

Plant species	Part used	Voucher specimen	Localities
Sarcostemma viminale (L) R. Br	Stem	MN Nkwanyana 11 ZULU	Lake Sibaya, Mseleni, garden of Bonani Qwabe, [2732 BC (Ubombo)]
Schotia brachypetala Sond.	Bark	MN Nkwanyana 25 ZULU	Tshongwe, garden of Doreen Mavundla, [2732 AD]
Sclerocarya birrea (A. Rich.) Hochst. subsp. *caffra* (Sond.)	Bark	MN Nkwanyana 12 ZULU	Lake Sibaya, Mseleni, garden of Bonani Qwabe, [2732 BC (Ubombo)]
		MN Nkwanyana 18 ZULU	Tshongwe, garden of Thembi Mhlongo, [2732 AD]
		MN Nkwanyana 34 ZULU	Mabibi, garden of Ms. Promise Mbonambi, [2632 DD (Bela Vista)]
Senna occidentalis (L) Link	Roots	MN Nkwanyana 2 ZULU	Lake Sibaya, Mseleni, garden of Jabhisa Zikhali, [2732 BC (Ubombo)]
		MN Nkwanyana 23 ZULU	Lake Sibaya, Mseleni, garden of Thembinkosi Mkhize, [2732 BC (Ubombo)]
Strychnos madagascariensis Pior.	Leaves	MN Nkwanyana 9 ZULU	Lake Sibaya, Mseleni, garden of Bonani Qwabe, [2732 BC (Ubombo)]
		MN Nkwanyana 15 ZULU	Tshongwe, garden of Hlambase Gumede, [2732 AD]
Syzygium cordatum Hochst. ex C. Krauss.	Bark	MN Nkwanyana 36 ZULU	Mabibi, garden of Fikile Mdletshe, [2632 DD (Bela Vista)]
Terminalia sericea Burch. ex DC.	Bark	MN Nkwanyana 16 ZULU	Tshongwe, garden of Thembi Mhlongo, [2732 AD]
Trichilia emetica Vahl	Bark	MN Nkwanyana 35 ZULU	Mabibi, garden of Promise Mbonambi, [2632 DD (Bela Vista)]
Vangueria infausta Burch. subsp. *infausta*	Bark	MN Nkwanyana 38 ZULU	Mabibi, [2632 DD (Bela Vista)]
Vernonia natalensis (DC) Sch. Bip. ex. Walp	Roots	MN Nkwanyana 33 ZULU	Mabibi, garden of Robert Zikhali, [2632 DD (Bela Vista)]

2.2. Muthi market collection

A pilot survey was undertaken on two KwaZulu-Natal muthi markets namely Mtuba muthi market in the square of Mtubatuba town and Mona muthi market approximately one kilometer outside Nongoma town. The muthi market sellers were asked if they sold any traditional medicine that is used to treat diarrhoea. The mentioned plant species were then bought for analysis. The collected plants were identified by using vernacular names and then confirming with scientific names. This type of identification is not reliable as different species might have the same vernacular name but this is the only way to identify muthi market specimens. Table 2.2 shows the plants collected from two different muthi markets.

Table 2.2 Plants collected from the Muthi markets for antidiarrhoeal screening.

Plant species	**Vernacular names**	**Part used**	**Muthi market localities**
Sarcophyte sanguinea Sparrm. Sparrm.	Umavumbuka , Ihlule	Roots	Mona
			Mtuba
Sclerocarya birrea (A. Rich.) Hochst. subsp. *caffra* (Sond.)	Umganu	Bark	Mona
Strychnos henningsii Gilg	Umqalothi	Bark	Mtuba
Ximenia caffra Sond.	Umthunduluka, uMgwenya	Roots	Mona

2.3 Sample extraction

The collected plant samples were dried at ambient temperature and ground into fine powder with a hammer mill (Scientec RSA hammer mill 430). The powder was then stored in airtight containers at ambient temperature for further use for the antimicrobial screening assays. Two types of plants extractions were prepared from the ground plant material. One organic and one aqueous extract for each plant species. The first extract (organic) was prepared by submerging 10 g of the dried macerated plant material into 100 ml of a 1:1 mixture of dichloromethane and

methanol. The extract was heated to 30 ºC for 24 hours. Thereafter it was filtered, evaporated and stored at 4 ºC. The aqueous extract was prepared to mimic actual preparation in the homesteads. An aqueous extract was prepared by mixing 10 g of the macerated plant material to 100 ml of hot, sterile, distilled water, which was then kept at ambient temperature overnight. Thereafter it was filtered and stored at -80 ºC before lyophillisation (Van Vuuren and Viljoen, 2006). The dried and lyophillised plant extracts were then re-dissolved in acetone or alternatively in dimethylsulfoxide (DMSO) if the plant didn't dissolve in acetone. The extracts that were dissolved in DMSO were: *Krauseola mosambicina, Strychnos madagascariensis, Syzygium cordatum* and one *Melia azedarach* sample. The reconstituted extracts were kept at 4 ºC for further antibacterial screening.

2.4 Antimicrobial assays

2.4.1 The microorganisms

The following microorganisms were selected for this study based on their association with stomach ailments and diarrhoea;

Bacillus cereus (ATCC 11778)

*Enterococcus faecali*s (ATCC 29212)

Escherichia coli (ATCC 8739)

Proteus vulgaris (ATCC 33420)

Salmonella typhimurium (ATCC 14028)

Shigella flexneri (ATCC 25875)

Staphylococcus aureus (ATCC 12600)

All ATCC reference numbers have been omitted in the following Chapters for the sake of brevity.

2.4.1.1 *Bacillus cereus*: is an endemic, soil-dwelling, Gram-positive, rod-shaped, beta hemolyticbacteria that causes food borne illness. These bacteria are facultative aerobes and like many members of the genus *Bacillus* can produce endospores (Ryan and Ray, 2004). *Bacillus cereus* is responsible for (2–5%) of all food borne illnesses and the symptomoms are severe nausea, vomiting and diarrhoea (Kotiranta *et al*., 2000). *Bacillus* related food borne illnesses occur as a result of survival of the bacterial endospores due to improperly cooked food (Turnbull, 1996). Germination of

the endospore can be observed mainly when food is improperly refrigerated (McKillip, 2000). Bacterial growth results in production of an enterotoxin and ingestion may lead to two types of illness, diarrhoeal and emetic (vomiting) syndrome (Ehling-Schulz *et al*., 2004). The first type is associated with a wide-range of food, has an 8–16 hour incubation time and is associated with diarrhoea and gastrointestinal pain. The long-incubation known form of *B. cereus* food poisoning makes it difficult to distinguish it from food poisoning caused by *Clostridium perfringens* (Todar, 2006). Rice that is improperly cooked and improperly refrigerated tends to be the main cause of food poisoning. Nausea and vomiting is normally observed as a sign of infection 1-5 hours after consumption. It is hard to differentiate it from other food borne pathogens like *Staphylococcus aureus* (Todar, 2006).

2.4.1.2 *Enterococcus faecalis*: formerly classified as part of the Group D *Streptococcus* system is a Gram-positive commensal bacterium that is a normal flora of the gastrointestinal tracts of humans and other mammals (Ryan and Ray, 2004). A commensal organism like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity (Ryan and Ray, 2004). *Enterococcus faecalis* can cause endocarditis, as well as bladder, prostate and epididymal infections; nervous system infections are less common (Pelletier, 1996; Ryan and Ray, 2004). In Nigeria *Enterococcus faecalis* has been identified as one of the causes of diarrhoea during its rainy season as it was isolated from drinking water and some vegetables (Nzeako and Okafor, 2002). *Enterococcus feacalis* is said to cause nosocomial infections such as intraabdominal infections (Franz *et al.,* 1999). Enterococci are opportunistic pathogens that may cause diarrhoea (Mavziutov *et al*., 2007).

2.4.1.3 *Escherichia coli:* is a Gram-negative, non-sporulating, facultatively aerobic bacilli that usually inhabit the gastrointestinal tract of humans and other warm-blooded animals. The pathogenic strains of these bacteria are the causative agents of diarrhoea and urinary tract infections. Some strains cause enterohemorrhagic gastroenteritis and meningitis in children/infants (Atlas, 1995). *Escherichia coli*

bacteria which produce enterotoxins are known as entero toxigenic *Escherichia coli* (ETEC). Travelers' Diarrhoea and approximately half of all diarrhoeal infections in high risk regions are mainly caused by entero toxigenic *Escherichia coli* (ETEC). ETEC infection leads to profuse, watery diarrhoea, which although usually self limiting can be very uncomfortable and disruptive to normal activities. One of the major ETEC toxins is similar in structure and function to the cholera toxin that causes the intestinal fluid imbalance leading to watery diarrhoea. In China, entero toxigenic *Escherichia coli* and *Proteus* spp. are the major pathogens responsible for diarrhoea, followed by *Shigella* spp. (Shen *et al*., 1996).

2.4.1.4 *Proteus vulgaris*: is a rod-shaped, Gram-negative bacterium that is a normal flora of the intestinal tracts of humans and animals. It is also found in soil, water and faecal matter. This pathogen is grouped with the enterobacteriaceae and is an opportunistic pathogen in humans. It is also known to be the culprit behind urinary tract infections (Mavziutov *et al*., 2007). As mentioned previously, *Proteus* spp. are also one of the major pathogenic groups to be responsible for diarrhoea in China (Shen *et al*., 1996).

2.4.1.5 *Salmonella typhimurium*: is a rod shaped, flagellated, Gram-negative bacterium that can lead to a form of human gastroenteritis sometimes referred to as salmonellosis (Giannella, 1996). Salmonellosis infections cases observed in the United States is caused mainly by *Salmonella typhimurium*. Salmonellosis leads to diarrhoea, fever, and abdominal cramps that are observed 12 to 72 hours after infection and the sickness may last for up to seven days. *Salmonella typhimurium* can be transmitted through the faeces of people or animals. The incidence of non-typhoid salmonellosis (which is caused by *Salmonella enterica typhi*) is increasing worldwide, causing millions of infections and many deaths in the human population each year (European Bioinformatics Informatics, 2009).

2.4.1.6 *Shigella flexneri*: is a non-motile, nonspore-forming, facultative anaerobic Gram-negative bacterium. Its non-motile characteristic means that this species doesn't have flagella to facilitate its movement like many other human enterobacteria. *Shigella flexneri* is a rod-shape bacterium which is lactose-

fermenting causing dysentery (Yang and Yang, 2005). *Shigella* is one of the major pathogens responsible for diarrhoea in China (Shen *et al*., 1996; Ying *et al*., 2005). *Shigella* spp. cause bacillary dysentery in human beings, accounting for 20% of the 4.6 million diarrhoea-associated deaths among children (Ying *et al*., 2005). It is a bacterium that is closely related to *E. coli*. Shigellosis that is caused by *Shigella flexneri* is an endemic infection that affects both developed and developing countries. *Shigella* continues to be a major food-borne threat to public health in many developed countries where the issues of sanitation are closely monitored. Uncooked food or contaminated water easily transmits this pathogen. Seventy persent of all cases of Shigellosis are caused by *Shigella sonnei* in the United States (Shiferaw *et al*., 2004).

2.4.1.7 *Staphylococcus aureus*: is a Gram-positive, aerobic, catalase positive, facultatively aerobic small cocci; which also inhabit the human intestines and throat (Atlas, 1995). The pathogenecity is mainly observed in the wide range of infections such as toxic shock syndrome and food poisoning. Staphylococci can be present in raw food of animal origin or in those handled by man. Many of staphylococcal infections are caused by *Staphylococcus aureus* and thus this pathogen is the most important pathogen among the staphylococci. Staphylococcal food-poisoning is caused by the enterotoxins that are produced during the growth period of the pathogens. There are seven identified enterotoxins and those are enterotoxins: A, B, Cl, C2, C3, D and E. Food poisoning is normally caused by the Enterotoxin A (SEA) with as little as 100 to 200 ng causing illness. Sympotoms of this type of food-poisoning are vomiting and diarrhoea. Symptoms manifest two to six hours after ingestion of food containing one or more of these staphylococcal enterotoxins (Ayulo *et al*., 1994).

2.5 Minimum inhibition concentration (MIC) assay

The National Committee for Laboratory Standards (NCCLS) methodology and guidelines as well as Eloff (1998), was used to determine the minimum inhibition concentration (MIC). Minimum inhibition concentration is the lowest concentration at which the plant extract inhibits the bacterial growth. Bacterial cultures were sub-cultured from stock agar plates and grown in Tryptone Soya broth overnight.

Microtitre plates were aseptically prepared by adding 100 µl sterile distilled water into each well. Then, 100 µl of the plant extracts at starting stock concentrations of 64 mg/ml were transferred into the microtitre plate. Serial dilutions were performed leading to a final volume of 100 µl per well in a microtitre plate. The overnight culture was diluted in fresh Tryptone Soya broth at a 1:100 ratio, yielding an approximate inoculums size of 1×10^6 colony forming units (CFU)/ml and 100 µl added to each well. The plates were then covered with a sterile plastic cover to prevent evaporation of volatile compounds. The plates were then incubated for 24 hours at 37 °C, ciprofloxacin (0.02 mg/ml) was used as a positive control while acetone (64 mg/ml) and DMSO (64 mg/ml) were used as negative controls. The positive control was included in each assay to confirm the antimicrobial susceptibility and the negative control was used to confirm the extract antimicrobial activity not the solvent activity. After 24 hours 40 µl of 0.2 mg/ml of *p*-iodonitrotetrazolium (INT) violet (Sigma) was added into all wells of the microtitre plates. The plates were then kept for six hours at ambient temperature before inspection for antibacterial activity. The INT was used as the bacterial growth inhibition indicator where by the pink purple or red colour represented bacterial growth while no change or colourless appearance represented growth inhibition. The lowest concentration at which the plant extract inhibited bacterial growth after six hours was considered as MIC value of the crude extracts. Tests were performed in duplicates and quadruplicates if the results indicated variance of more than one dilution factor.

2.6 The fractional inhibitory concentration (FIC)

Microtitre plates were aseptically prepared by adding 100 µl sterile distilled water into each well of a 96 well microtitre plate. A 1:1 combination was prepared from stock solutions (64 mg/ml for extracts) adding up to 100 µl in each well. An MIC value was determined for these combinations to establish any interaction. Serial dilutions were performed as with MIC assays (Section 2.5). Tests were performed in duplicates and triplicates. The fractional inhibitory concentration (FIC) was then calculated according to the following equation;

$$FIC_a = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

FIC_b = MIC (b) in combination with (a) / MIC (b) independently

a and b represents the two plants. The sum of the FIC, known as the ΣFIC or FIC index and was calculated as;

$FIC_{index} = FIC^{(a)} + FIC^{(b)}$

The FIC index (Schelz *et al*., 2006), was used to determine the correlation between the two plants and may be classified as either synergistic (≤ 0.5), additive (> 0.5-1.0), indifferent (> 1.0-4.0) or antagonistic (> 4.0). Conventional antimicrobials were included in all repetitions as controls and tests were undertaken in triplicate.

2.7 Isobologram studies

The isobologram ratio method (Berenbaum, 1978) was used in this part of the study and involved combining the plant combinations in nine ratios i.e. 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9. The MIC values were determined for all ratios and the plant samples independently. The MIC values (mg/ml) of plant samples in combination relative to the independent MIC values were plotted on an isobologram as a ratio, allowing for a graphical representation of the interaction of the various combinations. The isobologram can be interpreted by examining the data points of the ratios where the MIC values for each concentration is determined in relation to the independent MIC values (shown as a straight line) and extrapolating synergy (below the line), antagonism (above the line) and additive (in the vicinity closest to or on the line). Conventional antimicrobials were included in all repetitions. Tests were undertaken in duplicates and triplicates and the mean values plotted on the isobologram. Results for plant combinations were analysed using isobolograms on Graphpad Prism 5 ® software.

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Chapter 3

Ethnobotany of the study area

3.1 Introduction

Ethnobotany is the study of the way in which people use, manage or classify plants. This provides information about the conservation status and general knowledge of the uses of plants by the local people. It is the largest sub discipline of ethnobiology, and is generally defined as the “science of people's interaction with plants” (Martin, 2004). Ethnobotany includes the study of plants that have medicinal properties, which explain the partnerships between ethnobotanists and pharmacologists. While bioprospecting (exploring biodiversity for new sources of natural products) is still the objective of some pharmacologists, the field of ethnobotany is generally more concerned about the cultural perspective of the relationship between humans and plants than in harvesting for plant pharmaceuticals (McClatchey *et al*., 2009).

Since ancient times, mankind has been utilizing plants for dyes (e.g. indigo), flavours (e.g. vanillin), fragrances (e.g. rose oil), stimulants (e.g. caffeine), hallucinogens (e.g. morphine), insecticides (e.g. nicotine), vertebrate and human poisons (e.g. strychnine) and most importantly as therapeutic agents (e.g. quinine, codeine) (Van Wyk and Wink, 2004). This knowledge has been enriched through experimentation and through observations of animal behavior. For an example, some plants can induce diarrhoea in the browsers therefore the humans will only take that plant as a laxative or if the diarrhoeic animal eats a certain plant and it recovers that plant will be considered a cure (Huffman, 1996). To date more than 80 000 plants are used medicinally worldwide (Foster and Johnson, 2006).

There is evidence that people who live in the rural areas use plants as their primary health measures (Van Wyk *et al*., 2008; Dahlberg and Trygger, 2009: De Beer and Van Wyk, 2011). Many South Africans use traditional medicine as it is part of their culture and tradition. It is not considered an inferior alternative to western medicine. It is a belief that traditional medicine treats a wide range of health problems that western medicine does not treat (Mander *et al*., 2006).

Ethnobotanical studies done in South Africa on antidiarrhoeal plants have mostly concentrated on the knowledge of traditional healers and what has been documented in the literature (Lin *et al*., 2002; Mathabe *et al*., 2006; Appidi *et al*., 2008; Fawole *et al*., 2009). Recently, only two studies have focused on lay people in South Africa. One such study was conducted in the Eastern Cape Province (Appidi *et al*., 2008) and a case study which was restricted to the Mbazwana area (northern Maputaland) where ten homesteads were visited (De Wet *et al*., 2008). Both studies stressed the important role medicinal plants play in the primary health care of rural people. This was also confirmed by Dahlberg and Trygger (2009), where a survey was undertaken on the usage of medicinal plants in the community of Mnqobokazi (north-western part of the Mkuze wetlands on the coastal plain of KwaZulu-Natal).

As many of the rural people are illiterate or semi-illiterate, the knowledge on plant use is often restricted to verbal communications only. Njoroge and Kibunga (2007) have also noted that the upcoming generation is losing touch when it comes to utilization of plants. Urban migration, modernization and mortality rates of the aged result in the disappearance of this knowledge. It is thus important to systematically document the traditional medicinal knowledge of plants with therapeutic value.

3.2 Study area

The study area is situated between 32º22′ and 32º52′ latitudes and 27º15′ and 27º30′ longitudes. The 72 homesteads that were visited were mostly dominated by the following three types of vegetation: the Tembe Sandy Bushveld type, the Maputaland Coastal Belt type and the Maputaland Wooded Grass Land type (Mucina *et al*., 2005). This region is situated in the UMkhanyakude District Municipality at Umhlabuyalingana Local Municipality. According to the data available from the 2001 census, this Local Municipality has a population of approximately 140 952. Only 4% of the households have flushable toilets, and about 27% have pit toilets or buckets and other types of sanitary ablutions. About 69% have no toilets at all. Thirty four percent of the households do not have access to piped water and depend on boreholes, springs, rainwater tanks, dams, pools and river water (Municipal Demarcation Board South Africa, 2001).

3.3 Results

3.3.1 Social demographic information

Eighty percent of the interviewees were women. The average age of the interviewees' was 53 years. The people interviewed had basic education (up to grade 4) or no education at all and only knew how to write their names. In this survey it was found that interviewees with the most information, were elders and the youngsters have no information. Some of the interviewees (five) were deceased a year after the first interviews were conducted (February 2008/2009). The remaining family members have no knowledge on the uses of medicinal plants. Therefore it is very important that knowledge like this is passed on and preserved before it is lost. Most of the interviewees obtained their knowledge from their grandmothers (33%), elders (20%), mothers (19%), neighbours (11%), fathers (9%) and grandfathers (8%). The grandmothers proved to be the main information source of the interviewees. Tables 3.1-3.4 provides information of the interviewees, the localities of their homesteads and the plant species they use to treat diarrhoea. Information on nine of the 12 plant species collected in the Mbazwana area were included from a previous pilot study done in this area on plants used to treat diarrhoea.

Table 3.1 Interviewees and the plants mentioned to treat diarrhoea in the Mbazwana area. Information was taken from a previous study where the GPS readings were not taken (* = plants that were not collected due to unavailability).

Homesteads	Household number	Age	Gender	Plants mentioned
1	67	80	F	*A. malvaceae**
2	66	47	F	*C. roseus*
3	69	73	F	*G. senegalensis*
4	70	37	F	*K. mosambicina*
5	64	56	F	*M. azedarach*
6	63	56	F	*P. guajava*
7	67	80	F	*S. birrea*
8	67	80 & 46	F & F	*S. filiformis**
9	66	54	F	*S. madagascariensis*
10	65	44	F	*S. cordatum*
11	67	80	F	*V. infausta*
12	68	69	F	*V. natalensis*

Table 3.2 Interviewees and the plants mentioned to treat diarrhoea in the Mabibi area (* = plants that were not collected due to unavailability). F= Female; M = Male

Homestead GPs reading	Household number	Age	Gender	Plants used to treat diarrhoea
S 27°20'046" E 32°43'154"	41	50	F	*S. cordatum, Plumbago auriculata**
S 27°20'150" E 32°43'198"	42	>65	F	*S. birrea, G. livingstonei, S. cordatum*
S 27°20'689" E 32°43'055"	43	52	F	*C. roseus*
S 27°20'693" E 32°43'136"	44	28	F	*P. guajava, C. ambrosioides*
S 27°20'692" E 32°43'135"	45	88	F	*S. cordatum, S. birrea*
S 27°20'467" E 32°43'027"	47	16	F	*C. ambrosioides, C. roseus*
S 27°20'448" E 32°43'044"	48	66	M	*Idumbe dumbe*, Strychnos henningsii*, B. transvaalensis, P. guajava*
S 27°20'257" E 32°43'080"	51	25	M	*Sarcophyte sanguinea*, S. henningsii*, C. roseus, M. indica*
S 27°19'865" E 32°43'173"	52	45	F	*Umthusi*. G. malvaceae*, Albizia adianthifolia**
S 27°19'795" E 32°43'233"	53	56	M	*S. henningsii**
S 27°19'793" E 32°43'199"	54	65	F	*S. birrea, T. emetica*
S 27°19'517" E 32°43'297"	55	45	F	*K. mosambicina, A. glabratum, S. serratulloides**
S 27°19'738" E 32°43'447"	56	24	F	*K. mosambicina, P. guajava, A. glabratum*
S 27°19'748" E 32°43'448"	57	>65	F	*C. hirta, A. glabratum, idumbe, G. Senegalensis*
S 27°19'787" E 32°43'568"	58	16	F	*P. guajava, S. birrea,T. emetica, K. mosambicina*
S 27°19'880" E 32°43'614"	59	61	F	*M. azedarach*
S 27°20'005" E 32°43'596"	60	19	F	*M. azedarach, P. guajava, T. emetica*

Table 3.3 Interviewees and the plants mentioned to treat diarrhoea in the Mseleni area (* = plants that were not collected due to unavailability).

Homesteads GPs reading	**Household number**	**Age**	**Gender**	**Plants mentioned**
S 27°22'069" E 32°31'769"	1	>65	F	*C. roseus, S. occidentalis*
S 27°21'030" E 32°31'826"	2	56	F	*M. azedarach, P. guajava, A. marlotti*, A. burkei*
S 27°21'937" E 32°31'805"	3	71	F	*M. azedarach, P. guajava, S. madagascariensis*
S 27°21'842" E 32°32'202"	4	63 & 51	M & F	*P. guajava, C. roseus, S. birrea, S. cordatum*
S 27°21'176" E 32°32'103"	5	76 & 75	M & F	*P. guajava, S. madagascariensis, C. roseus*
S 27°21'722" E 32°31'834"	6	45	F	*P. guajava, S. birrea, S. cordatum*
S 27°21'675" E 32°31'766"	7	57	F	*S. birrea*
S 27°21'699" E 32°31'903"	8	50	F	*P. guajava*
S 27°21'645" E 32°31'894"	9	>65	M	*M. azedarach, P. guajava*
S 27°20'918" E 32°31'497"	10	±30	M	*M. azedarach, P. guajava, S. madagascariensis*
S 27°21'032" E 32°31'379"	11	31	F	*S. madagascariensis, C. roseus, A. glabratum*
S 27°21'091" E 32°31'742"	12	63	M	*M. azedarach, P. guajava, S. madagascariensis, S. birrea, C. roseus, S.occidentalis , G. senegalensis, S. viminale*
S 27°21'288" E 32°31'676"	13	45	M	*P. guajava, S. occidentalis, K. mosambicina*
S 27°21'501" E 32°31'637"	14	72 & 72	M & F	*K. mosambicina, M. azedarach, P. guajava, C. roseus*
S 27°19'556" E 32°33'861"	16	>65	F	*K. mosambicina, M. azedarach, P. guajava, L. javanica*
S 27°19'565" E 32°34'026"	17	26	F	*K. mosambicina, M. azedarach, P. guajava, C. ambroisiodes*
S 27°19'590" E 32°33'909"	18	27	F	*C. roseus*
S 27°19'636" E 32°33'820"	19	>65	F	*C. roseus*
S 27°22'069" E 32°31'769"	20	25	F	*C. roseus, P. guajava*

Table 3.4 Interviewees and the plants mentioned to treat diarrhoea in the Tshongwe area (* = plants that were not collected due to unavailability).

Homesteads GPs reading	Household number	Age	Gender	Plants mentioned
S 27°25'101" E 32°22'390"	21	35	F	*A. glabratum, P. guajava, S. birrea*
S 27°25'819" E 32°22'599"	22	>65	F	*S. birrea, T. sericea, B. transvaalensis*
S 27°24'887" E 32°23'346"	23	>65	F	*S. birrea, S. madagascariensis, G. livingstonei, ubanda*, Erythrophleum iasianthum*, umanono**
S 27°25'265" E 32°23'064"	24	25	F	*S. henningsii*, E. iasianthum**
S 27°24'681" E 32°26'168"	25	29	F	*C. roseus, Sarcophyte sanguinea**
S 27°24'878" E 32°25'648"	26	20	M	*S. madagascariensis, P. guajava, S. birrea, C. roseus*
S 27°24'879" E 32°25'630"	27	63	F	*M. azedarach, C. roseus, S. birrea, A. burkei, S. sanguinea**
S 27°24'926" E 32°25'096"	28	81	F	*A. ambrosioides, S. madagascariensis, M. azedarach*
S 27°25'405" E 32°24'497"	29	30	F	*M. azedarach, S. madagascariensis, G. livingstonei, P. guajava*
S 27°25'565" E 32°24'560"	30	27	F	*P. guajava, M. azedarach, S. birrea, G. senegalensis*
S 27°24'759" E 32°24'763"	31	20	F	*S. birrea, P. guajava, C. roseus, K. mosambicina*
S 27°24'969" E 32°25'064"	32	66	F	*P. guajava, C. roseus , M. azedarach*
S 27°25'015" E 32°24'977"	33	30	F	*C. roseus, S. birrea, S. madagascariensis, isifici**
S 27°25'030" E 32°24'986"	34	38	F	*S. madagascariensis, S. birrea*
S 27°26'190" E 32°22'229"	35	>65	M & F	*P. guajava, C. roseus, M. azedarach*
S 27°26'138" E 32°22'19 "	36	25 & 44	F & F	*P. guajava, C. roseus, M. azedarach, S. madagascariensis, T. sericea, G. senegalensis*
S 27°26'202" E 32°21'884"	37	32 &>65	M & M	*P. guajava, C. roseus, M. azedarach, S. madagascariensis, C. hirta*
S 27°26'204" E 32°21'636"	38	56	F	*S. birrea, V. infausta, G. livingstonei, A. burkei, Acacia robusta*, S. sanguinea*, Ozoroa spp*, Peltophorum africanum**
S 27°26'296" E 32°21'275"	39	23	F	*A. glabratum, T. sericea*
S 27°26'329" E 32°21'311"	40	51	F	*S. birrea, S. brachypetala*

3.3.2 Plants used to treat diarrhoea

A number of plants were identified as possible treatments for diarrhoeal symptoms (Tables 3.1 to 3.4). As part of this study, the recorded uses for these plants are published by De Wet *et al.* (2010). A more detailed approach is given here where the botanical description, geographical description, recorded medicinal uses and ethnobotanical information obtained from the interviewees is provided. Unless otherwise stated all plants are not threatened or endangered with respect to their conservation status.

3.3.2.1 *Acacia burkei* Benth **(Fabaceae)**

Black Monkey Thorn (E), Umkhaya (Z), Swartapiesdoring (A)

Botanical description

This species is a medium size, deciduous tree (Figure 3.1). The main stem is usually branching from midway with a dense spreading crown and the thorns are in pairs. Leaves are unpredictable in size with the pinna and leaflets in pairs. The flowers are white with spikes (Pooley, 2003). Pods are flat, reddish to purplish brown and leathery (Van Wyk and Van Wyk, 1997).

Figure 3.1 *Acacia burkei* tree (photo: H de Wet).

Geographical distribution

It is distributed in the bushveld, wooded grassland and dry river valleys. It includes the northern parts of KwaZulu-Natal, Mpumalanga, Swaziland, Mozambique and Botswana (Pooley, 2003).

Recorded medicinal uses

Roots and bark are used for eye and back complaints (Pooley, 2003).

Ethnobotanical information obtained from interviewees

Acacia burkei bark or leaves are used for the treatment of diarrhoea. The bark or leaves are crushed and mixed with cold or hot water and sieved. Half a mug (+/-125 ml) of the sieved infusion is taken twice a day until diarrhoea subsides. No side effects have been reported. (Gumede N., 2008, pers. comm.; Gumede S., 2008, pers. comm.; Gumede S*, 2008, pers. comm.; Khanyile M., 2008, pers. comm.).

3.3.2.2 *Acanthospermum glabratum* (DC) Wild **(Asteraceae)**

[= *Acanthospermum australe* auct.]

Five seeded starbur (E), Inamathela (Z)

Botanical description

Figure 3.2 shows the leaves and flowers of *Acanthospermum glabratum*.This is an erect or prostrate annual herb, often mat-forming (Herman *et al.*, 2000). The leaves are elliptic, broadly ovate to almost round, base truncate or shortly cuneate, margins sub-entire to dentate, apex acute or obtuse, pubescent but quickly glabrescent, densely glandular. The florets are yellow or greenish cream. Fruits are usually oblong, with a large pore at the apex and have glands (Beentje *et al.*, 2005)**.**

Figure 3.2 *Acanthospermum glabratum* leaves and flowers (photo: H de Wet).

Geographical distribution

Originally from tropical America, *Acanthospermum glabratum* is now naturalized as a weed in southern Africa (Beentje *et al.*, 2005).

Recorded medicinal uses

The whole plant is crushed and mixed with warm water to treat chills fever, runny nose, chest pain sore throat and/or headaches. The mixture is drunk by adults and taken as enema for kids. The whole plant can be boiled withthe leaves of *Tetradenia riparia* and *Lippia javanica* to curb coughs and tiredness (York *et al*., 2011).

Ethnobotanical information obtained from interviewees

This herb grows readily in the homesteads and is used as a remedy for children inflicted with diarrhoea. The whole plant is crushed and mixed with warm water and sieved. The infusion is taken orally. A quarter of a cup for adults and a spoonful (+/- 10 ml) for children are taken three times a day until diarrhoea subsides. It can be administered anally as well. This herb is also used to treat colds and flu. It can also be used in combination with *K. mosambicina* or *P. guajava*. No side effects have been reported.(Nxumalo S., 2008, pers. comm.; Mhlongo T., 2008 pers. comm.; Mehlomakhulu N., 2008 pers. comm.; Ndlovu T., 2008 pers. comm.; Mthembu T., 2008 pers. comm.; Ndlovu M., 2008 pers. comm.).

3.3.2.3 *Brachylaena transvaalensis* E. Phillips & Schweick **(Asteraceae)**

Forest Silver Oak (E), IPhahla, uMphahla (Z), Bosvaalbos, Vaalboom (A)

Botanical description

This plant is a small sized tree of up to 10 m in height. It is a tree with well-spaced leaves. The leaves are hairless above, finely velvety and paler below (Figure 3.3). The leaves are sometimes regularly toothed, more usually toothless. Inflorescences are terminal and lateral, elongated, much-branched with numerous crowded stalkless flower-heads. There are few florets; male florets have sterile unbranched protruding styles and female florets have forked styles (Swaziland flora database, 2010).

Figure 3.3 *Brachylaena transvaalensis* leaves (photo: H de Wet).

Geographical distribution

This species is found in the following provinces: Mpumalanga, Limpopo, Gauteng, Swaziland and Kwazulu-Natal (Boon, 2010).

Recorded medicinal uses

The South African traditional healers use the leaves of *Brachylaena transvaalensis* to treat oral candidiasis (Motsei *et al.*, 2003).

Ethnobotanical information obtained from interviewees

Bark or leaves are crushed and mixed with cold or hot water and sieved. The infusion is then administered anally or orally. Half a mug is taken twice a day until diarrhoea subsides. It can also be used in combination with *S. birrea* or *P. guajava*. No side effects have been reported (Nhlozi M., 2008, pers. comm.; Mhlongo D., 2008, pers. comm.; Zikhali R., 2008, pers. comm.).

3.3.2.4 *Catharanthus roseus* (L.) G. Don. **(Apocynaceae)**

Periwinkle (E), Imbali, ikhwinini (Z)

Botanical description

Figure 3.4 shows a *Catharanthus roseus* plant. This plant is a perennial herb of up to

1 m in height. The leaves are glossy dark green and the midrib is clearly visible. Flowers are either pink or white (Van Wyk *et al.*, 2009).

Figure 3.4 Flowers and leaves of *Catharanthus roseus* (photo: H de Wet).

Geographical distribution

Catharanthus roseus originates from Madagascar but has become naturalized in tropical and subtropical parts of the world. In South Africa it is found along the roads and disturbed areas in KwaZulu-Natal and Mpumalanga (Van Wyk *et al*., 2009).

Recorded medicinal uses

The Siddha medical practitioners of India use*Catharanthus roseus*to treat urinary ailments (Chellappandian *et al.,* 2012). Muda residents of Mozambique use this herb to treat venereal diseases (Bruschi *et al*., 2011). *Catharanthus roseus* is medicinally used to treat diabetes and rheumatism. It is used to treat some forms of cancer including breast and lung cancer, uterine cancer, melanomas and Hodgkin's and non-Hodgkin's lymphoma (Pooley, 2005; Van Wyk *et al*., 2009). It is also used for treating insect bites, warts and gonorrhoea. Tea from the flowers is used for blood cleansing and the milk sap is used for insect bites and warts (Roberts 1983; Hutchings *et al*., 1996; Pooley, 2005). Leaves are used for menorrhagia and rheumatism in parts of southern Africa and as a galactagogue in Madagascar where leaves and roots are used as purgatives, emetics and as a depurative (Watt and Breyer-Brandwijk, 1962). Investigators attempting to confirm antidiabetic properties

in the plant were unable to do so, but Canadian workers, in 1955, found that the extracts produce leukopenic action in rats (Trease and Evans, 1983).

Ethnobotanical information obtained from interviewees

The *Catharanthus roseus* is known for its bitterness when administered orally. The roots are the main plant part used for medicinal purposes, but the leaves and stems of the herb can also be used. The plant parts are crushed and mixed with hot or cold water and sieved. A teaspoon (+/-5 ml) of the infusion is taken twice a day until diarrhoea subsides. No side effects have been reported. (Mabika M., 2008, pers. comm.; Gumede H., 2008, pers. comm.; Nxumalo F., 2008, pers. comm.; Gumede M., 2008, pers. comm.; Mbuthu T., 2008, pers. comm.; Nsibande N., 2008, pers. comm.; Mhlongo M., 2008, pers. comm.; Nkanini G., 2008, pers. comm.; Qwabe B., 2008, pers. comm.; Gumede N., 2008, pers. comm.; Gumede T., 2008, pers. comm.; Mageba H., 2008, pers. comm.; Qwabe X., 2008, pers. comm.; Ngubane L., 2008, pers. comm.; Maphanga D., 2008, pers. comm.; Ngobe L., 2008, pers. comm.; Mthethwa N., 2008, pers. comm.; Mthembu N., 2008, pers. comm.; Ndlovu A., 2008, pers. comm.; Ngubane M., 2008, pers. comm.; Gumede K., 2008, pers. comm.; Mnguni L., 2008, pers. comm.; Zikhali J., 2008, pers. comm.; Ndlovu T., 2008, pers. comm.; Thwala N., 2008, pers. comm.; Mkhonto P., 2008, pers. comm.; Ngubane H., 2008, pers. comm.; Mlambo S., 2008, pers. comm.; Mabika T., 2008, pers. comm.; Mahlangu R., 2008, pers. comm.).

3.3.2.5 *Chenopodium ambrosioides* L. (Chenopodiaceae)

Sandworm plant (E), Unukani, Ikhambi* (Z), Galsiektebos (A)

(*= new Zulu vernacular name for this species)

Botanical Description

Chenopodium ambrosioides (Figure 3.5) is a common weed which grows up to 1 m in height. Stems are drooping and slender. Leaves are long, narrow and grey-green. Flowers are small, inconspicuous and in spikes. This plant has a very strong characteristic scent (Pooley, 2005; Van Wyk *et al*., 2009).

Geographical distribution

It originates from South and Central America (Van Wyk *et al*., 2009). This plant is commonly found as a weed in disturbed soil in South Africa (Pooley, 2005).

Figure 3.5 Stems and leaves of *Chenopodium ambrosioides* (photo:http://shaman-australis.com.au/shop/index.php?cPath=28_301).

Recorded medicinal uses

This herb is used for a number of ailments such as coughs, stomach complaints, diarrhoea, eczema and sandworm. It is also used as an insecticide (Pooley, 2005; Rahecho *et al*., 2011). *Chenopodium ambrosioides* is used for intestinal parasites particularly ectoparasites, ascaris and hookworm in adults and children (Van Wyk and Wink, 2004). It is also used against snake bite, stomach ache and as a tonic. Leaf infusions are rubbed on the face, burnt and the smoke inhaled to treat insanity and convulsions. Leaf infusions are instilled in the vagina for uterine pain. An ointment of the leaves is prepared and rubbed on the body of an infant to treat high fever (Hutchings *et al.*, 1996; Van Wyk and Gerické, 2000). This herb has been said to be used by the community of Porvenir, Bajo Paraguá Indian Reservation, Bolivia for abortion, heal fractures and bruises (Hajdu and Hohmann, 2012).

Ethnobotanical information obtained from interviewees

Chenopodium ambrosioides is used mainly to treat children with stomach complaints including diarrhoea. It is prepared by crushing the whole plant and mixing it with cold, warm or hot water and sieved. The infusion is then administered orally or anally. Orally, only a quarter of the cup is taken twice a day until diarrhoea subsides. This herb is also used to treat colds and flu (Mavundla V., 2008, pers. comm.; Khumalo T., 2008, pers. comm.; Zikhali Z., 2008, pers. comm.; Thwala N., 2008, pers. comm.).

3.3.2.6 *Cissampelos hirta* Klotzch **(Menispermaceae)**

Umanyokane (Z)

Botanical description

Figure 3.6 shows *Cissampelos hirta* plant. This herb is a slender liane with branchlets striate and glabrescent. Leaves are simple, alternate with a shiny adaxial side. Leaf-lamina is ovate-subtriangular, broadly cordate, sometimes deeply cordate, subobtuse or subtruncate at the base, acute and slightly mucronate at the apex, puberulous or glabrescent on the lower side, except the nerves, sparsely puberulous on the upper sidelong. Flowers are unisexual, small and inconspicuous (Troupin, 1960)**.**

Figure 3.6 *Cissampelos hirta* in flower (photo: H de Wet).

Geographical distribution

This species is limited to Mozambique and northern KwaZulu-Natal in South Africa (De Wet, 2005).

Recorded medicinal uses

The root of this herb is used for a number of disorders such as infant stomach complaints, stomach pains, lower stomach pains, draining “green stuff” also known in Zulu as “umphezulu” from a newborn baby, stops vomiting and back pain. “Umphezulu” is when a baby’s faeces contain green threads (Dahlberg and Trygger, 2009). The leaves are used to treat ringworm and itching of the genital area (De Wet and Van Wyk, 2008).

Ethnobotanical information obtained from interviewees

The whole plant or roots are used to treat diarrhoea, it is crushed and mixed with cold water and sieved. A quarter of a cup of the infusion is administered orally. This is taken twice a day until diarrhoea subsides. No side effects have been reported (Ndlovu M., 2008, pers. comm.; Mlambo S., 2008, pers. comm.; Mabika T., 2008, pers. comm.).

3.3.2.7 *Garcinia livingstonei* T. Anderson **(Clusiaceae)**

African Mangosteen (E), Umphimbi (Z), Laeveldse geelmelkhout (A)

Botanical description

This plant is a sturdy small to medium size semi-deciduous tree of up to 8 m in height (Figure 3.7). Leaves are leathery, stiff and blue-green with a yellowish white midrib. Flowers are small, yellowish green and in clusters in leaf axils. The fruits are luminous orange-pink when ripe (Pooley, 2003).

Geographical distribution

This species is found in the bushveld and coastal grassland in northern KwaZulu-Natal, Mpumalanga, Swaziland, Mozambique, Botswana, Namibia and into tropical Africa (Pooley, 2003).

Figure 3.7 Collecting bark form *Garcinia livingstonei* (photo: H de Wet).

Recorded medicinal uses

People of Muda, Mozambique use the decoction and inhalant made from leaves to treat conjunctivitis (Bruschi *et al*., 2011). Powdered roots are used as an aphrodisiac (Pooley, 2003). The root decoctions are taken against abdominal pain and during pregnancy in Somalia and East Africa (Hutchings *et al*., 1996). Fruits and stems are used for coughs, fevers and parasitic diseases in Africa (Hutchings *et al.*, 1996).

Ethnobotanical information obtained from interviewees

The fruits of the *Garcinia livingstonei* plant are edible. The roots or bark are crushed and mixed with warm or hot water. This is administered orally or anally to treat diarrhoea. Dosages of 125 ml (half a mug) can be administered orally three times a day until diarrhoea subsides. No side effects have been reported (Gumede H., 2008, pers. comm.; Gumede S., 2008, pers. comm.; Masuku N., 2008, pers. comm.; Mabika J., 2008, pers. comm.).

3.3.2.8 *Gymnosporia senegalensis* (Lam.) Loes. **(Celastraceae)**

Red Spike-thorn (E), uBuhlangwe (Z), Rooipendoring (A)

Botanical description

Figure 3.8 show a branch of *Gymnosporia senegalensis***.** This species occur as

shrubs or small multi-stemmed trees, unarmed or with spines. The flowers are small creamy-green in dense axillary and terminal clusters, sweetly scented. The fruits are small, creamy-green and in dense clusters (Pooley, 2003).

Geographical distribution

Gymnosporia senegalensis is found in the bushveld, grassland, coastal and dune bush in KwaZulu-Natal, Mpumalanga, Swaziland, Mozambique, southern Spain and India (Pooley, 2003).

Figure 3.8 A branch of *Gymnosporia senegalensis* (photo: H de Wet).

Recorded medicinal uses

The Shangana tribe of South Africa uses the roots of *G. senegalensis* for the treatment of haemoptysis (coughing up blood from the respiratory tract). The plant is also used as a poultice (dressing) and root decoctions are used in porridge for intercostals (between the ribs) pains (Watt and Breyer-Brandwijk, 1962). The Luvale people of Zambia use roots and leaves for snake bite. Roots and leaves are used for dysentery, diarrhoea and colic (Irvine, 1961; Hutchings *et al*., 1996). Root infusions are taken for heavy menstruation, uterine cramps and to prevent an incipient abortion. The plant is also used for sore throat, headache, earache, fever, measles, abdominal pains, sexually transmitted infections and epilepsy (Van Wyk and

Gerické, 2000; Mulaudzi *et al*., 2012). The roots are taken in Zimbabwe as a powder or as an infusion for cough, bronchitis, pneumonia and tuberculosis (Watt and Breyer-Brandwijk, 1962; Mulaudzi *et al*., 2012). The genus *Gymnosporia* is also known for its maytansine macrolides with antileukaemic activity. The plant is thought to contain a bitter principle of celastrin (Watt and Breyer-Brandwijk, 1962). *Gymnosporia senegalensis* is known for a wide spectrum of uses in traditional medicine (Pooley, 2003). The Baskoure people of Kourittega province, Burkina Faso use this plant to treat malaria (Nadembega *et al*., 2011).

Ethnobotanical information obtained from interviewees

The leaves are used to treat diarrhoea and are either chewed or crushed and mixed with cold water. A quarter of a cup (75 ml) of the infusion is sieved and taken orally. The infusion is taken three times a day until diarrhoea subsides. No side effects have been reported (Mafuleka T., 2007, pers. comm.; Nkanini G. 2008, pers. comm.; Qwabe B. 2008, pers. comm.; Qwabe X. 2008, pers. comm.; Nxumalo N., 2008, pers. comm.; Ndlovu M., 2008, pers. comm.; Zikhali R., 2008, pers. comm.).

3.3.2.9 *Krauseola mosambicina* (Moss.) Pax & K. Hoffm. **(Caryophyllaceae)**

Isihlaza/ Isihlazi (Z)

Botanical description

Krauseola mosambicina (Figure 3.9) is an annual or perennial herb of up to 40 cm in height branching near the base. The stems and the leaves are sparse with soft hairs. The flowers are arranged in 3-9 terminal or axillary cymes. The flowers are cream in colour. The capsules are broadly ovoid, smooth, brown and are surrounded by persistent sepals. The capsules contain seeds that are reddish-brown (Wild, 1961).

Geographical distribution

Krauseola mosambicina is endemic to northern KwaZulu-Natal andextends up into Mozambique (Jordaan, 2000). It occurs in open scrub on coastal dunes (Wild, 1961).

Figure 3.9 *Krauseola mosambicina* plant with flowers (photo: H de Wet).

Recorded medicinal uses

Krauseola mosambicina is used medicinally by villagers in northern KwaZulu-Natal. The specific medicinal uses were not mentioned in the literature (Dahlberg and Trygger, 2009). The Maputaland community in South Africa uses this herb to treat diarrhoea, respiratory infections and sexually transmitted diseases (York *et al*., 2011; De Wet *et al*., 2012).

Ethnobotanical information obtained from interviewees

The whole plant or leaves are crushed and mixed with cold water and sieved. A quarter of a cup (75 ml) of the infusion is taken orally. The infusion is taken twice a day until diarrhoea subsides. It can also be administered as an enema. It is sometimes used in combination with *A. glabratum.* No side effects have been reported (Mhlongo M., 2008, pers. comm.; Ngubane M., 2008, pers. comm.; Maphanga D., 2008, pers. comm.; Mkhize T., 2008, pers. comm.; Khumalo T., 2008, pers. comm.; Nxumalo E., 2008, pers. comm.; Mehlomakhulu N., 2008, pers. comm.; Mthembu T., 2008, pers. comm.; Mbonambi P., 2008, pers. comm.).

3.3.2.10 *Lippia javanica* (Burm.f.) Spreng. **(Verbenaceae)**

Fever tree, Lemon bush (E), Umsuzwane (Z)

Botanical description

The structure of *Lippia javanica* is shown on Figure 3.10. This plant is an erect, woody shrub of up to 2 m in height. The leaves are hairy with conspicuous veins. The leaves have a strong lemon smell. Flowers are small, yellowish and are produced in dense rounded heads (Van Wyk *et al.*, 2009).

Figure 3.10 Leaves and flowers of *Lippia javanica* (photo: H de Wet).

Geographical distribution

This species is found in large parts of South Africa from the Eastern Cape Province northwards extending into tropical Africa including Botswana, Swaziland, Mozambique, Malawi, Zambia, Tanzania, and Kenya (Van Wyk *et al.*, 2009).

Recorded medicinal uses

Lippia javanica infusions are drunk as a tea, to treat coughs, colds, fever and bronchitis. Chest diseases, influenza, measles, rashes, malaria, stomach ailments as well as headaches can be treated using *L. javanica*. A tea is made from weak infusions but strong infusions can also be used topically for scabies and lice (Hutchings *et al*., 1996; Pooley, 2005; Viljoen *et al*., 2005; Van Wyk *et al*., 2009; Corrigan *et al*., 2011). The Kikuyu people in Kenya use *L. javanica* to manage diarrhoea in the Thika urban area (Njoroge and Kibunga, 2007). The infusions are also used to bathe marasmic (malnutrition) infants. The Vhavenda tribe uses the leaf

infusions as antihelminthics, for respiratory and febrile ailments and also as a prophylactic for dysentery, diarrhoea and malaria. Powder from burnt roots is used for food poisoning, bronchitis and sore eyes in Botswana. In West Africa leaves and roots are used for fevers, headaches and skin diseases (Hutchings *et al*., 1996; Duraipandiyan and Ignacimuthu, 2011; Van Wyk, 2011), as well as by the Zulu people (York *et al.,* 2011). Muda residents in Mozambique use the decoction made from *Lippia javanica* for venereal diseases (Bruschi *et al*., 2011). Traditional birth attendants of Machakos District in Kenya use *Lippia javanica* powder smear to prevent and manage postpartum haemorrhage after labour (Kaingu *et al*., 2011). UMkhanyakude district residents in South Africa use the smoke from fresh, burnt leaves to repel mosquitos (Mavundza *et al*., 2011).

Ethnobotanical information obtained from interviewees

Lippia javanica is used mainly for colds, flu, headaches and diarrhoea. For diarrhoea the leaves of the plant are crushed and mixed with cold or hot water. The mixture is then sieved and a quarter of a cup (75 ml) is taken twice a day until diarrhoea subsides. The herb is also burnt to chase away mosquitoes. No side effects have been reported (Nxumalo E., 2008, pers. comm.).

3.3.2.11 *Mangifera indica* L. (Anacardiaceae)

Mango (E), Umango (Z)

Botanical description

Figure 3.11 shows a mature *Mangifera indica* tree. This is a tree of up to 15 m in height with a dense heavy crown (Pooley, 2003). The leaves are drooping, glossy and oblong. The flowers are small, red or yellow and very numerous. The fruits hang outside the foliage canopy. The fruits are oval to kidney-shaped, usually turns yellow, orange or reddish when ripe (Van Wyk, 2005).

Geographical distribution

Mangifera indica is native to southern Asia, Burma, Indomalaysia and eastern India. It spread early on to Malaya, eastern Asia and eastern Africa. Mangos were

introduced to California (Santa Barbara) in 1880. It is now also naturalized along the KwaZulu-Natal coastal areas in South Africa (Pooley, 2003).

Recorded medicinal uses

The Kikuyu people in Kenya and Tamilnadu residents use leaves of *Mangifera indica* to treat diarrhoea (Njoroge and Kibunga, 2007; Beverly and Sudarsanam, 2011), *Mangifera indica* is used to manage diabetes in India and Brazil (Patil *et al*., 2011; Trojan-Rodriguez *et al*., 2012; Thirumalai *et al*., 2012). Communities of the Sub-Himalayan region of Uttarakhand, India used the bark decoction of Mango for the management of jaundice (Sharma *et al*., 2012). Muda residents in Mozambique use this plant to manage hernia (Bruschi *et al*., 2011). The Baskoure people of Kourittega province, Burkina Faso use this plant to treat malaria and cough (Nadembega *et al*., 2011). Residents from UMkhanyakude district in South Africa use the smoke from dried, burnt seeds to repel mosquitos (Mavundza *et al*., 2011).

Figure 3.11 A mature *Mangifera indica* tree (photo: H de Wet).

Ethnobotanical information obtained from interviewees

The leaves of this plant are crushed and boiled in water. The leaves can be mixed with *Sarcophyte sanguinea*. The infusion is cooled down and sieved and a quarter of

a cup (75 ml) is taken twice a day until diarrhoea subsides. No side effects have been reported (Ngubane L., 2008, pers. comm.).

3.3.2.12 *Melia azedarach* L. (Meliaceae)

Syringa (E), Umsilinga (Z), Maksering (A)

Botanical description

Melia azedarach (Figure 3.12) is a medium to large deciduous tree. The leaves are clustered towards the end of branches, with 3-pairs of pinnae which are glossy and dark green. The flowers are pale lilac, bisexual and the fruit are yellow drupes (Van Wyk and Van Wyk, 1997).

Figure 3.12 Leaves, flowers and fruits of *Melia azedarach* (photo: H de Wet).

Geographical distribution

This plant is exotic but is now naturalized in disturbed areas in South Africa. It is found in KwaZulu-Natal and Eastern Cape (Transkei) (Pooley, 2003). It is native to India, southern China and Australia (Van Wyk and Van Wyk, 1997).

Recorded medicinal uses

A cold-water infusion is made from a handful of leaves and half a cup of water (125 ml) and is taken for abdominal pain. Leaf, flower, root and bark preparations are also used in other parts of Africa for various skin diseases. The Luo people in Kenya prepare ointments from leaves and fruits which are applied externally for convulsions, spasms, constipation and stomach ache (Johns *et al*., 1990; Hutchings *et al.,* 1996). Leaf decoctions are also traditionally used for constipation and stomach ache by the Luo people in Kenya (Johns *et al*., 1990). Infusions are used for asthma and eczema (Watt and Breyer-Brandwijk, 1962). The seeds are used to treat diabetes in India (Thirumalai *et al*., 2012). Muda residents in Mozambique use this plant for the treatment of venereal diseases (Bruschi *et al*., 2011). The decoction of *Melia azedarach* is believed to cure measles by Suba district dwellers in Kenya (Nagata *et al*., 2011). The Tamilnadu residents use the juice of the leaves for treatment of jaundice (David and Sudarsanam, 2011). UMkhanyakude district residents in South Africa use the smoke from dried, burnt leaves to repel mosquitos (Mavundza *et al*., 2011).

Ethnobotanical information obtained from interviewees

Melia azedarach leaves are used to treat diarrhoea in the homesteads. The leaves are crushed and mixed with cold water and sieved. Half a mug (125 ml) of the infusion is taken twice a day until diarrhoea subsides. The infusion is bitter. No side effects have been reported (Gumede C. 2008, pers. comm.; Mavundla V., 2008, pers. comm.; Gumede N. 2008, pers. comm.; Mbuthu T., 2008, pers. comm.; Nkanini G., 2008, pers. comm.; Qwabe B. 2008, pers. comm.; Gumede T. 2008, pers. comm.; Gumede K. 2008, pers. comm.; Qwabe X. 2008, pers. comm.; Maphanga D., 2008, pers. comm.; Ngubane M., 2008, pers. comm.; Khumalo T., 2008, pers. comm.; Nxumalo E. 2008, pers. comm.; Mthembu N., 2008, pers. comm; Ntuli J. 2008, pers. comm.; Ndlovu S., 2008, pers. comm.; Skhakhane Z., 2008, pers. comm.; Khanyile M., 2008, pers. comm.; Dlamini T., 2008, pers. comm.; Ntuli L. 2008, pers. comm.; Mabika J. 2008, pers. comm.; Nxumalo N. 2008, pers. comm.; Mlambo S., 2008, pers. comm.; Mabika T. 2008, pers. comm.; Zikhali R., 2008, pers. comm.).

Conservation status

This species is not threatened or endangered, but this species is one of the problem plants of South Africa as it replaces the indigenous vegetation and obstructs waterways (Bromilow, 2001).

3.3.2.13 *Psidium guajava* L. **(Myrtaceae)**

Guava (E), Ugwava (Z), Koejawel (A)

Botanical description

Figure 3.13 shows the *Psidium guajava* plant with its raw fruits. It is a shrub or small tree, usually 4 m in height. The leaves are large and in pairs with prominent veins on the lower side. Flowers are small with numerous stamens produced in the early summer. The yellow fruits are round or pear shaped with many seeds. The fruits are delicious and contain vitamin C (Van Wyk *et al.*, 2009).

Figure 3.13 Fruits and leaves of *Psidium guajava* (photo: SF van Vuuren).

Geographical distribution

Psidium guajava is found naturally in central America but has become naturalized in many parts of the world which include Africa, India, Asia and Australia. In South

Africa it is found in the warm subtropical areas of KwaZulu-Natal, Mpumalanga and Limpopo Provinces (Van Wyk *et al.*, 2009).

Recorded medicinal uses

The leaves are used as a remedy for diarrhoea. It is also used for other ailments such as diabetes, fever, coughs, ulcers, boils and for wound healing. In Africa, *P. guajava* is used to treat malaria (Van Wyk *et al.*, 2009; Ghorbani *et al*., 2011; Maroyi, 2011; Thomas *et al*., 2011; Alonso-Castro *et al*., 2012; Hajdu and Hohmann, 2012; Madikizela *et al*., 2012). Lin *et al.* (2002) showed that *P. guajava* has inhibitory properties on the growth of some diarrhoeagenic pathogens. Known to South Africans as *umgwava*, unripe fruit show significant antidiarrhoeal activity, and the leaf infusions are administered as enemas for severe diarrhoea (Hutchings *et al.* 1996; Alonso-Castro *et al*., 2012; Hajdu and Hohmann, 2012; Nunkoo and Fawzi Mahomoodally, 2012). The Kikuyu people in Kenya also use *P. guajava* to manage diarrhoea (Njoroge and Kibunga, 2007; Tangjang *et al*., 2011; Neamsuvan *et al*., 2012). Leaf and root decoctions are used for diarrhoea in Madagascar (Andriantsoa, 1983). Fruits are also used for blood stools (dysentery) in the Eastern Cape (Transkei). Roots are used for sexually transmitted infections by the Vhavenda people of South Africa (Limpopo province). *Psidium guajava* is used as an antidiabetic treatment in Taiwan and India (Hsu and Cheng, 1992; David and Sudarsanam, 2011; Alonso-Castro *et al*., 2012; Thirumalai *et al*., 2012). York *et al*. (2011) in a recent report demonstrated that this plant is used to treat respiratory infections. The Huasteca Potosina residents of Mexico utilize *Psidium guajava* to curb Herpes, rash, gastritis, vermifuge and vomiting (Alonso-Castro *et al*., 2012). The Bulu and the Kaulong tribes of Papua, New Guinea use this plant species as a laxative (Prescott *et al*., 2012).

Ethnobotanical information obtained from interviewees

The ethnobotanical information on the utilization of *P. guajava* for curbing diarrhoea indicated that this species is the most trusted remedy in the study area. This is because of the high percentage of interviewees who mentioned it as the plant of choice for the treatment of diarrhoea. The leaves of *P. guajava* are crushed and added to hot, warm or cold water to form a mixture which is then sieved and given to

the patient orally. The patient drinks half a cup (125 ml) of the infusion three times a day until the illness subsides. The leaves can also be chewed to treat diarrhoea. It can also be used in combination with *B. transvaalensis or A. glabratum.* No side effects have been reported (Mabika M. 2008, pers. comm.; Mbuthu T., 2008, pers. comm.; Mhlongo T. 2008, pers. comm.; Nkanini G., 2008, pers. comm.; Qwabe B. 2008, pers. comm.; Gumede H. 2008, pers. comm.; Gumede M. 2008, pers. comm.; Qwabe X. 2008, pers. comm.; Maphanga D., 2008, pers. comm.; Ngubane M. 2008, pers. comm.; Mkhize T., 2008, pers. comm.; Mthethwa N., 2008, pers. comm.; Khumalo T., 2008, pers. comm.; Nxumalo E. 2008, pers. comm.; Mthembu T. 2008, pers. comm.; Gumede C. 2008, pers. comm.; Ntuli L. 2008, pers. comm.; Ndlovu S., 2008, pers. comm.; Zikhali Z. 2008, pers. comm.; Mtshali K., 2008, pers. comm.; Ngubane M., 2008, pers. comm.; Gumede T. 2008, pers. comm.; Mnguni L. 2008, pers. comm.; Mnguni M. 2008, pers. comm.; Skhakhane Z., 2008, pers. comm.; Khanyile M., 2008, pers. comm.; Dlamini T., 2008, pers. comm.; Zikhali L. 2008, pers. comm.; Ntuli S. 2008, pers. comm.; Mhlongo M. 2008, pers. comm.; Mhlanga 2008, pers. comm.; Mabika J., 2008, pers. comm.; Mthembu N. 2008, pers. comm.; Nxumalo N. 2008, pers. comm.; Mkhonto P., 2008, pers. comm.; Nhlozi M., 2008, pers. comm.; Mbonambi P., 2008, pers. comm.; Mlambo S., 2008, pers. comm.; Mabika T. 2008, pers. comm.).

Conservation status

This species is not threatened or endangered, but is one of the problem plants of South Africa as it is known to attract fruit flies that affect orchards of other fruits. It also replaces indigenous vegetation and transforms the landscape (Bromilow, 2001).

3.3.2.14 *Sarcostemma viminale* (L) R. Br **(Apocynaceae)**

Coustic vine (E), Melktou (A), Amabhelebhele, Ingotshwa (Z)

Botanical description

Figure 3.14 shows the flowers of *Sarcostemma viminale.*This species is a vine which can grow up to 7 m in length. The stems are succulent and the leaves are reduced to scales. Flowers are lobes and sweetly scented. The shape of the fruits resembles the shape of a chilli pepper with blunt endings (Pooley, 2005).

Geographical distribution

This species is found in dry areas in Africa (Botswana, Namibia and southern Africa up to Kenya), Asia and Australia. In South Africa it is common in most grassland and bushveld areas (Pooley, 2005; Latti, 2010).

Recorded medicinal uses

This species is used traditionally to treat heartburn, sexually transmitted infections, ulcers and septic sores. It is also used as a diuretic and to increase milk during lactation (Pooley, 2005). Latex is applied to eyes to relieve pain caused by the latex of *Euphobia ingens* (Watt and Breyer-Brandwijk, 1962). Unspecified parts are taken in emetics for heartburn (Hutchings *et al.*, 1996). In southern Africa and Madagascar, it is applied for uterine bleeding, septic wounds, varicose ulcers, and sores. In Madagascar, the stems and leaves are used for blennorrhagia (any excessive discharge of mucus) (Hutchings *et al.*, 1996). The Baskoure people of Kourittega province, Burkina Faso use this plant to treat vertigo (Nadembega *et al*., 2011).

Figure 3.14 *Sarcostemma viminale* (photo:http://ru.wikipedia.org/wiki/Sarcostemma).

Ethnobotanical information obtained from interviewees

Sarcostemma viminale grows in the bush surrounding the homesteads. The very bitter fruits are eaten raw or cooked as a stew. The stems of this plant are used as an anti-diarrhoeal. They are crushed and mixed with cold water and sieved. A quarter of a cup of the infusion is taken twice a day until diarrhoea subsides. No side effects have been reported (Qwabe B., 2008, pers. comm.).

3.3.2.15 *Schotia brachypetala* Sond. **(Fabaceae)**

Weeping boer-bean (E), Huilboerboon (A), Umgxamu (Z)

Botanical description

Schotia brachypetala is a medium to large tree with a wide-spreading, densely branched, rounded crown. It has a single trunk that sometimes branches low down. Trees can reach a height of 22 m, with a spread of 10 to 15 m. The bark is rough, with a brown to grey brown colour. The leaves are compound, with 4 to 6 pairs of leaflets, each with an entire, wavy margin (Figure 3.15). The foliage is reddish to coppery when young, turning bright green and maturing to a glossy dark green. In warm frost-free areas this tree is evergreen, but in colder regions it is deciduous, losing its leaves for a short period in winter to spring. The flowers are rich deep red, and are produced in masses, in dense branched heads on the old wood during spring. The fruit is a hard, flattened, woody, dark brown pod containing flattened seeds with a conspicuous yellow aril. The pods split on the tree and mature during late summer to autumn (Mbambezeli and Notten, 2001; Van Wyk *et al.*, 2009).

Geographical distribution

Schotia brachypetala occurs in warm dry areas in the bushveld, deciduous woodland and scrub forest. The genus is most often found on the banks of rivers and streams or on old termite mounds. It grows at lower altitudes, in the UMthatha region of the Eastern Cape Province, through to KwaZulu-Natal, Swaziland, Mpumalanga, Northern Province and into Mozambique and Zimbabwe (Mbambezeli and Notten, 2001; Van Wyk *et al.*, 2009).

Figure 3.15 *Schotia brachypetala* leaves and pods (photo: H de Wet).

Recorded medicinal uses

A decoction of the bark is taken to treat heartburn and hangovers. Bark and root mixtures are used to strengthen the body and purify the blood, to treat nervous heart conditions and diarrhoea, as well as for facial saunas. Bark mixtures are also used to strengthen the body. The Venda's use the bark and roots for nervous heart conditions (Van Wyk *et al.*, 2009). The plant is used by KwaNibela residents in South Africa to treat flu (Corrigan *et al*., 2011).

Ethnobotanical information obtained from interviewees

Schotia brachypetala is used for treating diarrhoea. The crushed bark is boiled in water and allowed to cool down. The infusion is then sieved and half of a mug is taken twice a day until diarrhoea subsides. It can also be administered anally as an enema. No side effects have been reported (Mavundla D., 2008, pers. comm.).

3.3.2.16 *Sclerocarya birrea* (A. Rich.) Hochst. subsp. **caffra** (Sond.)
(Anacardiaceae)

Marula (E), Umganu (Z), Maroela (A)

Botanical description

A mature *Sclerocarya birrea* tree is shown in Figure 3.15. *Sclerocarya birrea* is a deciduous tree growing up to 18 m in height. The bark is rough, flaky and mottled. The leaves are divided into a number of pairs of leaflets. The leaves are dark green above and pale below with a sharp pointed tip. The tree produces unisexual flowers from September to November. The flowers are small with red sepals and yellow petals that are borne in small, oblong clusters. It bears fruits from January to March (Pooley, 2003; Van Wyk *et al.*, 2009).

Figure 3.16 A mature *Sclerocarya birrea* tree (photo: H de Wet).

Geographical distribution

Sclerocarya birrea occurs from KwaZulu-Natal south coast northwards to Mpumalanga, Mozambique, Swaziland and into tropical Africa. It grows in the bushveld, woodland and on forest margins at low altitudes (Pooley, 2003).

Recorded medicinal uses

The powdered bark is used to determine the gender of an unborn baby. If a pregnant woman wishes to have a girl, she will take a preparation from the female plant and for a boy she will take the male plant (Mutshinyalo and Tshisevhe, 2003). The Nigerians use the plant to facilitate child birth (Attah *et al*., 2012). A decoction of the

bark is used to treat dysentery, diarrhoea, rheumatism and has a prophylactic effect against malaria. The bark is used as a remedy for haemorrhoids. Roots and bark are also used as laxatives. A drink made from *S. birrea* leaves is used for the treatment of gonorrhoea (Mutshinyalo and Tshisevhe, 2003; Corrigan *et al*., 2011; Nadembega *et al*., 2011). Bruschi *et al*. (2011) and York *et al*., (2011) revealed that this plant can be used to curb cough.The lay people of Maputaland in the KwaZulu-Natal Province in South Africa use the bark to treat sexually transmitted infections (De Wet *et al*., 2012). The people in Nhema communal area, Zimbabwe use this plant species to treat sore eyes and earache (Maroyi, 2011). Residents from UMkhanyakude district in South Africa use the smoke from dried, burnt seeds to repel mosquitos (Mavundza *et al*., 2011).

Ethnobotanical information obtained from interviewees

Sclerocarya birrea fruits are well known for making beer (*utshwala bamaganu*) which has a very high alcoholic content. As for the treatment of diarrhoeal infection, the bark of the tree is ground and mixed with hot, warm or cold water. The infusion is then sieved and administered orally or anally. Half a cup of the infusion is taken three times a day until diarrhoea subsides. It can also be used in combination with *B. transvaalensis*. No side effects have been reported (Mahlangu H., 2007, pers. comm.; Mabika J. 2008, pers. comm.; Gumede H. 2008, pers. comm.; Gumede N. 2008, pers. comm.; Mavundla D., 2008, pers. comm.; Gumede S., 2008, pers. comm.; Mhlongo T. 2008, pers. comm.; Gumede S. 2008, pers. comm.; Zikhali B. 2008, pers. comm.; Qwabe B., 2008, pers. comm.; Mabika M. 2008, pers. comm.; Mtshali 2008, pers. comm.; Mnguni L., 2008, pers. comm.; Mnguni M. 2008, pers. comm.; Zikhali R., 2008, pers. comm.; Mthembu D., 2008, pers. comm.; Mhlongo D. 2008, pers. comm.; Masuku N., 2008, pers. comm.; Nxumalo N., 2008, pers. comm.; Mkhonto P., 2008, pers. comm.; Mbonambi 2008, pers. comm.; Mdletshe F., 2008, pers. comm.; Zikhali T. 2008, pers. comm.).

3.3.2.17 *Senna occidentalis* (L.) Link **(Fabaceae)**

Septic weed (E), Ikhoshokhosho (Z)

Botanical description

Senna occidentalis (Figure 3.17) is a small annual shrub of up to 1.2 m in height. The stems are ribbed and glabrous. The petiole has a gland at the base. The leaves are up to 15 cm long, with usually 4-6 pairs of elliptic or lanceolate acuminate glabrous leaflets, the terminal pair is the largest. The flowers are in racemes and yellow, pods are linear, glabrous, 10-15 cm long and about 6 mm wide, with somewhat thickened margins. It contains 20-30 flat, ovate, brown seeds each 3 mm long (Stone, 1970).

Figure 3.17 Flowers and pods of *Senna occidentalis* (photo: SF van Vuuren).

Geographical distribution

This plant is originally from South America but is now naturalized in Africa. In South Africa it is found in the northern part of coastal KwaZulu-Natal (Stone, 1970).

Recorded medicinal uses

Senna occidentalis has been used as a natural medicine in the rainforest and other tropical areas for centuries. Its roots, leaves, flowers, and seeds have been employed in herbal medicine around the world. The roots of this plant species are said to be a diuretic and a decoction is made to treat fevers in Peru. The seeds are

brewed into a coffee-like beverage to aleviate asthma, and a flower infusion is used to treat bronchitis in the Peruvian Amazon (Stone, 1970).

In Brazil the roots are used as a tonic to reduce fever, as a diuretic, gastrointestinal problems, for menstrual problems, tuberculosis, anemia, liver complaints, general weakness and illness (Stone, 1970; Nagata *et al*., 2011; Pandikumar *et al*., 2011). The leaves are also used in Brazil for gonorrhoea, urinary tract disorders, edema, and menstrual problems. The Miskito Indians of Nicaragua Central America use a fresh plant decoction for general menstrual and uterine pain, as well as constipation in babies. In Panama, a leaf tea is used for stomach colic. The crushed leaves are used as a poultice and an anti-inflammatory. Furthermore the crushed fresh leaves are taken orally to get rid of intestinal worms and parasites (Stone, 1970). In many countries around the world, the fresh and or dried leaves of *S. occidentalis* are crushed or brewed into a tea and applied externally for skin disorders, wounds, skin diseases, abscesses, and as a topical analgesic and anti-inflammatory natural medicine (Stone, 1970). In the Ngazidja Island of the Comoro islands, a decoction of the entire plant is used to treat malaria. It is also massaged on the eyes to treat conjunctivitis. The roots are used for rheumatism gonorrhoea and asthma (Kaou *et al*., 2008). Agonlin residents of Benin use this shrub to cure icterus (jaundice) (Allabi *et al*., 2011). Tinea (fungus infection) is cured using this plant species in Bolivia (Hajdu and Hohmann, 2011)

Ethnobotanical information obtained from interviewees

This herbaceous plant grows mainly in the homestead area. The roots of this plant are used to treat diarrhoea. The roots are crushed and mixed with cold water and sieved. A quarter of a cup of the infusion is taken twice a day until diarrhoea subsides. No side effects have been reported (Qwabe B., 2008, pers. comm.; Mkhize T., 2008, pers. comm.; Zikhali J., 2008, pers. comm.).

3.3.2.18 *Strychnos madagascariensis* Pior. **(Strychnaceae)**

Black Monkey Orange (E), Umkwakwa (Z), Swartklapper (A)

Botanical description

This is a medium tree which can grow up to 8 m in height. It is multi-stemmed, with a densely branched, spreading canopy. Leaves are thick, shiny, opposite and clustered towards the end of twigs. Flowers are very small. Fruits are grey-green when unripe (Figure 3.18) turning yellowish-orange when ripe with a shiny hard, woody shell (Pooley, 2003).

Geographical distribution

Strychnos madagascariensis is found in the bushveld and warm, dry, rocky areas in KwaZulu-Natal, Mpumalanga, Swaziland, Mozambique and northwards into tropical Africa (Pooley, 2003).

Recorded medicinal uses

Africans use the roots of the *Strychnos madagascariensis* tree as an emetic. The roots are ground up, mixed with hot water and taken orally. A paste is made from the fruit for treating *Tunga penetrans,* commonly known as jigger fleas (Pooley, 2003). The people in Nhema communal area, Zimbabwe use this plant species to curb sore eyes (Maroyi, 2011).

Figure 3.18 Leaves and the fruit of *Strychnos madagascariensis* (photo: H de Wet).

Ethnobotanical information obtained from interviewees

This species is well known by the interviewees for its fruit which are edible. The pulp of pounded seeds is used to season porridge (*amahewu*). The bark, roots and leaves are crushed and mixed with cold water; the mixture is then sieved and administered orally. The mixture is taken by drinking a quarter of a glass (75 ml) twice a day until diarrhoea subsides. The bark of the stem can also be chewed to curb diarrhoea. No side effects have been reported (Zikhali J. 2007, pers. comm.; Mabika M. 2008, pers. comm.; Nxumalo F., 2008, pers. comm.; Mavundla V., 2008, pers. comm.; Gumede H. 2008, pers. comm.; Nkanini G., 2008, pers. comm.; Qwabe B. 2008, pers. comm.; Zikhali T. 2008, pers. comm.; Qwabe X. 2008, pers. comm.; Ndlovu T., 2008, pers. comm.; Gumede M. 2008, pers. comm.; Ngubane H., 2008, pers. comm.; Gumede C. 2008, pers. comm.; Skhakhane Z., 2008, pers. comm.; Mabika J. 2008, pers. comm.; Mlambo S., 2008, pers. comm.; Mabika T. 2008, pers. comm.).

3.3.2.19 *Syzygium cordatum* Hochst. ex. C. Krauss. **(Myrtaceae)**

Water Berry (E), Umdoni (Z), Waterbessie (A)

Botanical description

Syzygium cordatum is a medium-sized tree which can grow up to 15 m in height, and has a rough dark brown bark (Figure 3.19). Leaves are broad and sometimes almost circular, with a bluish-green colour. The flowers are cream to pinkish with numerous stamens that are produced in clusters. The fruits are egg-shaped and red to dark purple in colour (Van Wyk *et al*., 2009).

Geographical distribution

This species occurs along stream banks from KwaZulu-Natal northwards to Mozambique. It grows in forest margins or in open grassy areas (Pooley, 2003; Van Wyk *et al*., 2009).

Recorded medicinal uses

In central Africa and the study area the tree is used as a remedy for stomach ache and diarrhoea (Van Wyk *et al*., 2009). The leaf extract is effective against mild

Figure 3.19 *Syzygium cordatum* tree (photo: S F van Vuuren).

diabetes and in the case of glucose tolerance impairment (Musabayane *et al*., 2005). The bark has an antifungal activity against *Candida albicans* (Steenkamp *et al*., 2007). Unspecified parts are used for respiratory ailments, including tuberculosis and for stomach complaints and as an emetic (Watt and Breyer-Brandwijk, 1962). The Vhavenda people of South Africa use leaves for stomach ailments, colds and fever while the bark and roots are used for headaches, amenorrhoea and wounds (Mabogo, 1990). The Bemba people of Zambia use cold leaf infusions for various stomach ailments including diarrhoea (Hutchings *et al.,* 1996). The lay people of Maputaland in the KwaZulu-Natal Province in South Africa usethis plant to treat gonorrhoea (De Wet *et al*., 2012). It is also used to treat respiratory tract infections (York *et al*., 2011). The sap of this plant is used to disinfect wounds and treat snake bites (Carrigan *et al*., 2011).

Ethnobotanical information obtained from interviewees

This tree is found mainly in and around the homesteads. The fruits are edible. The bark is used to treat diarrhoea. The bark is crushed and mixed with water and boiled. The infusion is allowed to cool down. A quarter of a cup of the infusion is taken three times a day until diarrhoea subsides. No side effects have been reported (Mahlangu

N., 2007, pers. comm.; Mtshali K., 2008, pers. comm.; Mnguni L., 2008, pers. comm.; Mnguni M., 2008, pers. comm., Mdletshe S., 2008, pers. comm.; Zikhali B., 2008, pers. comm.; Masuku N., 2008, pers. comm.; Mdletshe F., 2008, pers. comm.).

3.3.2.20 *Terminalia sericea* Burch. ex DC. **(Combretaceae)**

Silver-cluster leaf (E), Vaalboom (A), UmKhonono (Z)

Botanical description

This tree (Figure 3.20) is a small to medium tree of about 5 to 8 m of height with a wide spreading crown. The leaves are silver-haired and grow out near the branch tips. The flowers are cream colored with an obnoxious smell. The fruits have two papery wings adjoining the thickened central part (Van Wyk *et al.*, 2009).

Figure 3.20 Mature *Terminalia sericea* tree (photo: H de Wet).

Geographical distribution

This species grow in the sandy savanna areas of South Africa. It is found in Mpumalanga, Maputaland, Swaziland, Mozambique, Zimbabwe and Tropical Africa (Pooley, 2003; Van Wyk *et al*., 2009).

Recorded medicinal uses

The root decoctions are used as a traditional Tswana remedy for stomach ailments as well as diarrhoea. Decoctions and infusions are used as eye lotion and to treat pneumonia. The bark is used for diabetes and topically for wounds (Van Wyk *et al*., 2009; Bruschi *et al*., 2011). The tree is also used for respiratory complains (Pooley, 2003; York *et al*., 2011). This tree has medical application against various sexually transmitted infections such as gonorrhoea and syphilis (De Wet *et al*., 2011). *Terminalia sericea* is also used to treat hypertension and even cancer (Fyhrquist *et al*., 2002). Traditional healers and lay people of Muda, Mozambique use this plant species to treat and manage infertility, menstrual cycle troubles and *feticeria* (Bruschi *et al*., 2011).

Ethnobotanical information obtained from interviewees

This tree grows both in and around the homesteads. The bark or roots of the tree are used to curb diarrhoea. The roots or bark are crushed and mixed with boiling or cold water and sieved. The infusion is either drunk or administered anally. A quarter of a cup (75 ml) is taken twice a day until the patient is well. No side effects have been reported (Nxumalo S., 2008, pers. comm.; Nkanini G., 2008, pers. comm.; Qwabe X., 2008, pers. comm. Mhlongo D., 2008, pers. comm.).

3.3.2.21 *Trichilia emetica* Vahl **(Meliaceae)**

Natal Mahogany (E), Umkhuhlu (Z), Rooiessenhout (A)

Botanical description

Trichillia emetica is an evergreen tree of about 10 m in height and has a dense, rounded crown and smooth, greyish-brown bark. The leaves are compound, dark glossy-green above, hairy below and the leaflets are tapering towards the base (Figure 3.21). Flowers are velvety with a yellowish-green colour and sweetly scented. The fruits are velvety and round in shape with a greenish brown colour. The fruits split into three segments (Pooley, 2003; Van Wyk *et al*., 2009).

Geographical distribution

Trichillia emetica occurs from Durban, KwaZulu-Natal northwards in a narrow zone

along the eastern border of South Africa. It is also found in Mpumalanga, Swaziland, Mozambique, Zimbabwe, Botswana and northwards towards tropical Africa (Pooley, 2003; Van Wyk *et al*., 2009).

Figure 3.21 *Trichillia emetica* leaves (photo: H de Wet).

Recorded medicinal uses

This plant is used as a remedy for intestinal complaints, dysentery, diarrhoea, kidney problems, indigestion and parasites. A root decoction is used for fever and as a purgative.Leaf or fruit poultices are applied topically for bruises and eczema. The seed oil is used for rheumatism (Van Wyk *et al*., 2009). The Baskoure people of Kourittega province, Burkina Faso use this plant to treat stomach ache and obesity (Nadembega *et al*., 2011). Residents from UMkhanyakude district in South Africa use the smoke from dried, burnt seeds to repel mosquitos (Mavundza *et al*., 2011). The tree is also used to treat respiratory infections (York *et al*., 2011). A recent review by Komane *et al*. (2011) reveals that this plant has a number of traditional medicinal uses.

Ethnobotanical information obtained from interviewees

The tree is normally found in the homesteads as a shade tree. The fruit can be eaten. For diarrhoea the bark is used. The bark is crushed and mixed with either hot

or cold water and sieved. The infusion is then administered anally twice a day. No side effects have been reported (Dlamini T., 2008, pers. comm.; Mthembu D., 2008, pers. comm.; Mkhonto P., 2008, pers. comm.; Mbonambi P., 2008, pers. comm.).

3.3.2.22 *Vangueria infausta* Burch. subsp. *infausta* (Rubiaceae)

Wild Medlar (E), uMviyo (Z), Wildemispel (A)

Botanical description

Vangueria infausta is shown in Figure 3.22. This is a small deciduous tree of up to 5 m, with a short, knobby stem. The leaves are large, thickly furry, opposite, light green and net veined. The flowers are small, greenish-white and velvety. The fruits are green and when ripe they are yellowish brown (Pooley, 2003).

Geographical distribution

This species is found in the bushveld, in the rocky hillside in the scrub of KwaZulu-Natal and Eastern Cape (Transkei). It is also found in Mpumalanga, Swaziland, Mozambique and tropical Africa (Pooley, 2003).

Figure 3.22 *Vangueria infausta* leaves (photo: H de Wet).

Recorded medicinal uses

In Zimbabwe some tribes use the root infusions to treat abdominal pains, diarrhoea, dysmenorrhoea and vaginal discharges (Gelfand *et al*., 1985; Bruschi *et al*., 2011; Maroyi, 2011). Bark or root is used to treat diarrhoea in the study area (De Wet *et al.,* 2010). Also in South Africa the root decoctions are reported to be widely used for menstrual complaints (Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996). An infusion of the roots and leaves has been used to treat malaria, chest ailments, as a purgative and to treat ringworms. An infusion of the leaves is used for the relief of toothache. For the treatment of swelling of the limbs the affected parts are bathed in a decoction of the pounded leaves and small twigs (Behr, 2004; Bruschi *et al*., 2011). In Mozambique, Muda area *Vangueria infausta* is used to treat cough, skin blisters and to induce the delivery process during child birth (Bruschi *et al*., 2011).

Ethnobotanical information obtained from interviewees

This plant is known for its fruits that are edible and can also be used to season *amahewu* (liquid porridge). For diarrhoea, the bark or roots are crushed and mixed with cold or hot water and sieved. A mug of the infusion is taken three times a day until diarrhoea subsides. No side effects have been reported (Mahlangu H., 2007, pers. comm.; Gumede S., 2008, pers. comm.).

3.3.2.23 *Vernonia natalensis* (DC) Sch. Bip. ex. Walp **(Asteraceae)**

Silver vernonia (E), Uhlambihloshane, Isibhaha (Z)

Botanical description

Figure 3.23 shows a *Vernonia natalensis* plant. This species is a herbaceous perennial with erect, flowering branches which develop from a woody rootstock. The leaves are narrower and have similar colour on both sides suspended from a very short stalk. The flowers are bright violet and are borne in large groups towards the branch tips (Notten, 2005).

Geographical distribution

It is widespread in the grassland regions of South Africa: Eastern Cape, Free State, KwaZulu-Natal, Gauteng, Mpumalanga, Limpopo Province and the North West

Province. It also grows in Lesotho, Swaziland, Angola, Zambia, Zimbabwe and Malawi (Notten, 2005; Van Wyk *et al*., 2009).

Figure 3.23 *Vernonia natalensis* plant (photo: SF van Vuuren).

Recorded medicinal uses

Vernonia natalensis is used in traditional medicine to treat coughs, malaria and other feverish conditions, headache, pains in the loins and pain in the kidneys, and to bathe haemorrhoids. It is an ingredient in medicines known as *isihlambezo* that are prescribed for pregnant women and taken during pregnancy to ensure a healthy mother and baby. A mixture of leaves and roots is sometimes used by the Vhavenda people to induce abortion, a treatment that has occasionally also killed the mother (Watt and Breyer-Brandwijk 1962; Pooley, 2005 and Notten, 2005).

Ethnobotanical information obtained from interviewees

The roots of this plant (boiled with water, left to cool and sieved) are used to treat diarrhoea. A quarter of a cup (75 ml) of the infusion is taken twice a day until diarrhoea subsides. No side effects have been reported (Qwabe M., 2007, pers. comm.; Zikhali R., 2008, pers. comm.).

3.3.3 Antidiarrhoeal plants collected from muthi markets

The plants collected were: *Sarcophyte sanguinea, Sclerocarya birrea, Strychnos henningsii and Ximenia caffra.* Three species: *Sarcophyte sanguinea, Strychnos henningsii and Ximenia caffra* were not mentioned by the homestead dwellers as a treatment for diarrhoea and hence are discussed further here with respect to their botanical description, geographical description, recorded medicinal uses and ethnobotanical information obtained from the interviewees. Unless otherwise stated all plants are not threatened or endangered with respect to their conservation status.

3.3.3.1 ***Sarcophyte sanguine*** Sparrm. (**Balanophoraceae)**

Wolwekos (A), Umavumbuka, Ihlule (Z)

Botanical description

This species (Figure 3.24) is a parasitic plant on the roots of trees and shrubs especially *Acacia* species. The male plant usually grows up to 300 mm, while the female plant is shorter. The leaves are reduced to scales. The inflorescence is branched with the male inflorescence narrower and taller than the female which is shorter, rounder and fleshy. The anthers are white. This plant species resembles fungi rather than flowering plant. This plant species has a foul smell that attracts a number of different insects (Pooley, 2005).

Figure 3.24 *Sarcophyte sanguinea* samples bought on the muthi market (photo: H de Wet).

Geographical distribution

In Africa it is found in Ethiopia, Somalia, Kenya, Tanzania, Malawi, Mozambique and Zambia. In South Africa it is found in the Western province, KwaZulu-Natal province and Mpumalanga province (USDA, 2010).

Recorded medicinal uses

The homestead women of the Mbazwana area (KwaZulu-Natal province) use the stem of *Sarcophyte sanguinea* to treat wounds (De Wet *et al.,* 2008). This plant is also used traditionally to treat dysentery, diarrhoea, swollen glands, and irregular menstruation (Pooley, 2005).

Ethnobotanical information obtained from interviewees

This plant is used to treat diarrhoea and dysentery. Crushed leaves of *Mangifera indica* can be mixed with *Sarcophyte sanguinea* and boiled. The infusion is cooled down and sieved and a quarter of a cup (75 ml) is taken twice a day until diarrhoea subsides. No side effects have been reported (Ngubane L., 2008, pers. comm.; Mtuba muthi market seller pers. comm., 2008; Mona muthi market seller pers. comm., 2008).

3.3.3.2 *Strychnos henningsii* Gilg (**Loganiaceae)**

Rooibitterbessie (A), Red bitterberry (A), Umqalothi (Z)

Botanical description

Figure 3.25 shows the dried bark of *Strychnos henningsii.* This species varies from a big shrub to a tall tree of up to 15 m in height. At a younger stage the bark of this tree is usually pale grey and smooth, but at the matured stage the bark becomes flaky and dark brown. The leaves are glossy and bright green. The flowers are yellow and are produced along the branches. The fruits are glossy orange. The pulp of the fruits is edible but the seeds are bitter and poisonous (Van Wyk *et al.*, 2009).

Figure 3.25 *Strychnos henningsii* bark samples bought on the muthi market (photo: H de Wet).

Geographical distribution

The plant is normally found occurring along the east coast of South Africa and Northwards into the Kruger National Park (Van Wyk *et al.*, 2009).

Recorded medicinal uses

Strychnos henningsii is used as an antimalarial remedy in Marracuene, Mozambique (Jurg *et al.*, 1991). The bark of this plant is traditionally used to treat nausea, stomach complaints, rheumatic fever, dysmenorrhoea and as a bitter tonic. It is also taken as a colic remedy and as a purgative. The unripe fruit and the root bark are used to treat snake bites (Van Wyk *et al.*, 2009). In Kenya, this plant is used to treat chronic joint pains (Wambugu *et al*., 2011).

Ethnobotanical information obtained from interviewee

The bark of this species is used to treat diarrhoea (Mtuba muthi market seller pers. comm., 2008). No further information could be obtained from the seller.

3.3.3.3 *Ximenia caffra* Sond. (**Olacaceae)**

Suurpruim (A); Natal sourplum (E.); umThunduluka-obomvu, UmGwenya, UmThunduluka (Z)

Botanical description

Figure 3.26 shows the dried bark of *Ximenia caffra* bought at the muthi market. *Ximenia caffra* is a deciduous spiny tree up to 6 m tall with an untidy open crown. The bark is dark grey and rough, but pale green or brown on younger branches. Branchlets are spine-tipped. Sapwood is white and heartwood is hard and reddish brown. The leathery, dark green leaves are often in clusters (fascicles) on short spur branchlets. *Ximenia caffra* has dense reddish hairs on the leaves and branchlets. The flowers are small, sweet-scented and creamy green and borne in single stem clusters in the axils of the spines or on the dwarf branchlets. The fruit are thinly fleshy, oval, attractive and glossy deep red with white spots (Pooley, 2003; Baloyi and Reynolds, 2004).

Figure 3.26 *Ximenia caffra* sample bought on the muthi market (photo: T York).

Geographical distribution

The tree is found in woodlands and grasslands and on rocky outcrops and sometimes on termites mounds. It occurs from Tanzania in the north to KwaZulu-Natal in the south. In South Africa the two varieties have a different distribution pattern with var. *caffra* occurring in the northern and central regions of Limpopo and

var. *natalensis* is found further east and south in Mpumalanga, Limpopo and KwaZulu-Natal (Pooley, 2003; Baloyi and Reynolds, 2004).

Recorded medicinal uses

In Zambia the traditional healers use *Ximenia caffra* to treat sexually transmitted infections (Ndubani and Höjer, 1999). A decoction from the leaves is used as a wash to soothe inflamed eyes. Infusions of the roots are used as a remedy for dysentery and diarrhoea and together with the leaves, they are taken for abdominal pain and bilharziasis. Powdered roots are applied to sores to speed up healing; used in soup, and in beer as an aphrodisiac. Powdered dried leaves are taken orally for fever and infertility. Extracts of the leaves are used as a gargle for tonsillitis, and as a vermifuge. Porridge is made of a decoction of the roots, and eaten once a day for nausea in pregnancy. The root decoction is also taken for infertility (Baloyi and Reynolds, 2004; Maroyi, 2011). The decoction of *Ximenia caffra* is either gargled or drunk for toothache, mouth infection or stomach ache by the Luo tribe mothers and children in the Bondo district, Kenya (Geissler *et al.*, 2002). De Wet *et al*. (2012) in a recent study revealed that this species can be used to treat gonorrhoea. Backache can be cured by a decoction made from this plant species (Maroyi, 2011). Bruschi *et al*. (2011) revealed that this plant can be used to cure stomach ache, constipation, intestinal worms in children, weakness in children, female infertility, menstrual cycle troubles, venereal diseases, tuberculosis, cough, leprosy and propitiatory.

Ethnobotanical information obtained from interviewee

The bark of this species is used to treat diarrhoea (Mona muthi market seller pers comm., 2008). The sellar did not know how to prepare and what dosage is needed to treat diarrhoea.

3.4 Ethnobotanical review summary and discussion

Twenty three plant species were collected for antidiarrhoeal testing from the ethnobotanical survey, which comprises of 15 families (Table 3.5). Each homestead used at least one plant species to treat diarrhoea. Species mostly used to treat diarrhoea belongs to the families Asteraceae (three species) and Fabaceae (three species). Four of the 23 species collected namely: *Acacia burkei, Brachylaena*

transvaalensis, Cissampelos hirta and Sarcostemma viminale were recorded for the first time as a treatment for diarrhoea (De Wet *et al.,* 2010). The 23 plants collected consisted of 14 trees, two shrubs, six herbs and one succulent climber. There are seven species that are naturalized exotics to South Africa, namely: *Acanthospermum glabratum, Catharanthus roseus, Chenopodium ambrosioides, Mangifera indica, Melia azedarach, Psidium guajava and Senna occidentalis.* The other 16 species are indigenous.The leaves (eight species) are the main parts used, followed by bark (seven species), the whole plant (five species) and roots (three species). The whole plant is used if a herb is administered.

Table 3.5 Plant families which represented the species obtained from the ethnobotanical survey.

Plant families	Plant species
Anacardiaceae	*M. indica, S. birrea*
Apocynaceae	*C. roseus, S. viminale*
Asteraceae	*A. glabratum, B. transvaalensis, V. natalensis*
Caryophyllaceae	*K. mosambicina*
Celastraceae	*G. senegalensis*
Chenopodiaceae	*C. ambrosioides*
Clusiaceae	*G. livingstonei*
Combretaceae	*T. sericea*
Fabaceae	*A. burkei, S. brachypetala, S. occidentalis*
Meliaceae	*M. azedarach, T. emetica*
Menispermaceae	*C. hirta*
Myrtaceae	*P. guajava, S. cordatum*
Rubiaceae	*V. infausta*
Strychnaceae	*S. madagascariensis*
Verbenaceae	*L. javanica*

The mode mostly used for administration of the infusions (cold or hot water) was orally. An enema was also employed as a form of administration since it is believed to be more effective and works faster. Seven plants are used in five different combinations for better antidiarrhoeal efficacy. These include the combination of *Brachylaena transvaalensis* with *Psidium guajava*/*Sclerocarya birrea*, *Acanthospermum glabratum* in combination with *Krauseola mosambicina*/*Psidium guajava* and *Mangifera indica* in combination with *Sarcophyte sanguinea*.

Table 3.6 shows the number of occurrences at which the plant species were mentioned by the interviewees. *C. roseus* (22), *P. guajava* (31), *M. azedarach* (20), *S. birrea* (20) and *S. madagascariensis* (14) were the most mentioned plant species for the treatment of diarrhoea in the Maputaland homesteads.

Table 3.6 The number of occurrences at which the plant species were mentioned to treat diarrhoea by the 72 interviewees.

Plant species	Times mentioned
Acacia burkei	4
Acanthospermum glabratum	6
Brachylaena transvaalensis	3
Catharanthus roseus	22
Chenopodium ambrosioides	4
Cissampelos hirta	2
Garcinia livingstonei	4
Gymnosporia senegalensis	6
Krauseola mosambicina	7
Lippia javanica	1
Mangifera indica	1
Melia azedarach	20
Psidium guajava	31
Sarcostemma viminale	1
Schotia brachypetala	1
Sclerocarya birrea	20
Senna occidentalis	3
Strychnos madagascariensis	14
Syzygium cordatum	7
Terminalia seritia	3
Trichilia emetica	4
Vangueria infausta	2
Vernonia natalensis	2

Traditional medicine is the major aide in treating diseases in the rural communities of developing countries (Tabuti *et al*., 2003).This is also true for the Maputaland area where this study was conducted. Similar ethnobotanical surveys on antidiarrhoeal plants have been conducted in other parts of the world such as India (Tetali *et al*., 2009) and Africa (Nigeria) (Njoroge and Kibunga, 2007).

In India, twenty-eight medicinal plants were recorded to be used traditionally to treat diarrhoea in the Parinche valey (Tetali *et al*., 2009), none of which matched the antidiarrhoeal plants identified in this study. In an urban area Thika in Kenya (Africa), 41 medicinal plants are used to treat diarrhoea, of which four species have been recorded in this study. These species are: *Lippia javanica, Mangifera indica, Psidium guajava* and *Strychnos henningsii*. The first three species, which are all exotics to South Africa are all being used by the homestead inhabitants, however, *S. henningsii* was only mentioned by some medicinal plant sellers in the muthi markets visited. Other plants documented to treat diarrhoea in Africa were: *Epinetrum* v*illosum*. (Excell) Troupin, *Croton mubango* Müll, *Cissus rubiginosa* (Welw. ex. Bak) Planch, *Quassia africana* Baill, *Roureopsis obliquifoliolata* (Gild) Schellenb and *Vernonia amygdalina* Del (Otshudi *et al.*, 2000), *Acacia nilotica* (L.) Willd. *ex* Delile, *Acanthospermun hispidum* DC., *Carpolobia lutea*G. Don, *Gmelina arborea* Roxb., *Parkia biglobosa* (Jacq.) R.Br. ex G. Donand *Vitex doniana* L. (Agunu *et al.,* 2005; Nwafor and Bassey*,* 2007).

Previous studies done on diarrhoea plants in South Africa have focused mainly on information obtained from traditional healers and literature in the form of publications (Lin *et al.*, 2002; Mathabe *et al.*, 2006; Fawole *et al*., 2009). There is only one documented study on antidiarrhoeal plants that investigated the knowledge specifically from rural dwellers in the Eastern Cape (Bizana). This study revealed 34 plants species which were used to treat diarrhoea, among those noted was *Psidium guajava* which was also mentioned in this study (Madikizela *et al*., 2012). Another study which included the knowledge of both rural dwellers as well as those of the herbalist where 17 plant species were identified for the treatment of diarrhoea was documented by Appidi *et al*. (2008). None of these species correspond with the 23 plant species collected in this study. Possible reasons may be the difference in the

vegetation types between the two study areas and the difference in the ethnic groups living in the two areas. Appidi's and Madikizela's study area is inhabited by Xhosa's whereas Zulu's inhabit the Maputaland area. Another study by Lin *et al.* (2002) comprises of four medicinal plants (*Aritea* spp*., Bridelia micrantha* (Hochst.) Baill*., Eleutherina bulbosa* (Miller) Urb. and *Psidium guajava*) used by Zulu traditional healers in KwaZulu-Natal to treat diarrhoea. *Psidium guajava* is the only plant which is used by both the traditional healers as well as the rural people in this survey. *Psidium guajava* is also one of the most recorded plant species used to treat diarrhoea in developed countries (Gutiérrez *et al*., 2008). In a more recent study done by Fawole *et al*. (2009) on plants used for gastrointestinal disorders, information was obtained mostly from Hutchings *et al.* (1996). In Fawole's study only *Vernonia natalensis* correspond with our findings as one of the plants used by the Maputaland inhabitants. Mathabe *et al*. (2006) survey on antidiarrhoeal plants was conducted with traditional healers (North Sotho ethnic group) in the Limpopo Province. Only four of the 21 plant species mentioned to treat diarrhoea are also used by the rural community of Maputaland. These plants are: *Gymnosporia senegalensis*, *Schotia brachypetala*, *Sclerocarya birrea* and *Syzygium cordatum*.

Mathabe *et al*. (2006) recorded that one of the healers he interviewed used three of the plant species in two different combinations to treat diarrhoea. It is surprising that so little information on plant combinations have been documented in ethnobotanical antidiarrhoeal studies when it is known that it is general practice in traditional healing to combine plants for increased efficacy. The Maputaland survey revealed that seven plant species are being used in five different combinations for better efficacy against diarrhoea. Some plants are taken as enemas. An enema is an infusion that is administered anally (rectally). This may have a scientific importance as medicine is very rapidly absorbed through the delicate mucosa of the rectum and the large bowel, and the effects of the acidity of the stomach on the plant extract are avoided by just applying it rectally (Van Wyk *et al*., 2009).

Previous studies done on antidiarrhoeal plants documented that root and bark were mostly used (Mathabe *et al*., 2006), whereas in our survey the people used mostly the leaves, which is favourable for sustainability of the medicinal plants. The wide

variety of plant species used for the treatment of diarrhoea in this study can possibly be explained by the rich plant biodiversity in this area. KwaZulu-Natal Province consists of over 6000 vascular plant species and 1258 genera (70% of the genera in southern Africa) (Scott-Shaw, 1999). The choice of plants used to treat diarrhoea also depends on the plant availability in an around the homesteads.

3.5 Conclusions

- Twenty three plants species from fifteen families were mentioned by the interviewees to be used for treating diarrhoea.
- Four plants species were named by the interviewees from muthi markets to be used for treating diarrhoea.
- *Acacia burkei, Brachylaena transvaalensis, Cissampelos hirta and Sarcostemma viminale* were recorded for the first time as a treatment for diarrhoea.
- *Psidium guajava* is the most mentioned plant to treat diarrhoea.
- Leaves and bark are the main plant parts used.
- Also recorded for the first time is the use of the following plant combinations to treat diarrhoea; Brachylaena *transvaalensis* with *Psidium guajava or Sclerocarya birrea*, *Acanthospermum glabratum* in combination with *Krauseola mosambicina* or *Psidium guajava* and *Mangifera indica* in combination with *Sarcophyte sanguinea*.
- The wide variety of plants that are used to treat diarrhoea in this area supports the traditional value that medicinal plants have in the primary health care system of the rural people in northern Maputaland area.
- One new vanacular name for *C. ambrosioides* “Ikhambi” was recorded.
- The grandmothers proved to be the main information source from the interviewees.

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Chapter 4

Antibacterial activity

4.1 Introduction

Recently (1997-2008) there has been a number of findings on the antimicrobial activity of plants used traditionally for treating different ailments in South Africa (Van Vuuren, 2008). This chapter focuses on the antibacterial activity of the plant samples collected from different regions in the Maputaland area (Mabibi, Mseleni, Mbazwana and Tshongwe). The antibacterial screening was performed to validate the use of the plants by homestead dwellers as a treatment for diarrhoea. The Minimum Inhibition Concentration (MIC) assays were performed on 23 plants species against seven diarrhoeal pathogens, as described in Chapter 2. This allowed for the quantitative determination of the antimicrobial activity of the plant extracts. The mean MIC of the dichloromethane:methanol and aqueous crude plant extracts are presented as bar graphs and tables (Figures 4.1 to 4.60 and Tables 4.1 to 4.2). Results also include comparisons between the same plant species collected from the different regions. Dichloromethane:methanol extracts from household 1-20 represent Mseleni region, 21-40 represent Tshongwe, 41-62 represent Mabibi and 63-68 represent Mbazwana region. Due to restricted collecting practices, only one plant sample per household was studied with respect to aqueous extracts. Interpretation of the MIC value was as follows; extracts having activities below 8.00 mg/ml were considered to have some antimicrobial activity (Fabry *et al*., 1998). The MIC values of extracts below 1.00 mg/ml were considered noteworthy (Gibbons, 2004; Rios and Recio, 2005).

4.2 Results

The controls have not been included in the bar graphs but are placed as footnotes at the bottom of the graphs. Ciprofloxacin was used as the positive control. Acetone was used as a negative control. Where plant extracts did not dissolve in acetone DMSO was used.

4.2.1 *Acacia burkei*

Only one household (household 2) from four households that use the plant species

Acacia burkei to treat diarrhoea had the actual plant growing in the homestead. The dichloromethane:methanol extracts for household 2, showed antibacterial activity against all seven diarrhoeal pathogens (Figure 4.1). The *Acacia burkei* sample showed noteworthy antimicrobial activity (values below the red line) on Figure 4.1 against *Shigella flexneri* (MIC value 0.25 mg/ml) and *Proteus vulgaris* (MIC value 0.50 mg/ml) and the least antimicrobial activity (MIC value 3.00 mg/ml) was for *Bacillus cereus* and *Salmonella typhimurium* (Figure 4.1).

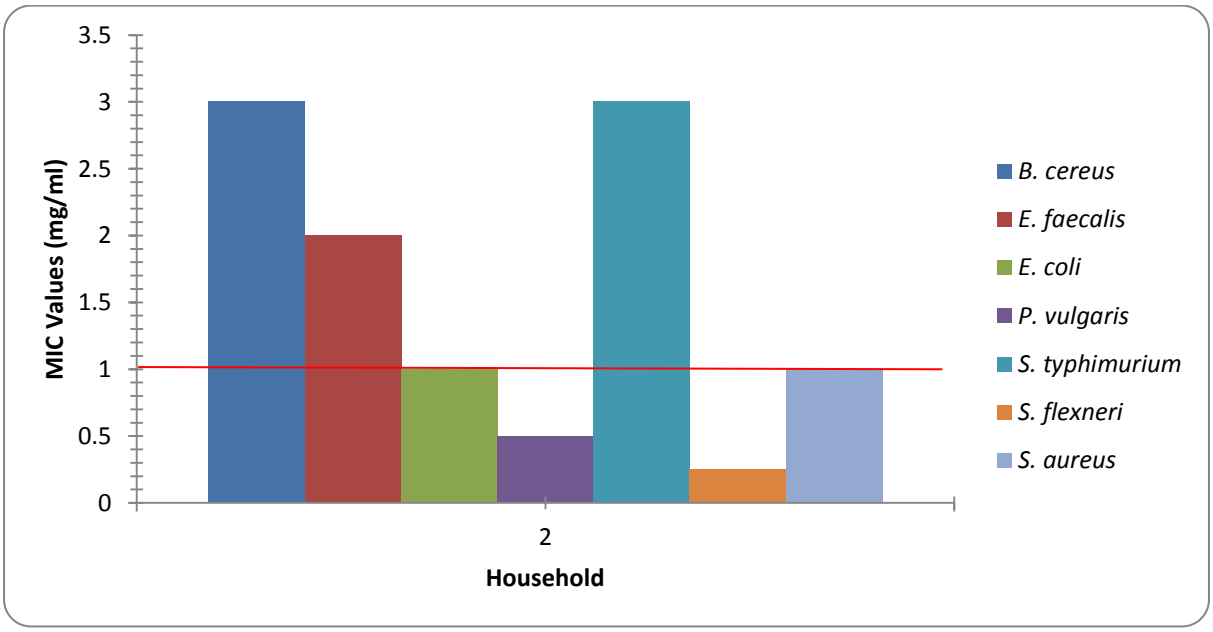

Figure 4.1 The mean MIC values of *Acacia burkei* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The aqueous extracts of *Acacia burkei* (household 2) exhibited antibacterial activity for all diarrhoeal pathogens (Figure 4.2). The noteworthy antibacterial activity was observed at the MIC value of 0.75 mg/ml against *Bacillus cereus*. The least activity observed was at the MIC value of 3.00 mg/ml against *Proteus vulgaris* and *Salmonella typhimurium*. The antibacterial activity of the dichloromethane:methanol crude extracts of *Acacia burkei* was nearly in the same range as that of the aqueous crude extracts.

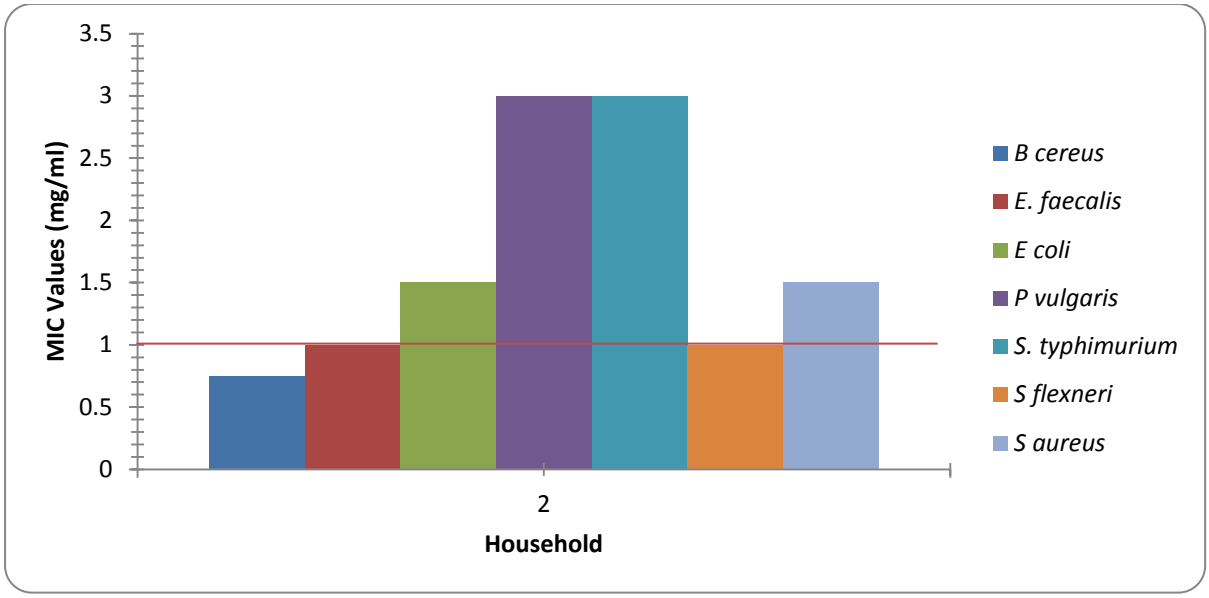

Figure 4.2 The mean MIC values of *Acacia burkei* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

To the best of my knowledge, these are the first recorded antimicrobial studies for *Acacia burkei*. This plant species showed noteworthy activity against three pathogens namely: *Bacillus cereus* (MIC value of 0.75 mg/ml), *Shigella flexneri* (MIC value 0.25 mg/ml) and *Proteus vulgaris* (MIC value 0.50 mg/ml). In the same genera *Acacia nilotica* (L.) Willd.ex Del. extracts showed significant antidiarrhoeal activity (Kambizi and Afolayan, 2001). The same constituents (tannins) responsible for antidiarrhoeal activity in *Acacia nilotica* could be responsible for the antibacterial activity of *Acacia burkei*.

4.2.2 *Acanthospermum glabratum*

Four households (household 11, 39, 44 and 55) use *Acanthospermum glabratum* to treat diarrhoea. The activity range of *Acanthospermum glabratum* was between 0.50 mg/ml and 6.00 mg/ml. Noteworthy activity was observed against *Bacillus cereus* (MIC value 0.50 mg/ml) and *Shigella flexneri* (MIC value of 0.25 mg/ml) for three households (household 11, 44 and 55). Four household samples of *Acanthospermum glabratum* were active against most pathogens except for *Enterococcus faecalis* (household 44, Mabibi region); *Escherichia coli* (household 39, Tshongwe region); *Proteus vulgaris* (household 11, 39 55) and *Staphylococcus*

aureus (household 39,Tshongwe region) (Figure 4.3). There was no major difference in the activities of extracts between households 11, 44 and 55 but household 39 extracts showed least activity against the pathogens.

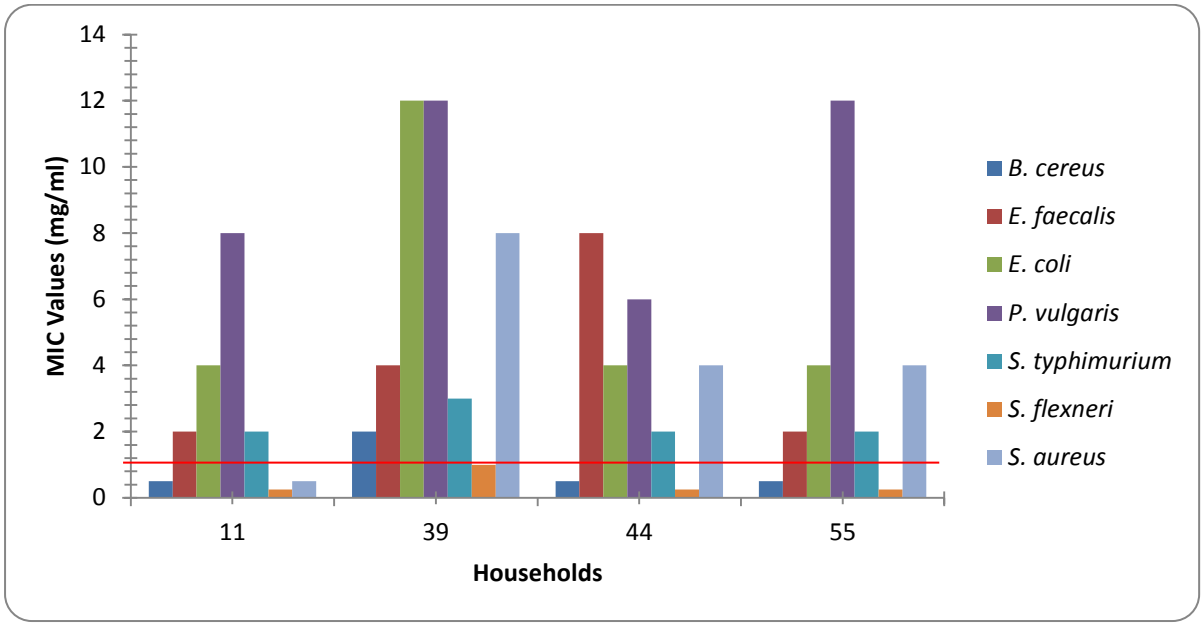

Figure 4.3 The mean MIC values of *Acanthospermum glabratum* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The aqueous extracts of *Acanthospermum glabratum* for household 55 (only one household sampled) were moderate to poorly active against four diarrhoeal pathogens namely *Bacillus cereus* (MIC value 5.33 mg/ml), *Salmonella typhimurium* (MIC value 6.00 mg/ml), *Shigella flexneri* (MIC value 4.00 mg/ml) and *Staphylococcus aureus* (MIC value 6.67 mg/ml) (Figure 4.4). The antibacterial activity of the dichloromethane:methanol crude extracts of *Acanthospermum glabratum* was higher than that of the aqueous crude extracts. This is often observed from the other studies (Motsei *et al.,* 2003; Mathabe *et al.,* 2006; Anas *et al*., 2008). Water which is an aqueous solvent and extracts only polar substances while dichloromethane:methanol which is an organic solvent extracts both polar and non-polar substances and hence increased activity is expected with dichloromethane:methanol extracts.

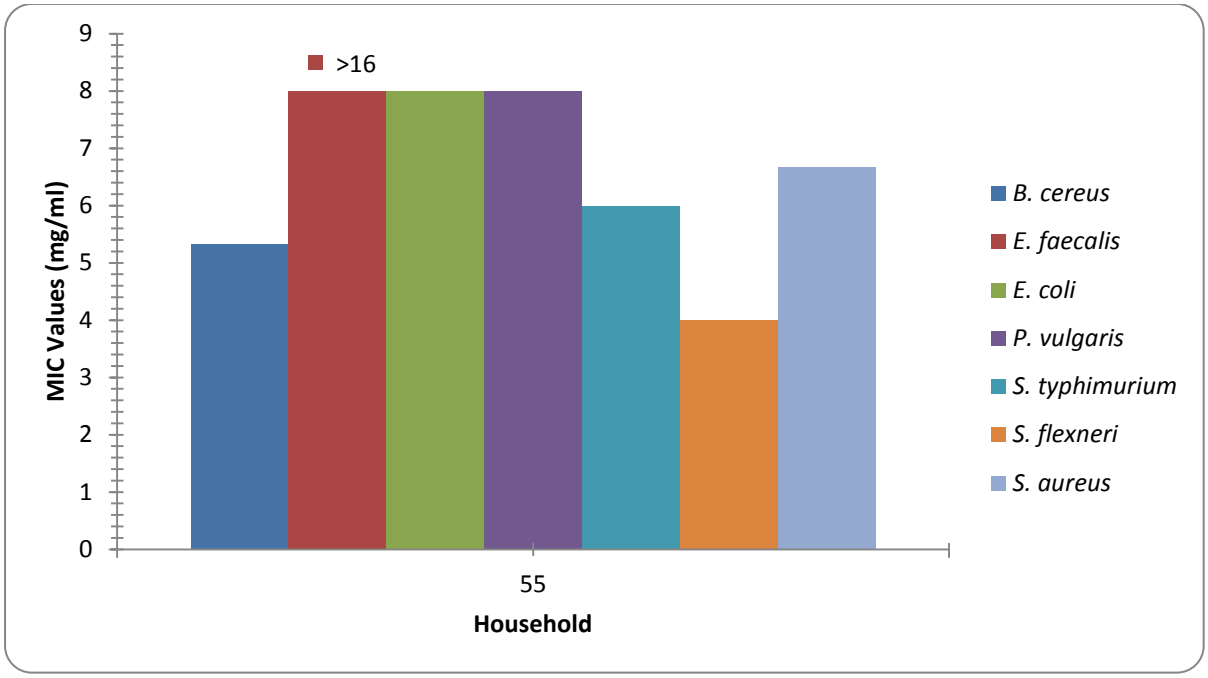

Figure 4.4 The mean MIC values of *Acanthospermum glabratum* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

York *et al*. (2012) demonstrated that dichloromethane:methanol extracts of this plant species showed moderate antibacterial activity when screened against *Staphylococcus aureus* at the mean MIC value of 2.00 mg/ml and the aqueous extracts yielded 16 mg/ml. This organic extract value is below the noteworthy value obtained in this study for household 11 (MIC value 0.50 mg/ml) while the aqueous value of this study showed moderate activity at 6.67 mg/ml. Dichloromethane:methanol and aqueous extractswere also screened for *Cryptococcus neoformans* (MIC values 0.30 mg/ml and > 16.00 mg/ml), *Klebsiella pneumoniae* (MIC values 3.33 mg/ml and 10.67 mg/ml), *Moraxella catarrhalis* (MIC values 4.00 mg/ml and > 16.00 mg/ml) and *Mycobacterium smegmatis* (MIC values 0.67 mg/ml and 8.00 mg/ml) (York *et al*., 2012). In another study of the species *Acanthospermum hispidum* DC. which is in the same genus as *A. glabratum*, showed antibacterial activity in disc diffusion method. The zones of inhibition were up to 19 mm for *Bacillus subtilis,* 21 mm for *Staphylococcus aureus,* 21 mm for *Streptococcus pyogenes,* 21 mm for *Salmonella typhi* and 20 mm for *Clostridium*

histolyticum (Fleischer *et al.*, 2003). This is very good activity and it shows the possibility of a positive antibacterial activity by the screened plant species.

4.2.3 *Brachylaena transvaalensis*

Two households (household 48 and 49, Tshongwe region) use *Brachylaena transvaalensis* to treat diarrhoea. The two household samples of dichloromethane:methanol extracts of *Brachylaena transvaalensis* showed antibacterial activity against all seven diarrhoeal pathogens (Figure 4.5). This species showed highest noteworthy antibacterial activity ranging from the MIC value of 0.13 mg/ml to 0.50 mg/ml against *Bacillus cereus* (both households), *Enterococcus faecalis* (household 48), *Shigella flexneri* (both households) and *Staphylococcus aureus* (household 48). The poorest antibacterial activity (MIC value 2 mg/ml) was against *Escherichia coli* (both households 48 and 49), *Enterococcus faecalis* (household 49), *Proteus vulgaris* (household 49) and *Staphylococcus aureus* (household 49).

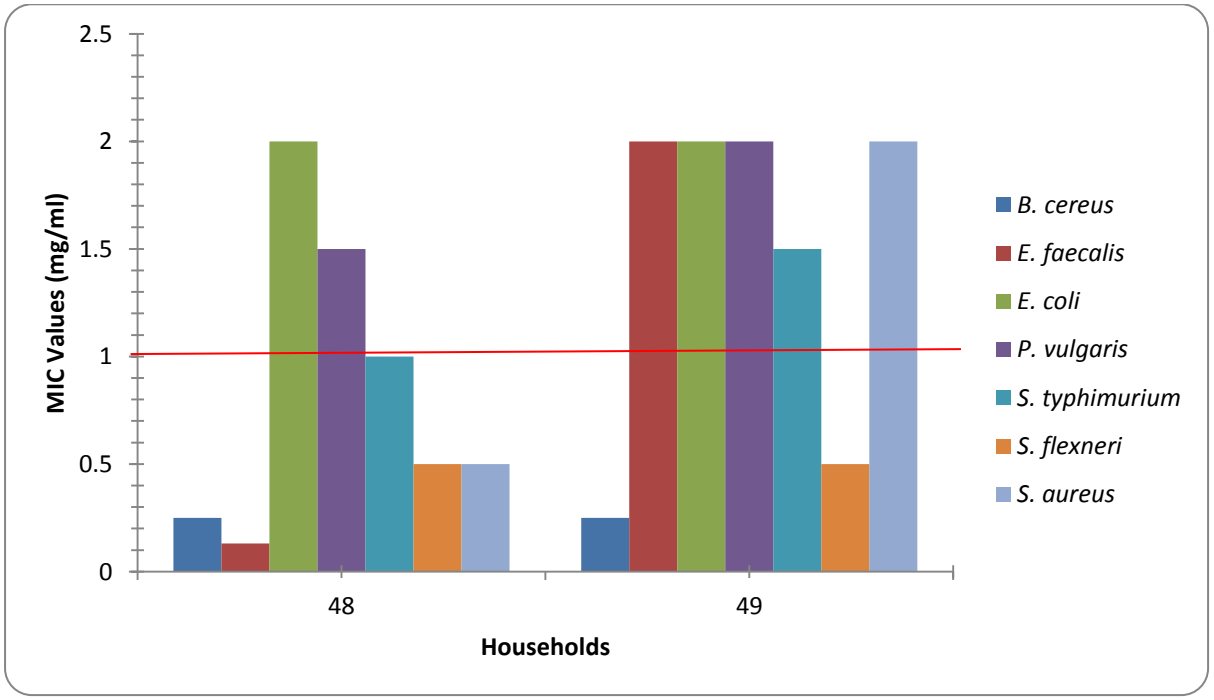

Figure 4.5 The mean MIC values of *Brachylaena transvaalensis* dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Only one pathogen (*Shigella flexneri*) showed susceptibility at MIC value of 4.00 mg/ml against aqueous extracts (Figure 4.6).

The antibacterial activity from this study is for the first time recorded against these pathogens. However, a previous study by Motsei *et al*. (2003) on *Brachylaena transvaalensis* demonstrated poor antifungal activity (MIC value > 8.35 mg/ml) against *Candida albicans* for ethanol, ethyl acetate, hexane and aqueous extracts.

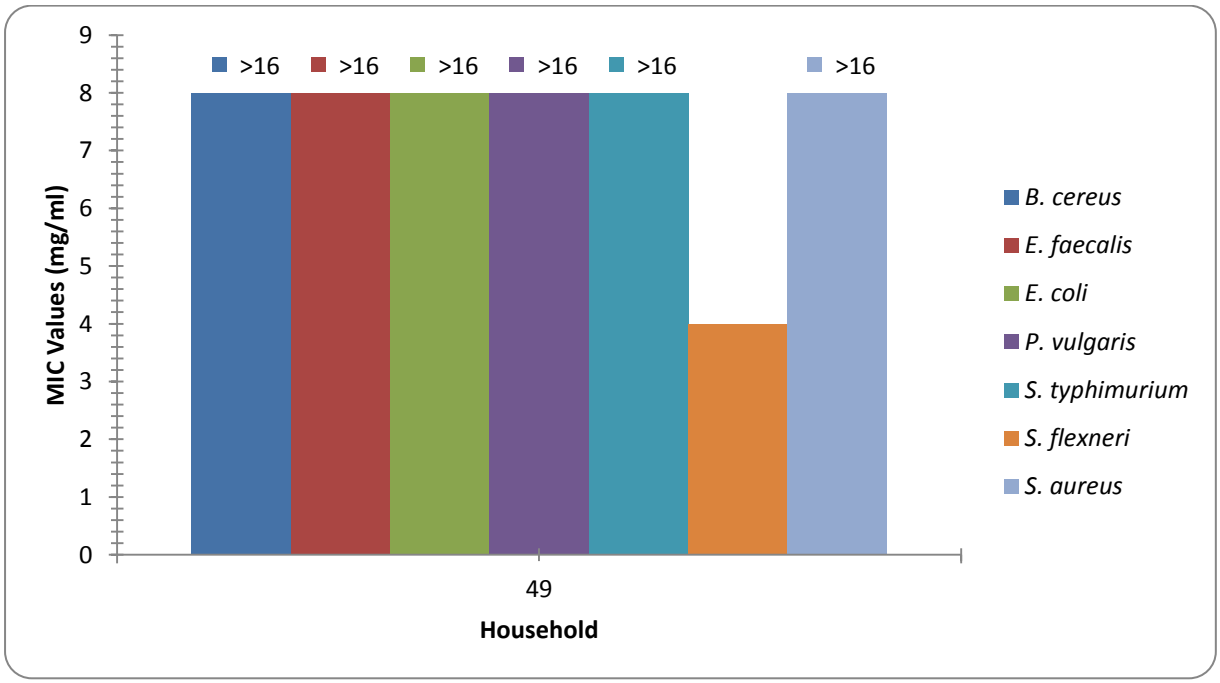

Figure 4.6 The mean MIC values of *Brachylaena transvaalensis* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.4 *Catharanthus roseus*

Six households (1, 4, 6, 14, 17 and 66) use *Catharanthus roseus* to treat diarrhoea (Figure 4.7). The dichloromethane:methanol extracts from the different households showed mostly moderate activity with the poorest MIC value of 8.00 mg/ml observed for *Escherichia coli*, *Proteus vulgaris, Staphylococcus aureus* (household 1, Mseleni) and *Salmonella typhimurium* (household 4, Mseleni). All households showed significant antibacterial activity against *Shigella flexneri* with MIC values ranging from 0.25 to 0.75 mg/ml.

The aqueous extract of *Catharanthus roseus* (household 66, Mbazwana/Olakeni) was moderately active against six diarrhoeal pathogens except *Staphylococcus aureus* (MIC value 8.00 mg/ml) (Figure 4.8).

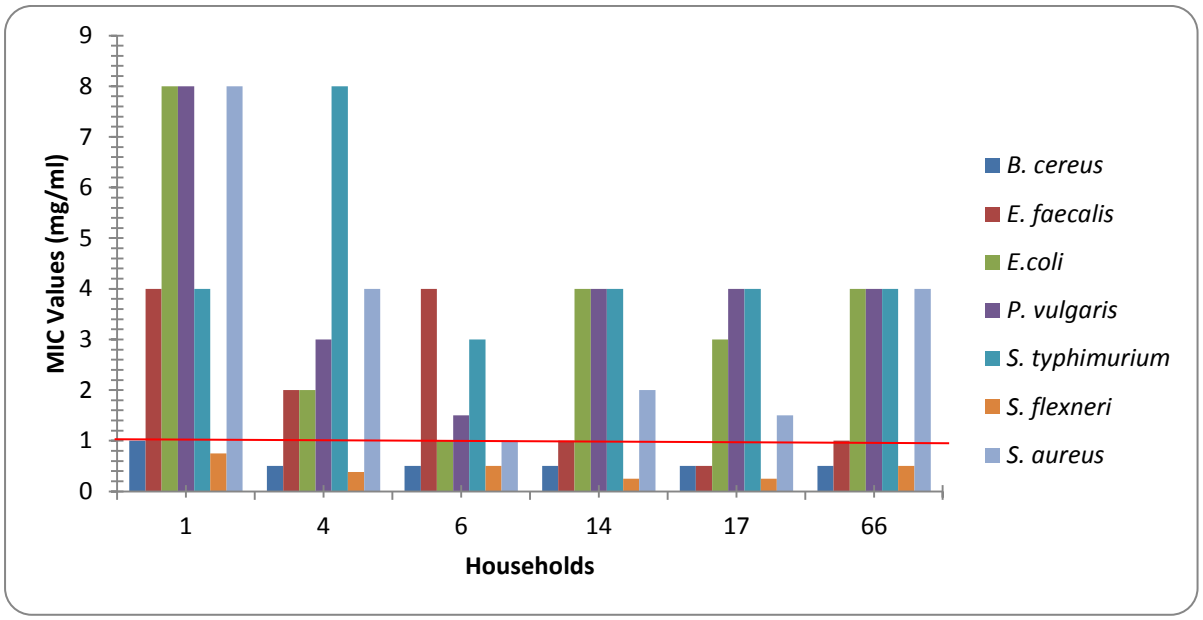

Figure 4.7 The mean MIC values of *Catharanthus roseus* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

In a previous disc diffusion assay studies, *Catharanthus roseus* showed moderate antibacterial activity against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Streptococcus pyogene*s (Ramya *et al.*, 2008; Govindasamy and Srinivasan, 2012). This activity correlates with the moderate activity shown in this study against *Escherichia coli* and *Salmonella typhi.* Activity against *Bacillus cereus* in the present study was more pronounced (Figure 4.7). Another study demonstrated some antimicrobial activity against *Staphylococcus aureus, Pythium ultimum*, and *Rhizoctonia solani* using the disc diffusion method (Goun *et al.*, 2003). In a more recent study by Van Vuuren and Naidoo (2010), it is demonstrated that *Catharanthus roseus* has antimicrobial activity against pathogens responsible for sexually transmitted infections.

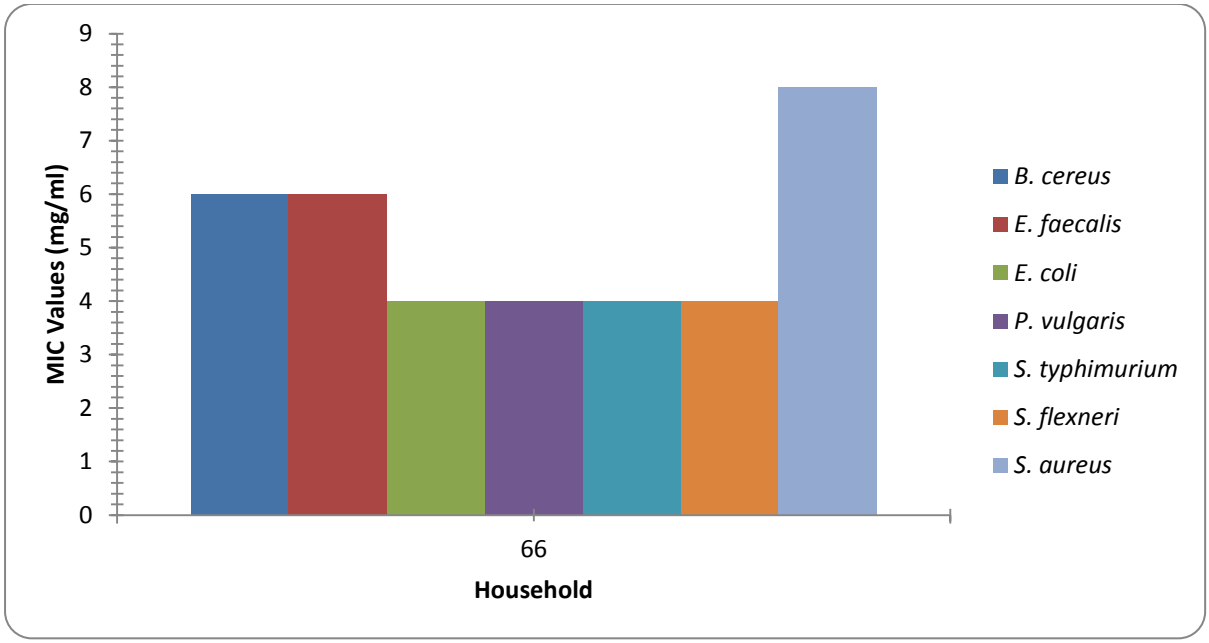

Figure 4.8 The mean MIC values of *Catharanthus roseus* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.5 *Chenopodium ambrosioides*

One household (household 28, Tshongwe) uses *Chenopodium ambrosioides* to treat diarrhoea. The dichloromethane:methanol extract showed antibacterial activity against six pathogens (Figure 4.9). Noteworthy antimicrobial activity was observed against *Proteus vulgaris* (MIC value of 0.25 mg/ml), *Staphylococcus aureus* (MIC value of 0.50 mg/ml) and *Shigella flexneri* (MIC value of 0.25 mg/ml). The aqueous extract of *Chenopodium ambrosioides* was found to be moderately active against three diarrhoeal pathogens i.e. *Bacillus cereus, Proteus vulgaris* and *Staphylococcus aureus* (Figure 4.10). The highest antimicrobial activity (lowest MIC value) was observed at the MIC value of 3.00 mg/ml against *Bacillus cereus* and *Proteus vulgaris*. The poorest activity observed was at the MIC value of 8.00 mg/ml against *Enterococcus faecalis, Escherichia coli, Salmonella typhimurium* and *Shigella flexneri*. The aqueous extract showed enhanced activity for *Bacillus cereus* at the MIC value of 3.00 mg/ml compared to the dichloromethane:methanol extract which showed a poor MIC value of 12.00 mg/ml.

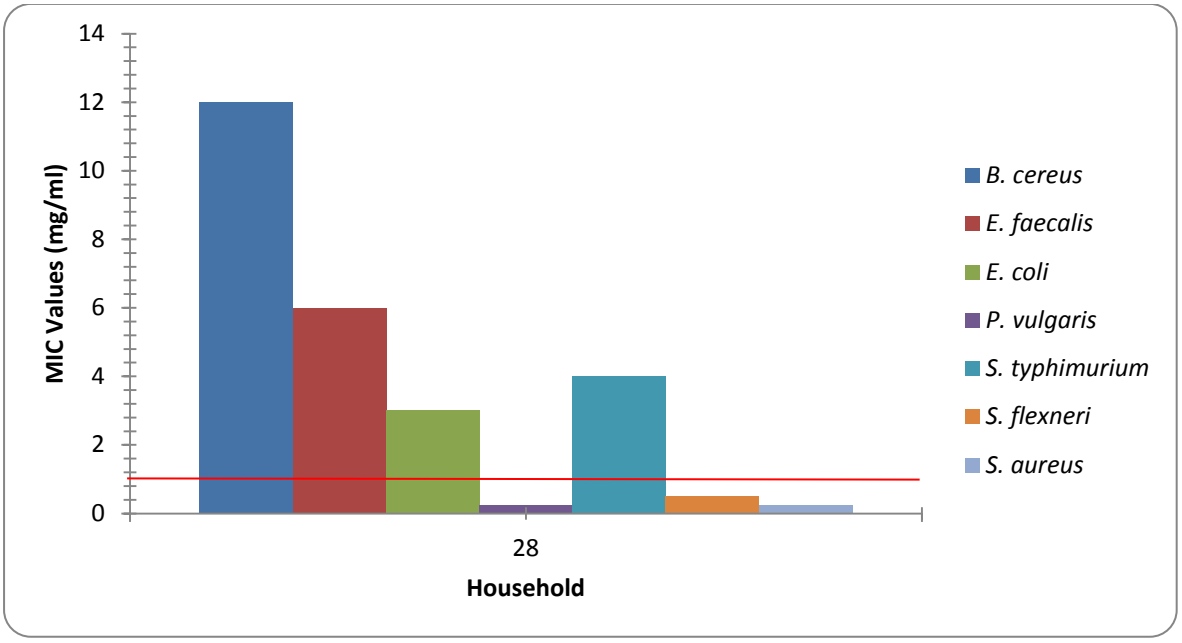

Figure 4.9 The mean MIC values of *Chenopodium ambrosioides* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

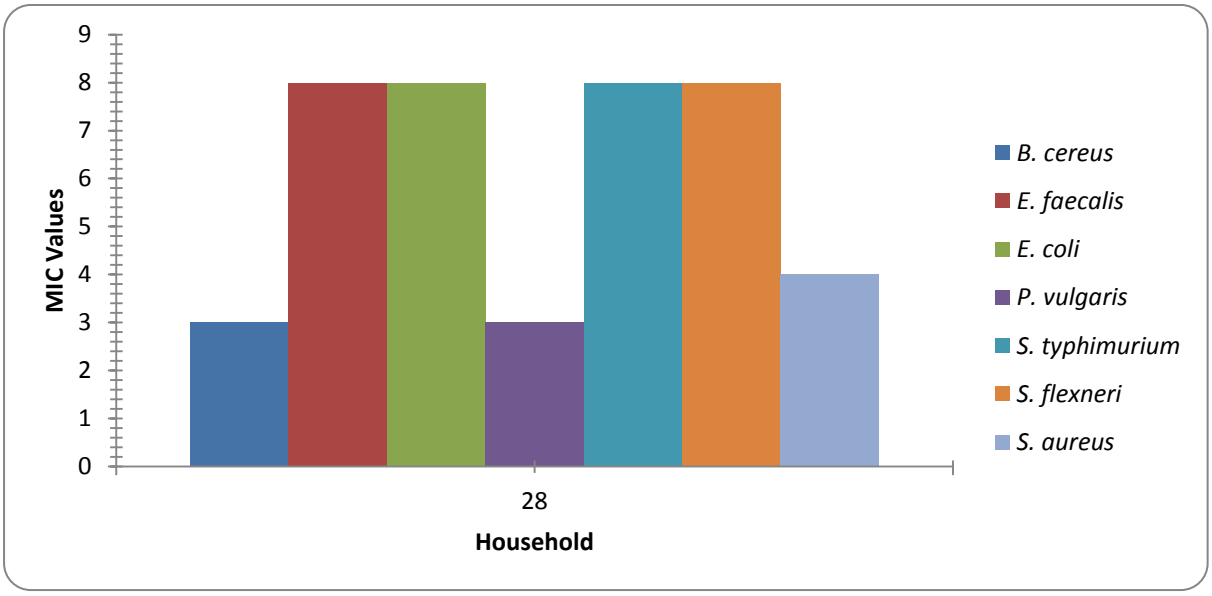

Figure 4.10 The mean MIC values of *Chenopodium ambrosioides* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Results from other antimicrobial studies show that ethanol extracts of this medicinal plant demonstrated poor antidiarrhoeal activity against the pathogen *Staphylococcus*

aureus (MIC value 8.00 mg/ml) (Bussmann *et al.*, 2010). Results from this study are much better than the latter study at MIC value of 0.25 mg/ml. This difference in activity may be due to different solvents used, geographical variation since the study was performed in Peru or the different strains of microorganisms used. *Mycobacterium tuberculosis* bacteria growth was inhibited at an MIC value of 0.5 mg/ml by *Chenopodium ambrosioides* (Lall and Meyer, 1999). In another study, *Chenopodium ambrosioides* showed no antifungal activity against *Aspergillus flavus* (Kumar *et al.*, 2007).

4.2.6 *Cissampelos hirta*

One household sample of dichloromethane:methanol extract (household 61, Mbazwana/ Olakeni) of *Cissampelos hirta* was tested against the seven diarrhoeal pathogens (Figure 4.11). This medicinal plant showed some antibacterial activity against six diarrhoeal pathogens with the exception of *Escherichia coli*. Noteworthy antimicrobial activity was observed against *Enterococcus faecalis* and *Shigella flexneri* with MIC values of 0.50 mg/ml.

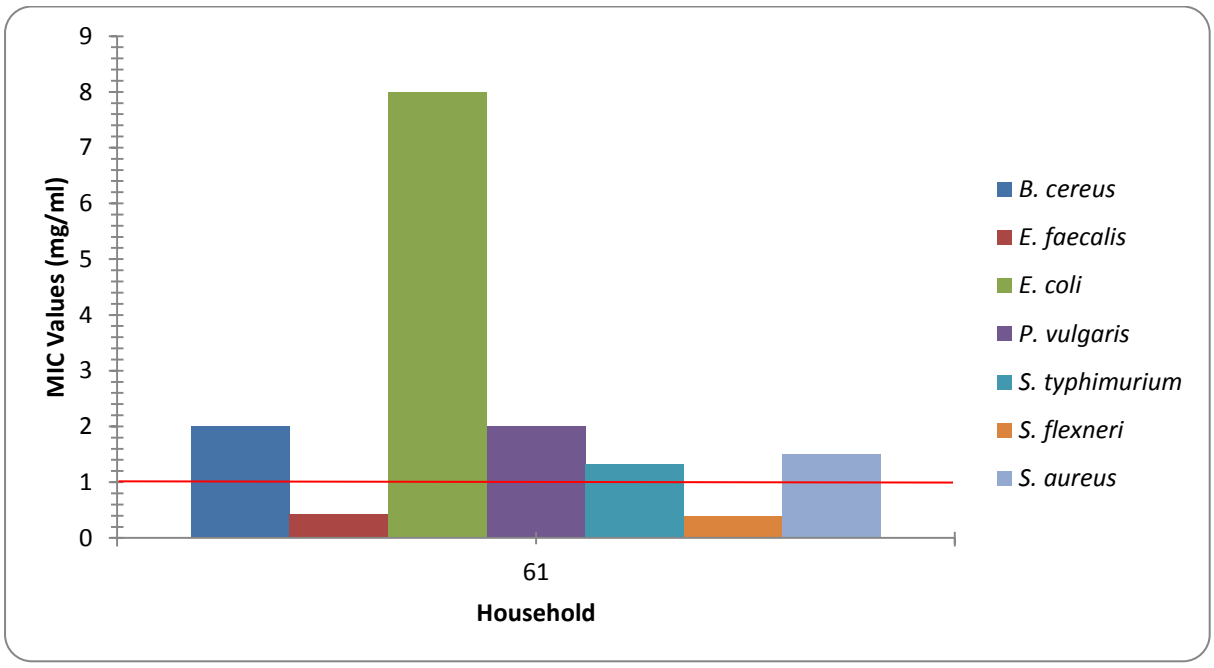

Figure 4.11 The mean MIC values of *Cissampelos hirta* (dichloromethane:methanol extract) against seven diarrhoeal pathogens.Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The aqueous extract of *Cissampelos hirta* (household 61) was moderately active against three diarrhoeal pathogens (Figure 4.12), namely *Bacillus cereus* and *Shigella flexneri* (MIC value 6.00 mg/ml) and *Enterococcus faecalis* (MIC value 4.00).

To the best of my knowledge, there are no recorded documents relating to antibacterial activity of *Cissampelos hirta* and hence this is the first antimicrobial report thereof.

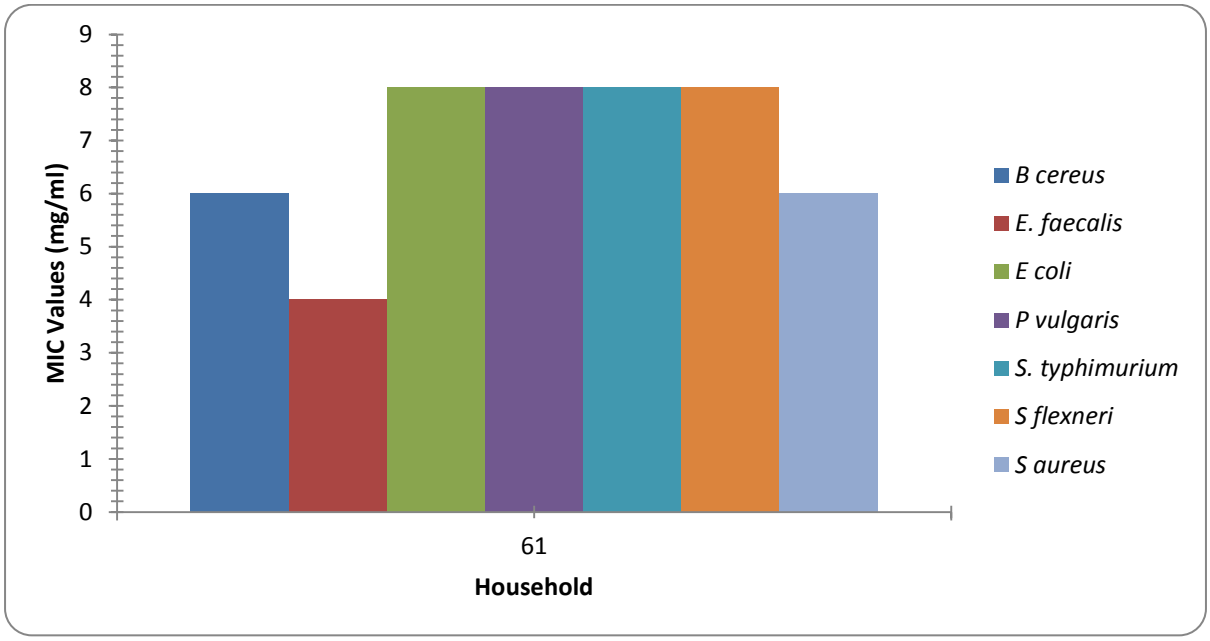

Figure 4.12 The mean MIC values of *Cissampelos hirta* (aqueous extract) against seven diarrhoeal pathogens.Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.7 *Garcinia livingstonei*

Two households (29, Tshongwe and 42, Mabibi) use *Garcinia livingstonei* to treat diarrhoea. Dichloromethane:methanol extracts for both households were active against all seven pathogens (Figure 4.13). Noteworthy antibacterial activity was observed for all diarrhoeal pathogens except *Escherichia coli* (household 42 Mabibi). The highest antimicrobial activity (lowest MIC value) was noted at the MIC value of 0.11 mg/ml against *Bacillus cereus* (household 42).No major difference was shown

by the two extracts from the two different locations except for MIC activity against *Escherichia coli* (MIC value 6.00 mg/ml) from household 42 Mabibi.

Only one household (household 42) was tested for aqueous extracts of *Garcinia livingstonei* against seven diarrhoeal pathogens. The aqueous extract of *Garcinia livingstonei* was active for all diarrhoeal pathogens (Figure 4.14). The most susceptible diarrhoeal pathogens were *Escherichia coli* and *Staphylococcus aureus* where growth was inhibited at noteworthy MIC values of 0.75 mg/ml. The least activity was observed at the MIC value of 2.00 mg/ml against *Enterococcus faecalis*, *Salmonella typhimurium* and *Shigella flexneri*.

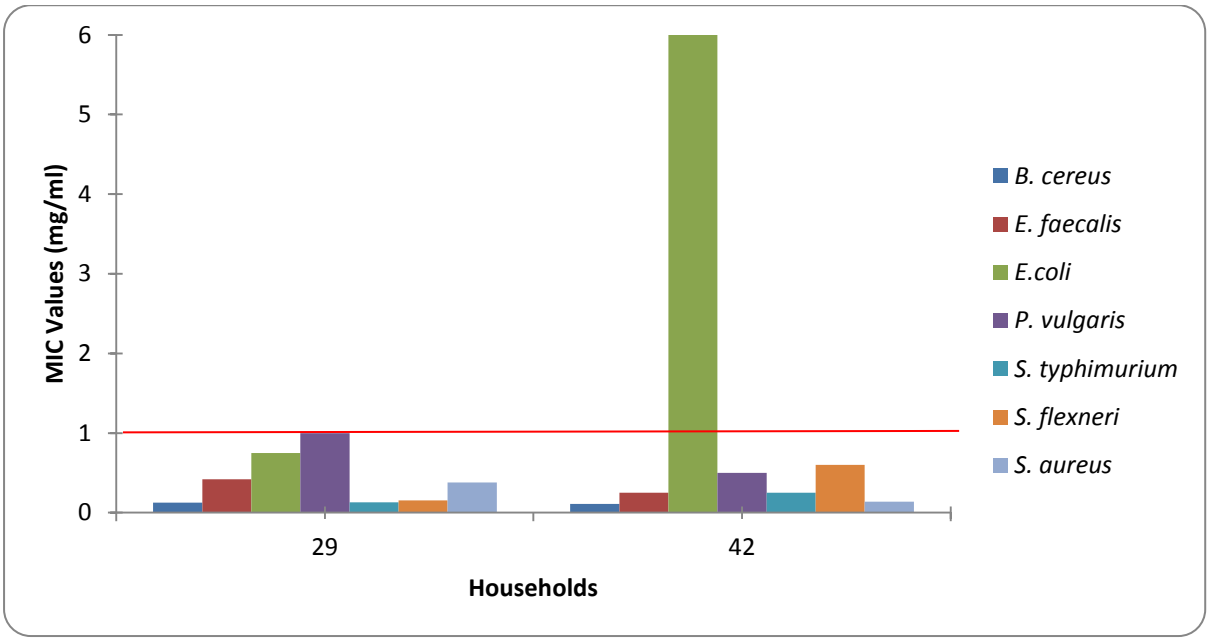

Figure 4.13 The mean MIC values of *Garcinia livingstonei* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The crude extracts of *Garcinia livingstonei* showed antibacterial activity against *Escherichia coli* (MIC value 1.57 mg/ml), *Staphylococcus aureus* (MIC value 0.50 mg/ml), *Pseudomonas aeruginosa* (MIC value 1.25 mg/ml), and *Enterococcus faecalis* (MIC value 0.55 mg/ml). The amentoflavone, compound isolated from *Garcinia livingstonei* also showed antibacterial activity against *Escherichia coli* (MIC value 0.04 mg/ml), *Staphylococcus aureus* (MIC value 0.04 mg/ml), *Pseudomonas*

aeruginosa (MIC value > 0.10 mg/ml), and *Enterococcus faecalis* (MIC value 0.06 mg/ml). Another isolated compound 4"-methoxy amentoflavone also showed antibacterial activity against *Escherichia coli* (MIC value 0.008 mg/ml), *Staphylococcus aureus* (MIC value 0.04 mg/ml), *Pseudomonas aeruginosa* (MIC value 0.060 mg/ml), and *Enterococcus faecalis* (MIC value 0.008 mg/ml) (Kaikabo *et al.,* 2009). Purified compounds (amentoflavone and 4 monomethoxyamentoflavone) extracted from this plant species showed antibacterial activity against *Mycobacterium smegmatis* at the MIC values of 0.60+/-0.70 mg/ml and 1.40+/-1.56 mg/ml respectively (Kaikabo and Eloff*,* 2011).

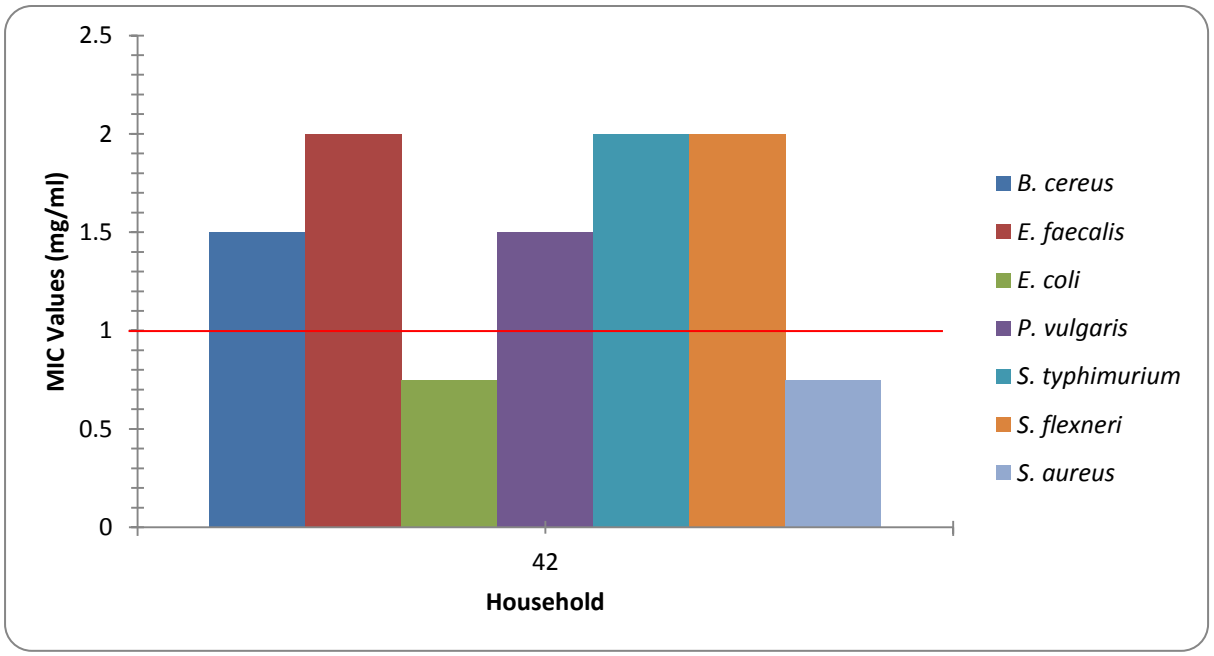

Figure 4.14 The mean MIC values of *Garcinia livingstonei* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.8 *Gymnosporia senegalensis*

Two households (12, Mseleni and 36, Tshongwe) use *Gymnosporia senegalensis* to treat diarrhoea. Dichloromethane:methanol extracts showed positive activity for five pathogens except against *Escherichia coli* (MIC value 8.00 mg/ml) (household 12) and *Proteus vulgaris* (MIC value ≥ 16.00 mg/ml) (household 36) (Figure 4.15). *Enterococcus faecalis* was the most susceptible with a noteworthy mean MIC value of 0.11 mg/ml (household 36). The aqueous extract of *Gymnosporia*

*senegalensis*was was poorly active with some activity against only one diarrhoeal pathogen, *Enterococcus faecalis* (MIC value 6.00 mg/ml) (Figure 4.16).

Previous studies of *Gymnosporia senegalensis* methanol, ethanol, acetone and aqueous crude extracts showed antibacterial activity with MIC values ranging from 0.08-0.31 mg/ml against a range of pathogens including *Staphylococcus aureus, Escherichia coli*, *Salmonella typhi* and *Shigella flexneri* (Mathabe *et al.,* 2006). The MIC values obtained from dichloromethane:methanol and aqueous extracts of *Gymnosporia senegalensis* from this study for *Escherichia coli* (4.00-12.00 mg/ml) *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus* is much poorer.

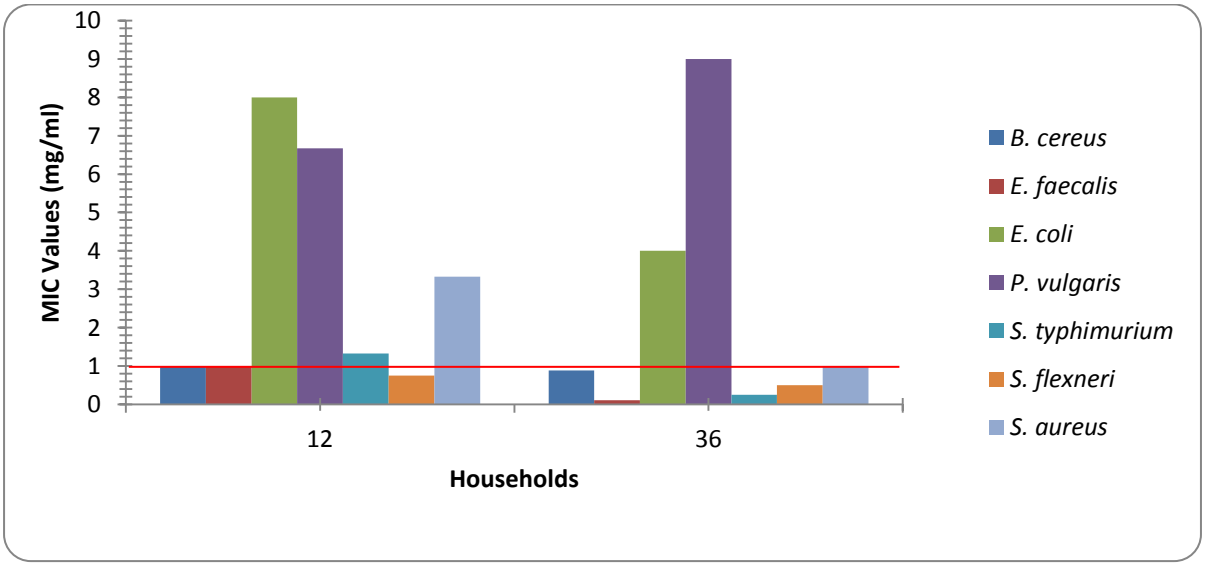

Figure 4.15 The mean MIC values of *Gymnosporia senegalensis* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Petroleum, ethanol, dichloromethane and aqueous crude root extracts of this plant species demonstrated antimicrobial activity against *Bacillus subtilis* (MIC value range 0.39 mg/ml to 1.56 mg/ml), *Escherichia coli* (MIC value range 0.10 mg/ml to 3.13 mg/ml), *Klebsiella pneumoniae* (MIC value range 1.56 mg/ml to 3.13 mg/ml) and *Staphylococcus aureus* (MIC value range 0.78 mg/ml to 6.25 mg/ml) (Mulaudzi *et al*., 2012). These results are better compared to this study for *Escherichia coli* (MIC value range 4.00 mg/ml to 9.00 mg/ml) but at the same range for *Staphylococcus*

aureus (MIC value range 1.00 mg/ml to 8.00 mg/ml). These minor differences may be due to different extractants used. Maytenonic acid derived from *Gymnosporia senegalensis* has shown antibacterial activity (0.10 mg/ml) against *Bacillus subtilis, Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (0.20 mg/ml) (Lindsey *et al.*, 2006). Results obtained from the latter study shows that *Gymnosporia senegalensis* has some significant activity compared to our findings.

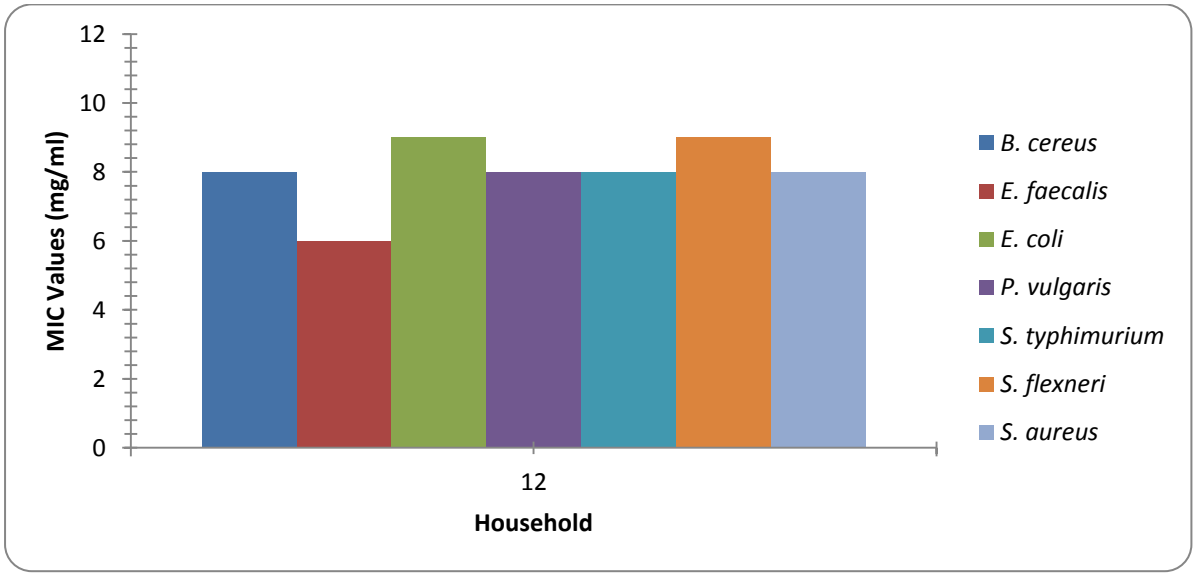

Figure 4.16 The mean MIC values of *Gymnosporia senegalensis* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.9 *Krauseola mosambicina*

Five households (4, Mseleni; 14, Mseleni; 17, Mseleni; 21 Tshongwe and 55 Mabibi) use *Krauseola mosambicina* to treat diarrhoea. The antibacterial activity of the dichloromethane:methanol extracts ranged between 1.00 mg/ml and ≥ 16.00mg/ml. The most susceptible pathogen was *Shigella flexneri* (MIC value of 1.00 mg/ml) (household 4, 17, 21 and 55) (Figure 4.17). The extracts from household 17 displayed the best antimicrobial activity.

One aqueous extract sample (household 17) was tested against the seven diarrhoeal pathogens. The aqueous extract of *Krauseola mosambicina* was active against three diarrhoeal pathogens namely *Enterococcus faecalis* (MIC value 5.33

mg/ml), *Salmonella typhimurium* (MIC value 6.67 mg/ml) and *Shigella flexneri* (MIC value 6.67 mg/ml) (Figure 4.18).

York *et al*. (2012), demonstrated that dichloromethane:methanol and aqueous extracts of this herb possess moderate to poor antibacterial activity against *Staphylococcus aureus* at the MIC values of 6.67 mg/ml and 16.00 mg/ml, respectively. These results narrowly correlate with the observation in this study but organic extract from household 17 showed good activity at the MIC value of 2.00 mg/ml. The aqueous extract showed better activity (MIC value 8.00 mg/ml) compared to the study by York *et al*. (2012). Dichloromethane:methanol and aqueous extracts were also screened for *Cryptococcus neoformans* (MIC values 1.00 mg/ml and 3.33 mg/ml), *Klebsiella pneumoniae* (MIC values 3.00 mg/ml and 16.00 mg/ml), *Moraxella catarrhalis* (MIC values 12.00 mg/ml and > 16.00 mg/ml) and *Mycobacterium smegmatis* (MIC values 1.00 mg/ml and 16.00 mg/ml) (York *et al*., 2012).

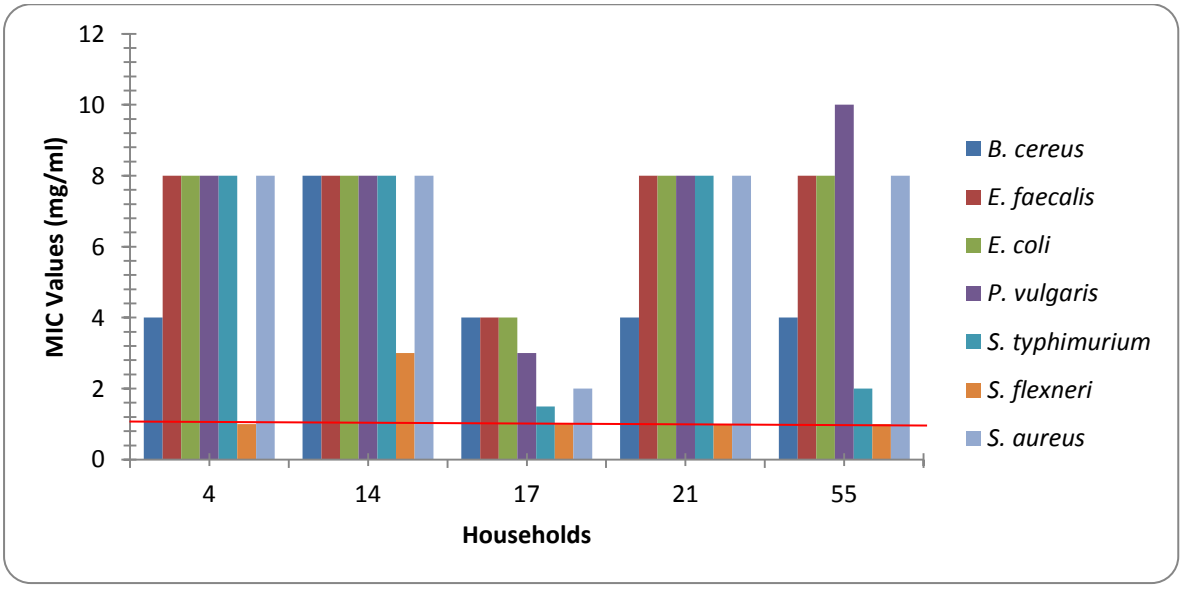

Figure 4.17 The mean MIC values of *Krauseola mosambicina* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

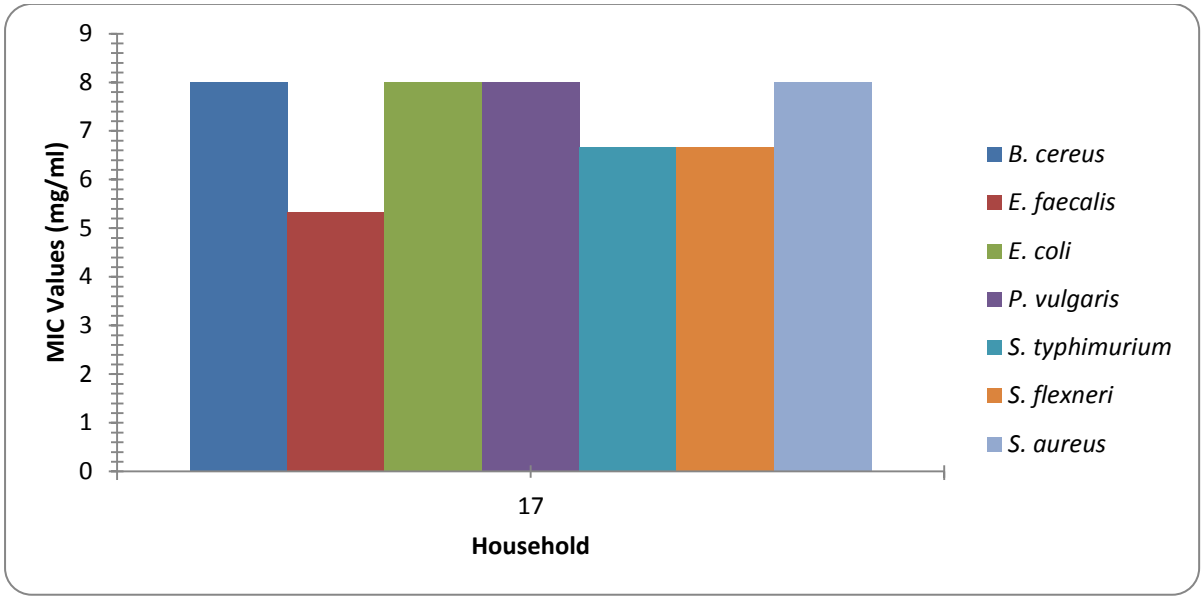

Figure 4.18 The mean MIC values of *Krauseola mosambicina* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.10 *Lippia javanica*

One household (16, Mseleni) uses *Lippia javanica* to treat diarrhoea. Dichloromethane:methanol extract exhibited antibacterial activity against all pathogens except *Proteus vulgaris* (MIC value 12.00 mg/ml) (Figure 4.19). Noteworthy activity was observed with *Shigella flexneri* at the MIC value of 0.50 mg/ml.

The aqueous extract of *Lippia javanica* was moderately active against four diarrhoeal pathogens namely: *Enterococcus faecalis* (MIC value of 4.00 mg/ml), *Proteus vulgaris*, (MIC value of 4.00 mg/ml), *Salmonella typhimurium* (MIC value of 6.00 mg/ml) and *Staphylococcus aureus* (MIC value of 6.00 mg/ml) (Figure 4.20).

Previous studies on *Lippia javanica* essential oil have shown positive antimicrobial activity using disc diffusion technique against *Staphylococcus aureus* (18 mm) and *Escherichia coli* (16 mm) (Manenzhe *et al.*, 2004). *Lippia javanica* essential oil also showed antimicrobial activity using time kill methods against *Bacillus cereus, Klebsiella pneumoniae* and *Cryptococcus neoformans* (Viljoen *et al.,* 2005).

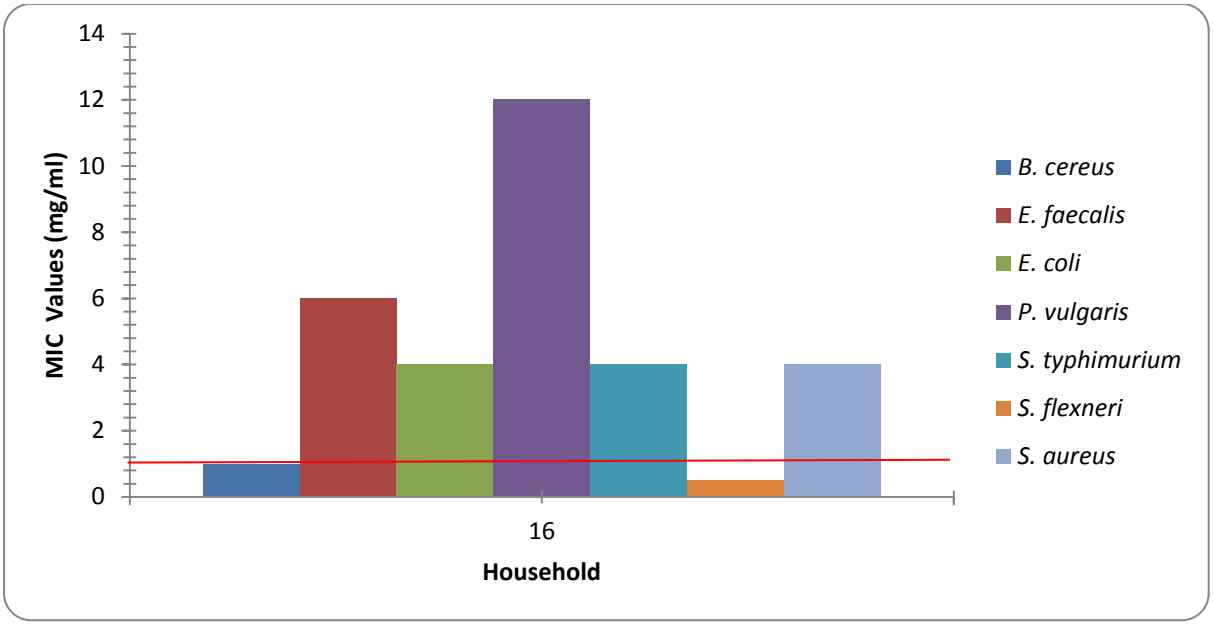

Figure 4.19 The mean MIC values of *Lippia javanica* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

A study by Shikanga *et al.* (2010), demonstrated that *Lippia javanica* crude extracts (water: acetone: methanol) possess antimicrobial properties against *Staphylococcus aureus* (MIC value 0.13 mg/ml), *Escherichia coli* (MIC value 0.30 mg/ml), *Enterococcus faecalis* (MIC value 0.14 mg/ml) and *Pseudomonas aeruginosa* (MIC value 0.42 mg/ml). In a recent study by York *et al*. (2012), it was shown that dichloromethane:methanol and aqueous underground part and leaf extracts of this plant possess moderate antibacterial activity against *Staphylococcus aureus* at the MIC value range of 1.33 mg/ml to 8.00 mg/ml. These results are better than the results obtained from this study but they are at the same range for dichloromethane:methanol extracts (MIC value 4.00 mg/ml) but not for aqueous extracts (MIC value 16.00 mg/ml). Dichloromethane:methanol and aqueous extracts were also screened for *Cryptococcus neoformans* (MIC value range 0.25 mg/ml to 16.00 mg/ml), *Klebsiella pneumoniae* (MIC value range 1.33 mg/ml to 8.00 mg/ml), *Moraxella catarrhalis* (MIC value range 3.33 mg/ml to > 16.00 mg/ml) and *Mycobacterium smegmatis* (MIC values 1.00 mg/ml to 16.00 mg/ml) (York *et al*., 2012). Methanol extracts of this medicinal plant have been found to possess antibacterial activity against *Mycobacterium tuberculosis* (Green *et al.,* 2010).

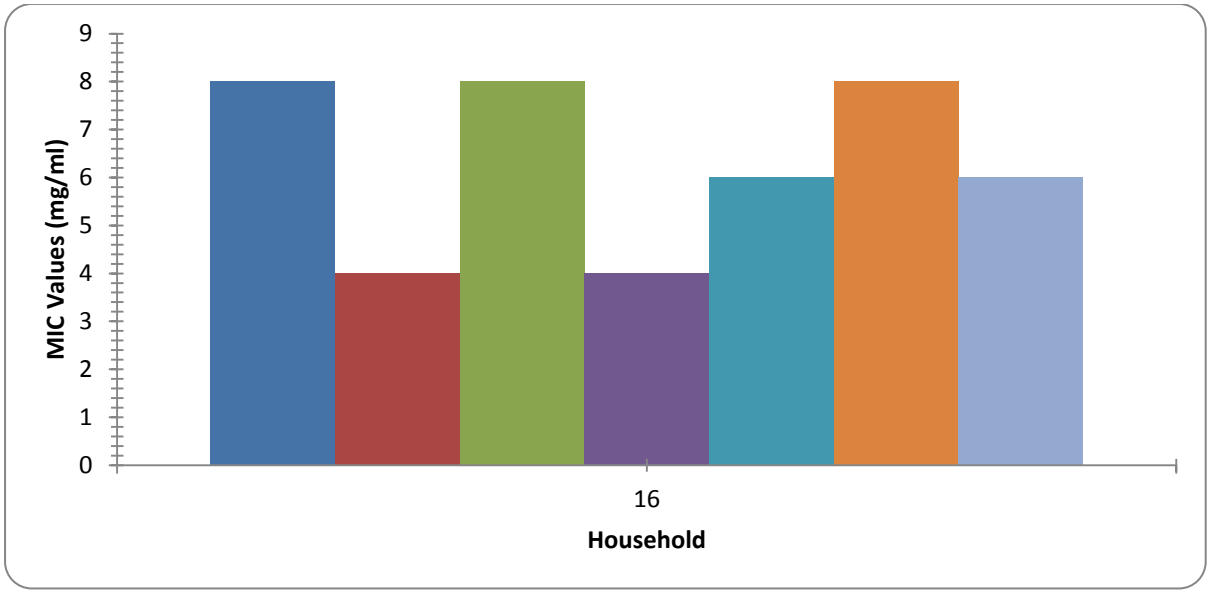

Figure 4.20 The mean MIC values of *Lippia javanica* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.11 *Mangifera indica*

One household (51, Mabibi) uses *Mangifera indica* to treat diarrhoea. The dichloromethane:methanol extract exhibited antibacterial activity, with MIC values ranging between 0.25 mg/ml and 2.00 mg/ml (Figure 4.21). The least susceptible diarrhoeal pathogen was *Salmonella typhimurium* at the MIC value of 2.00 mg/ml. The most susceptible pathogen was *Shigella flexneri* at the MIC value of 0.25 mg/ml.

The aqueous extract of *Mangifera indica* demonstrated antibacterial activity against four diarrhoeal pathogens (Figure 4.22), namely *Enterococcus faecalis* (MIC value 6.67 mg/ml), *Proteus vulgaris* (noteworthy activity with a MIC value of 0.50 mg/ml), *Shigella flexneri* (MIC value 4.00 mg/ml) and *Staphylococcus aureus* (MIC value 2.67 mg/ml).

In previous studies, Mango seed kernel ethanol extracts showed antibacterial activity at the MIC range of < 0.05 mg/ml to > 2.50 mg/ml, against a number of pathogens. Amongst those pathogens were the diarrhoeal strains screened in this study (Kabuki *et al.,* 2000). The results from the previous study correlate somewhat with the results

from this study. For some pathogens i.e. *Escherichia coli* and *Enterococcus faecalis,* poorer activities (2.5 mg/ml and 1.00 mg/ml respectively) were obtained.

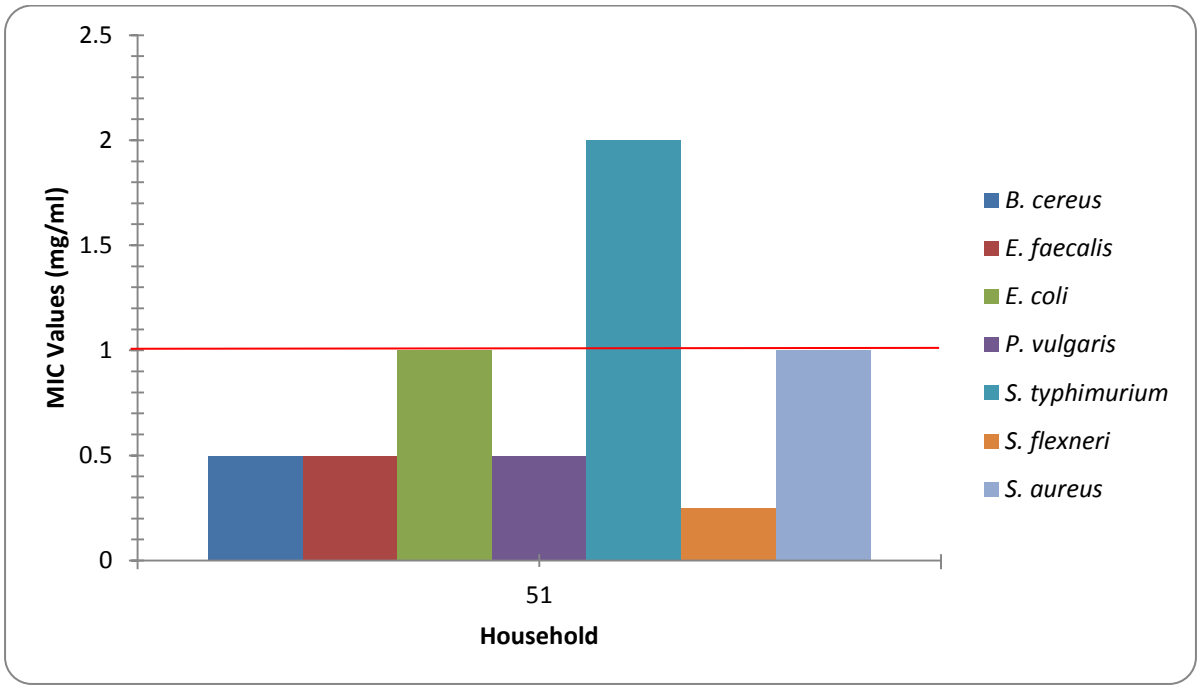

Figure 4.21 The mean MIC values of *Mangifera indica* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Crude methanolic extracts of mango seed kernel showed positive antibacterial activity when tested using the disc diffusion method. The zones of inhibition were observed for methicillin resistant *Staphylococcus aureus* (21.25 mm), *Escherichia coli* (17.70 mm) and *Vibrio vulnificus* (7.80 mm) (Kaur *et al.,* 2010). Disc diffusion of mangiferin showed some antibacterial activity against *Bacillus pumilus, Bacillus cereus, Salmonella virchow and Pseudomonas aeruginosa* (Singh *et al*., 2012). Seed kernel extracts of *Mangifera indica* showed positive antidiarrhoeal activity and antibacterial activity against *Proteus vulgaris* and *Staphylococcus aureus* (Sairam *et al.,* 2003). Another seed kernel extract of aqueous (MIC value 380.00 µg/ml), water (MIC value 215.00 µg/ml), chloroform (MIC value 95.00 µg/ml), ethyl acetate (MIC value 450.00 µg/ml), crude alcohol (MIC value 190.00 µg/ml), methanol (MIC value 130.00 µg/ml), benzene (MIC value 135.00 µg/ml) and hexane (MIC value 260.00 µg/ml) fractions showed antibacterial activity against *Shigella dysenteriae* (Rajan *et*

al., 2011). The phenolic compounds and fractions of *Mangifera indica* extracted with methanol demonstrated antibacterial activity against *Staphylococcus aureus* within the MIC range of 3.10 to 12.40 mmol/L (Engels *et al*., 2012). Seed kernel extracts of *Mangifera indica* showed positive antidiarrhoeal activity on castor oil induced diarrhoea in Swiss albino mice (Rajan *et al*., 2012). *Mangifera indica* also showed activity against *Entamoeba histolytica* at the MIC value of ≤ 7.81 µg/ml (Tona *et al.,* 1998). All these observations suggest that this plant species has potent antimicrobial potential.

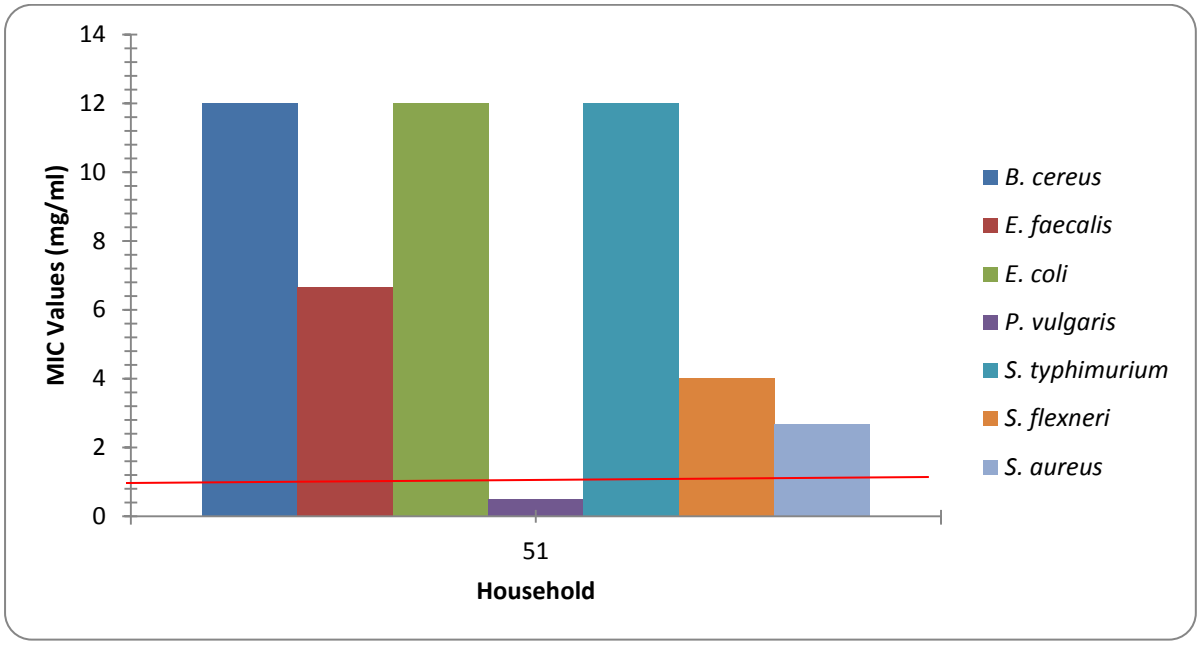

Figure 4.22 The mean MIC values of *Mangifera indica* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.12 *Melia azedarach*

Eleven households (3, 9, 10, 12, 17 from Mseleni; 27, 35, 36, 37 from Tshongwe; 49 from Mabibi and 64 from Mbazwana/Olakeni) use *Melia azedarach* to treat diarrhoea. The MIC values range for the different pathogens were: 1.00 mg/ml - 4.00 mg/ml for *Bacillus cereus*, 0.13 mg/ml - 2.00 mg/ml for *Enterococcus faecalis,* 0.50 mg/ml - 6.00 mg/ml for *Escherichia coli,* 0.50 mg/ml - 4.00 mg/ml for *Proteus vulgaris,* 1.00 mg/ml - 4.00 mg/ml for *Salmonella typhimurium*, 0.25 mg/ml - 1.00 mg/ml for *Shigella flexneri* and 0.33 mg/ml - 4.00 mg/ml for *Staphylococcus aureus.*

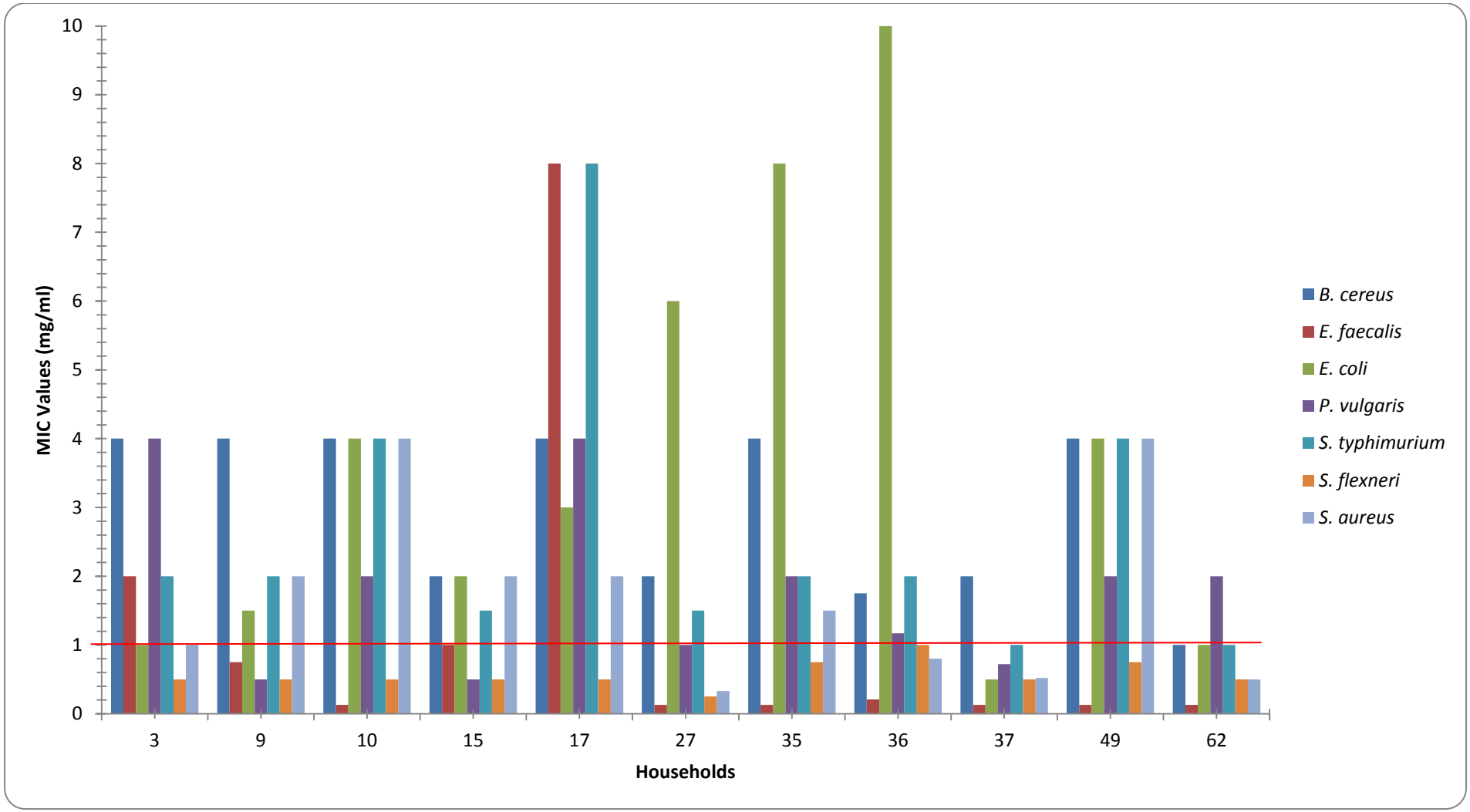

Figure 4.23 The mean MIC values of *Melia azedarach* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Dichloromethane:methanol crude extracts exhibited mostly noteworthy antibacterial activity against *Enterococcus faecalis* for seven households (Figure 4.23).

Noteworthy activity (MIC value < 1.00 mg/ml) was also observed against *Escherichia coli* (household 37), *Proteus vulgaris* (household 9, 15 and 37), *Shigella flexneri* (all households except 36), and *Staphylococcus aureus* (household 27, 36, 37 and 62). The least susceptible diarrhoeal pathogen was *Escherichia coli* at the MIC value of ≥ 16.00 mg/ml from household 36, Tshongwe. In general, the aqueous extract of *Melia azedarach* (household 36) demonstrated moderate antibacterial activity (MIC value 4.00 mg/ml) to poor activity (MIC value 12.00 mg/ml) (Figure 4.24).

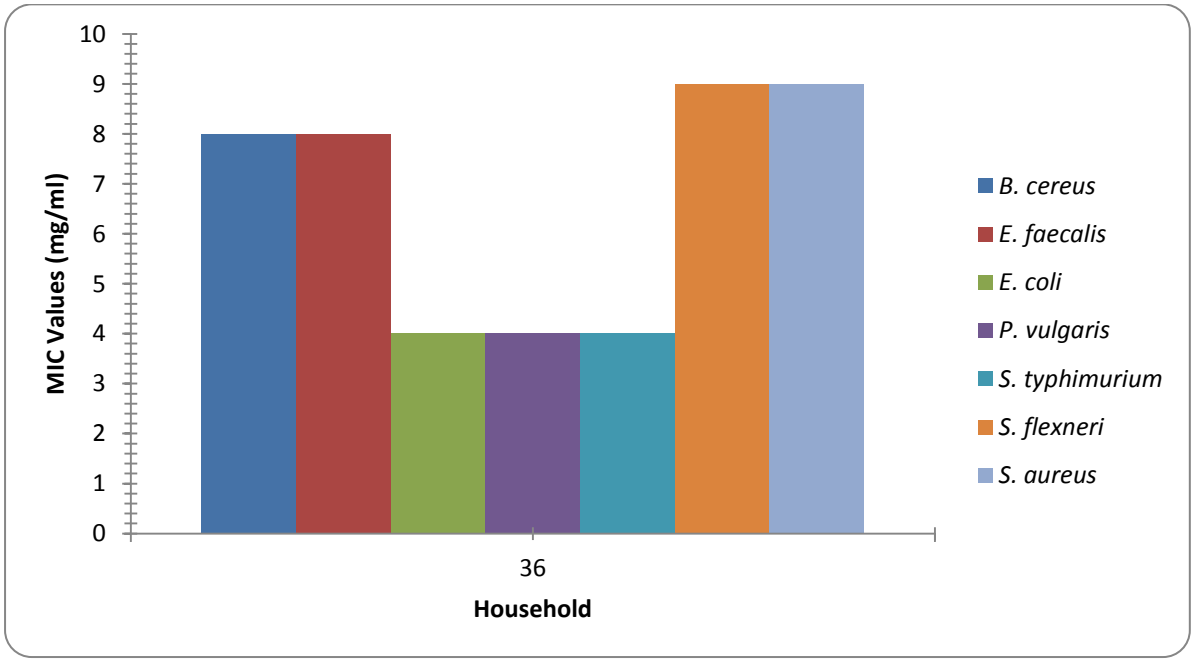

Figure 4.24 The mean MIC values of *Melia azedarach* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and Acetone (MIC value ≥ 16 mg/ml).

A previous disc diffusion study by Khan *et al*. (2001), Ali *et al*. (2001) and Khan *et al*. (2011) demonstrated that *Melia azedarach* possess antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis, Edwardsiella tarda, Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabillis, Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella boydii, Shigella dysenteriae, Shigella flexneri, Staphylococcus aureus, Staphylococcus epidermidis,* Group A *Streptococcus,* Group B *Streptococcus* and *Plesiomonas shigelloides*.

These results correlate with the results obtained in this study. The cream formulation prepared from the flowers of this medicinal plant is said to contain antimicrobial activity against skin diseases caused by bacteria including *Staphylococcus aureus* (Saleem *et al.,* 2008). The antimicrobial ability of this plant species is noteworthy as it shows positive activity for a number of pathogens.

4.2.13 *Psidium guajava*

Twelve households (2, 3, 4, 6, 9 and 12 from Mseleni; 21 and 35 from Tshongwe; 48, 58 and 60 from Mabibi; 63 from Mbazwana (Olakeni)) use *Psidium guajava* to treat diarrhoea. There was no major difference in the activity of samples collected from the different households, however, samples from household 60 (Mabibi region) indicated the poorest activity against *Escherichia coli* and *Proteus vulgaris*.

Dichloromethane:methanol extracts inhibited *Bacillus cereus* growth at an MIC range of 0.11- 1.25 mg/ml (Figure 4.25). Extracts from household 4 and 63, showed noteworthy activity at the MIC value of 0.11 mg/ml. *Bacillus cereus* was the most susceptible diarrhoeal pathogen tested. *Enterococcus faecalis* showed susceptibility as its growth was inhibited by *Psidium guajava* at the MIC range of 0.13 mg/ml to 2.00 mg/ml. Noteworthy activity was observed for nine (3, 4, 9, 12, 21, 35, 48, 58 and 60) out of twelve households. *Escherichia coli* growth was inhibited by *Psidium guajava* at the MIC range of 1.00 mg/ml to 4.00 mg/ml. Better activity was observed from the extracts from households 2, 4, 9, 21, 35, 48 at a mean MIC value of 1.00 mg/ml. *Proteus vulgaris* growth was inhibited at the MIC range of 0.50 mg/ml to 4.00 mg/ml. Significant activity was observed at the MIC value of 0.50 mg/ml (household 3 and 35). The least antibacterial activity was observed with the MIC value of 4.00 mg/ml from household 60. All homesteads samples showed noteworthy activity against *Salmonella typhimurium* (MIC value < 1.00 mg/ml) except samples from household 3 (MIC value 1.00 mg/ml) and household 21 (MIC value 1.50 mg/ml). All *Psidium guajava* crude extracts showed noteworthy antimicrobial activity against *Shigella flexneri,* inhibiting its growth at the MIC range of 0.20 mg/ml to 0.50 mg/ml. The growth of *Staphylococcus aureus* was also inhibited by *Psidium guajava* crude extracts at the MIC range of 0.31 mg/ml to 2.00 mg/ml. Noteworthy activity was observed for six households (4, 9, 21, 58, 60 and 63). There were minor differences observed in the activities of extracts especially for the extracts from household 60

against the pathogens *Escherichia coli* and *Proteus vulgaris* showed moderate activity (MIC value 4.00 mg/ml).

One aqueous extract of *Psidium guajava* (household 58) was tested against seven diarrhoeal pathogens (Figure 4.26). The best antibacterial activity was observed at the MIC value of 0.50 mg/ml against *Staphylococcus aureus*.

A review on *Psidium guajava* revealed that aqueous and alcoholic extracts of this plant species possess antimicrobial activity against a wide spectrum of pathogens. Activity was seen for the tested pathogens like *Bacillus cereus, Escherichia coli, Proteus spp., Shigella spp.* and *Staphylococcus aureus* (Gutiérrez *et al*., 2008).The MIC values from this study showed better results at the MIC value range of 1.00-4.00 mg/ml compared to the latter results.

Peptide, Pg-AMP1 from guava seeds demonstrated antibacterial activity against two *Escherichia coli* strains (ATCC 11229 and ATCC 35218) at the MIC value of 100 µg/ml for both and two *Staphylococcus aureus* strains (ATCC 3359-1 and ATCC 2921-3) at the MIC value of 50 and 100 µg/ml for both respectively (Tavares *et al*., 2012). *Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were inhibited at the MIC value of 100 µg/ml as well (Tavares *et al*., 2012). *Psidium guajava* has shown antibacterial activity in the disc diffusion assay (8.25 - 11.25 mm) against *Salmonella anatum* type strains which is the in same genus as *Salmonella typhimurium* using disc diffusion methods (Goncalves *et al.,* 2008).

Minimum inhibitory concentration (MIC) for methanolic and aqueous extracts of *Psidium guajava* were found to be at the range of 0.63 mg/ml to 9.03 mg/ml against *Staphylococcus aureus* (Abdelrahim *et al.,* 2002 and Anas *et al.,* 2008). Results obtained from the present study showed a much higher activity (0.31 mg/ml to 2.00 mg/ml) compared to the results from the above publications. York *et al*. (2012), demonstrated that dichloromethane:methanol and aqueous extracts of this plant species possess noteworthy antibacterial activity against *Staphylococcus aureus* at the MIC values of 0.50 mg/ml for both extractions. This observation correlates with the results obtained in this study however; there are minor differences in the organic

extracts as MIC values as high as 0.31 mg/ml were observed. A study by Elekwa *et al.* (2009), also demonstrated some antimicrobial activity for *Psidium guajava* (activity against *Bacillus subtilis* with a MIC value of 3.00 µg/ml). The minimum inhibitory concentration (MIC) for methanolic and aqueous extracts of *P. guajava* was found to be 9.03 mg/ml and 13.06 mg/ml against *Pseudomonas aeruginosa* (Abdelrahim *et al.,* 2002).

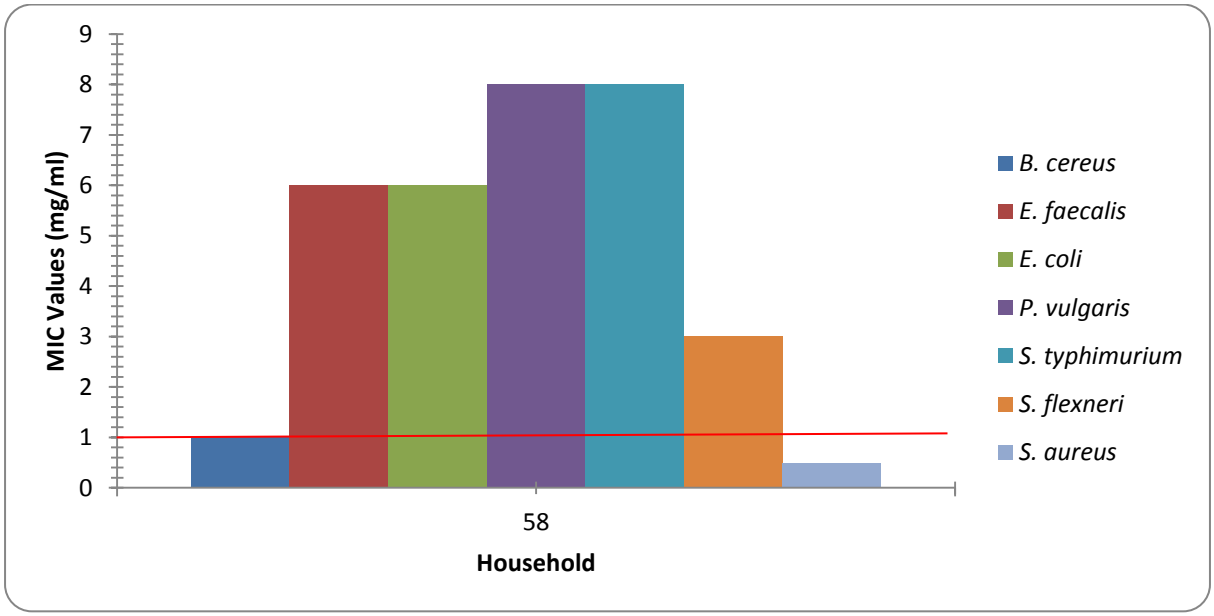

Figure 4.25 The mean MIC values of *Psidium guajava* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

In another study conducted by van Vuuren and Naidoo, (2010) it was revealed that the leaf extracts of this species also shows antimicrobial activity against sexual transmitted infections pathogens. Dichloromethane:methanol and aqueous extracts were also screened for *Cryptococcus neoformans* (MIC values 0.50 mg/ml and 6.00 mg/ml), *Klebsiella pneumoniae* (MIC values 1.00 mg/ml and 0.67 mg/ml), *Moraxella catarrhalis* (MIC values 1.00 mg/ml and 0.67 mg/ml) and *Mycobacterium smegmatis* (MIC values 2.00 mg/ml and 4.00 mg/ml) (York *et al*., 2012). Aqueous extracts and ethanol extracts of *Psidium guajava* demonstrated antibacterial activity against *Streptococcus mutans* (MIC value 500 µg/ml for aqueous and 250 µg/ml for ethanol) and *Phorphyromonas gingivalis* (MIC value 500µg/ml for aqueous and 500 µg/ml for ethanol) (Rosas-Piñól *et al*., 2012).

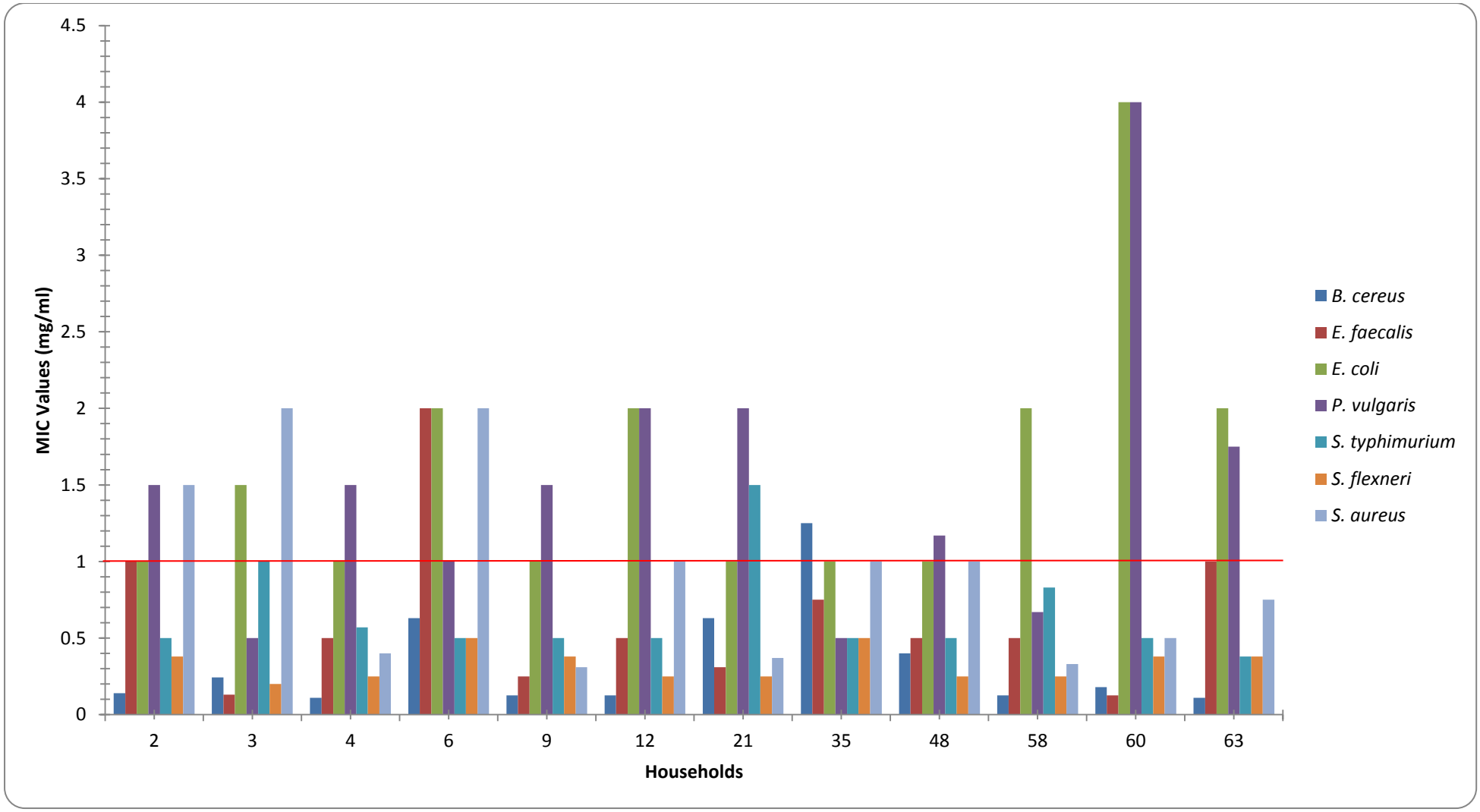

Figure 4.26 The mean MIC values of *Psidium guajava* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml)

4.2.14 *Sarcostemma viminale*

Only one household (12, Mseleni) uses *Sarcostemma viminale* to treat diarrhoea. The dichloromethane:methanol extract showed antibacterial activity against four pathogens *Bacillus cereus* (MIC value 1.00 mg/ml), *Salmonella typhimurium* (MIC value 2.00 mg/ml), *Shigella flexneri* (MIC value 0.50 mg/ml) and *Staphylococcus aureus* (MIC value 4.00 mg/ml) (Figure 4.27).

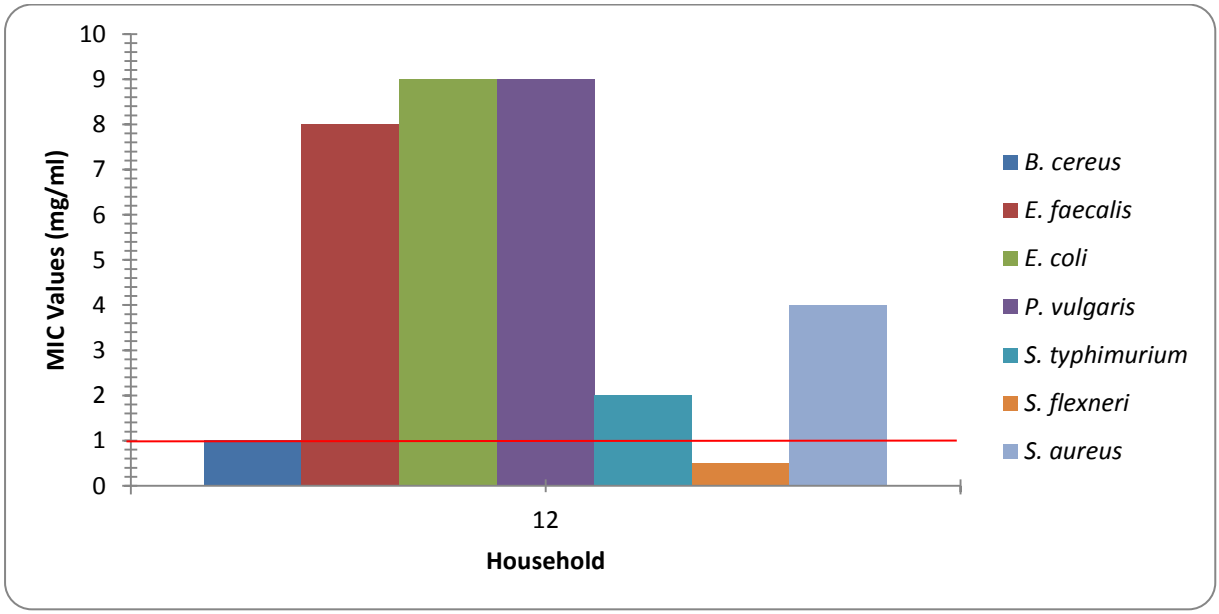

Figure 4.27 The mean MIC values of *Sarcostemma viminale* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The most noteworthy antibacterial activity was observed at the MIC value of 0.50 mg/ml against *Shigella flexneri*. The aqueous extracts of *Sarcostemma viminale* were moderately active against *Enterococcus faecalis* (MIC value 4.00 mg/ml) (Figure 4.28).

Previous studies on *Sarcostemma viminale* (dichloromethane and methanol extracts) showed antibacterial activity against *Staphylococcus aureus* (MIC value 1.25 mg/ml), *Escherichia coli* (MIC value 1.25 mg/ml) and *Pseudomonas aeruginosa* (MIC value 1.25 mg/ml) for both extracts (Luseba *et al.,* 2007). The study conducted by Luseba *et al.* (2007), showed better results than the current study, possibly due to differences in geographical variation and strains used.

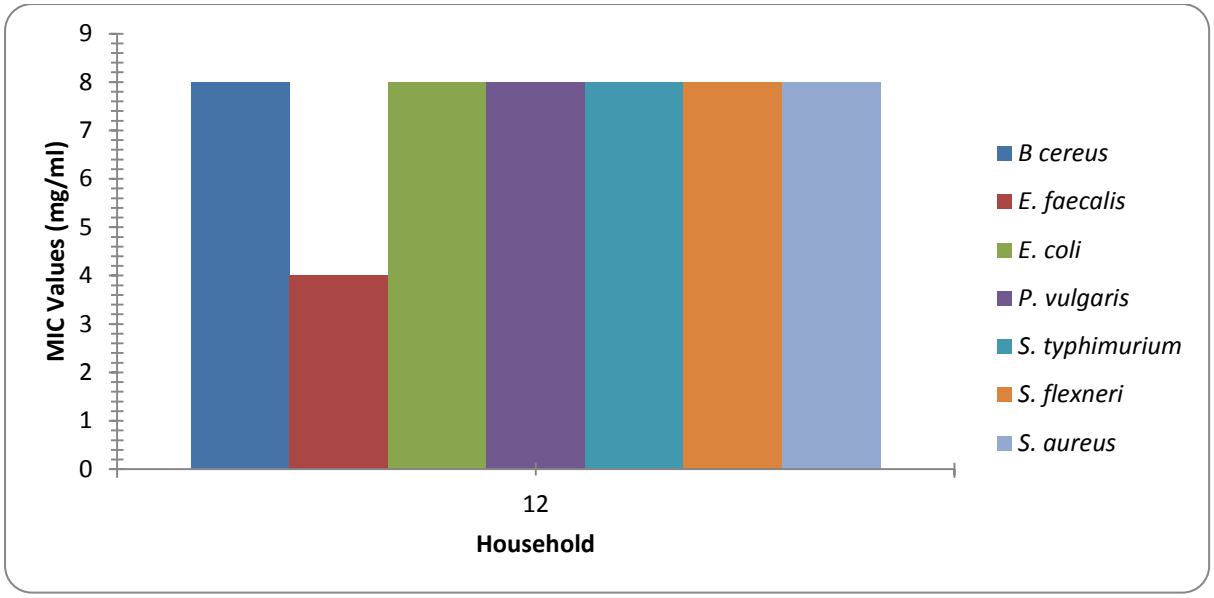

Figure 4.28 The mean MIC values of *Sarcostemma viminale* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and Acetone (MIC value ≥ 16 mg/ml).

4.2.15 *Schotia brachypetala*

Only one household (40, Tshongwe) uses *Schotia brachypetala* to treat diarrhoea. The dichloromethane:methanol extract (Figure 4.29) showed antibacterial activity for four diarrhoeal pathogens. The MIC range was between 0.58 - 8.00 mg/ml. The noteworthy antibacterial activity was observed at the MIC value of 0.58 mg/ml against *Shigella flexneri* and *Enterococcus faecalis* (MIC value 0.63 mg/ml). The poorest activity was observed at the MIC value of 8.00 mg/ml against *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium.*

When the *Schotia brachypetala* aqueous extract (household 40, Tshongwe) was tested against seven diarrhoeal pathogens, it demonstrated activity against six diarrhoeal pathogens (Figure 4.30). The best antibacterial activity was observed at the MIC value of 1.00 mg/ml against *Staphylococcus aureus.* The poorest activity was observed at the mean MIC value of 8.00 mg/ml against *Escherichia coli.* The aqueous extract showed better activity for some pathogens (*Bacillus cereus, Salmonella typhimurium, Shigella flexneri* and *Staphylococcus aureus*) compared to the dichloromethane:methanol extracts.

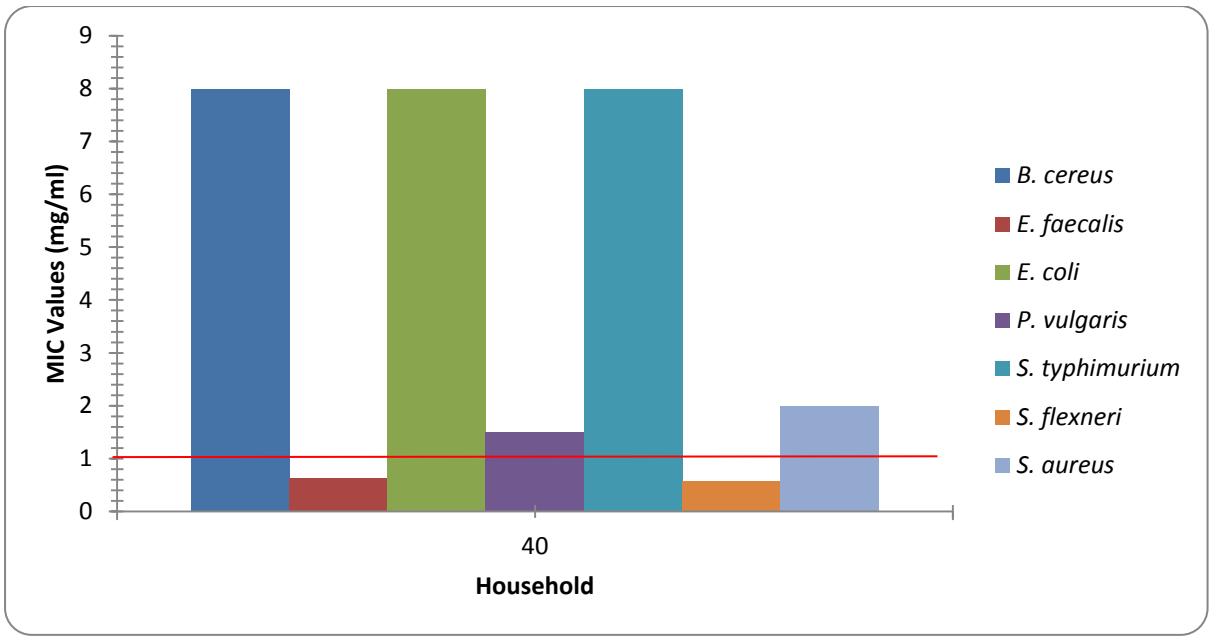

Figure 4.29 The mean MIC values of *Schotia brachypetala* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

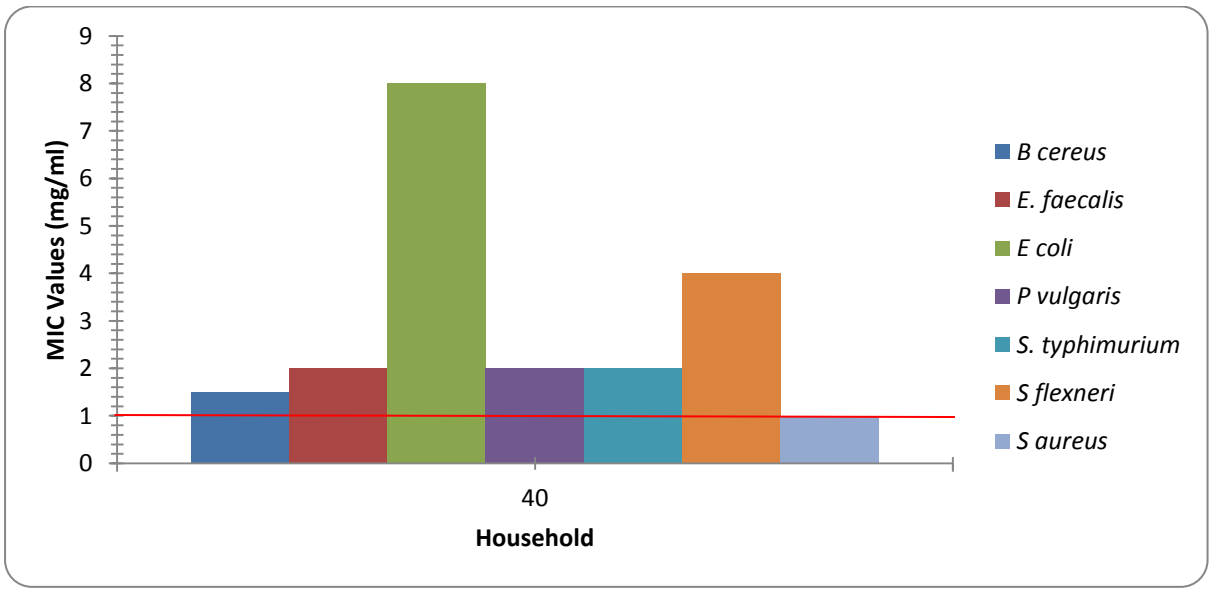

Figure 4.30 The mean MIC values of *Schotia brachypetala* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Previous studies have shown that *Schotia brachypetala* methanol, ethanol, acetone and aqueous crude extracts exhibit antibacterial activity against *Staphylococcus aureus* at the MIC range of (0.16 mg/ml to 0.31 mg/ml), *Vibrio cholerae* (0.16 mg/ml

to 0.31 mg/ml), *Shigella dysentery* (0.16 mg/ml to 0.63 mg/ml) and *Shigella flexneri* (0.16 mg/ml to 0.31 mg/ml) (Mathabe *et al.,* 2006). The activity against *Staphylococcus aureus* was lower in the present study (MIC values of 1.00 - 2.00 mg/ml) while that of *Shigella flexneri* was more or less in a similar range as the results found by Mathabe *et al*., 2006 (MIC value of 0.58 mg/ml). The differences may be due to the differences in extracts preparation, geographical variation or strains used. Green *et al.* (2010) demonstrated that acetone extracts of this medicinal plant has inhibitory activity against two *Mycobacterium tuberculosis* strains at the MIC value of 50 µg/ml.

4.2.16 *Sclerocarya birrea*

Six households (4 and 7 from Mseleni; 21 Tshongwe; 42, 50, and 58 from Mabibi) use *Sclerocarya birrea* to treat diarrhoea. Dichloromethane:methanol crude extracts showed good antibacterial activity against all diarrhoeal pathogens (Figure 4.31). The MIC range was between 0.13 mg/ml and 1.67 mg/ml. The best (noteworthy) antimicrobial activity was observed for samples from Mseleni region with a MIC value of 0.13 mg/ml against *Salmonella typhimurium* (household 7). The poorest antimicrobial activity was noted with samples from household 42 (Mabibi region) having an MIC value of 1.67 mg/ml against *Escherichia coli*. *Escherichia coli* and *Proteus vulgaris* were the least susceptible pathogens, while *Enterococcus faecalis* and *Bacillus cereus* were the most susceptible pathogens. There was no major difference in the activity of samples collected from different households within the same area, except household 42 samples where less activity was observed.

One aqueous extract from household (7) was used to test for antimicrobial activity against seven pathogens. The best (noteworthy) antibacterial activity was observed at the mean MIC value of 0.50 mg/ml against *Staphylococcus aureus* (Figure 4.32). The least activity was observed at the mean MIC value of 4.00 mg/ml against *Proteus vulgaris.*

Dichloromethane, petroleum and ethanol extracts of leaves, twigs and opercula (bud cap covering seeds) of this medicinal plant demonstrated antibacterial activity against *Bacillus subtilis* (MIC value range 0.098 mg/ml - 3.125 mg/ml), *Staphylococcus aureus* (MIC value range 0.195 mg/ml - 3.125 mg/ml), *Escherichia*

coli (MIC value range 0.390 mg/ml - 6.250 mg/ml), and *Klebsiella pneumoniae* (MIC value range 0.390 mg/ml - 6.250 mg/ml) (Moyo *et al.,* 2010; Moyo *et al*., 2011).

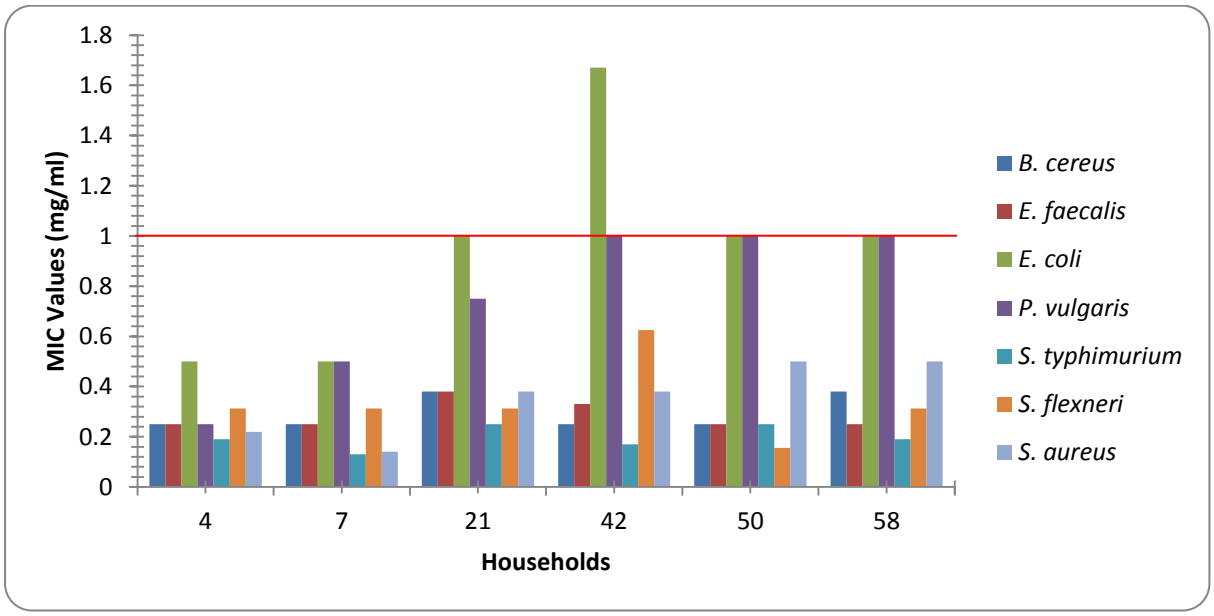

Figure 4.31 The mean MIC values of *Sclerocarya birrea* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

In a recent study by York *et al*. (2012), it was demonstrated that dichloromethane:methanol and aqueous extracts (young and mature bark) of this plant species possess noteworthy antibacterial activity against *Staphylococcus aureus* at the MIC range values of 0.25 mg/ml to 0.67 mg/ml. These results correlate with the results obtained in this study as it is in the same range (0.14 mg/ml to 0.50 mg/ml). Acetone extracts of *Sclerocarya birrea* were also found to be active against *Staphylococcus aureus* at the MIC value of up to 0.15 mg/ml, *Pseudomonas aeruginosa* 0.37 mg/ml, *Escherichia coli* 1.33 mg/ml and *Enterococcus faecalis* 0.60 mg/ml (Eloff, 2001). The results from the present study are more or less in the same range as the results obtained from the study by Eloff (2001). Dichloromethane:methanol and aqueous extracts were also screened by York *et al*. (2012) for *Cryptococcus neoformans* (MIC value range 0.04 mg/ml to 0.23 mg/ml), *Klebsiella pneumoniae* (MIC value range 0.67 mg/ml to 1.00 mg/ml), *Moraxella catarrhalis* (MIC value range 0.25 mg/ml to 0.50 mg/ml) and *Mycobacterium smegmatis* (MIC value range 1.33 mg/ml to 2.00 mg/ml). Disc diffusion of ethyl

acetate, acetone, ethanol, methanol and water crude extracts demonstrated that this plant species has antibacterial activity against *Helicobacter pylori* at the mean zone diameter range of 10 mm to 32 mm (Njume *et al*., 2011). In another study by Njume *et al*. (2011), drug resistant strains of *Helicobacter pylori* were inhibited by the volatile compounds of *Sclerocarya birrea* at the MIC value range of 310 µg/ml to 2500 µg/ml.

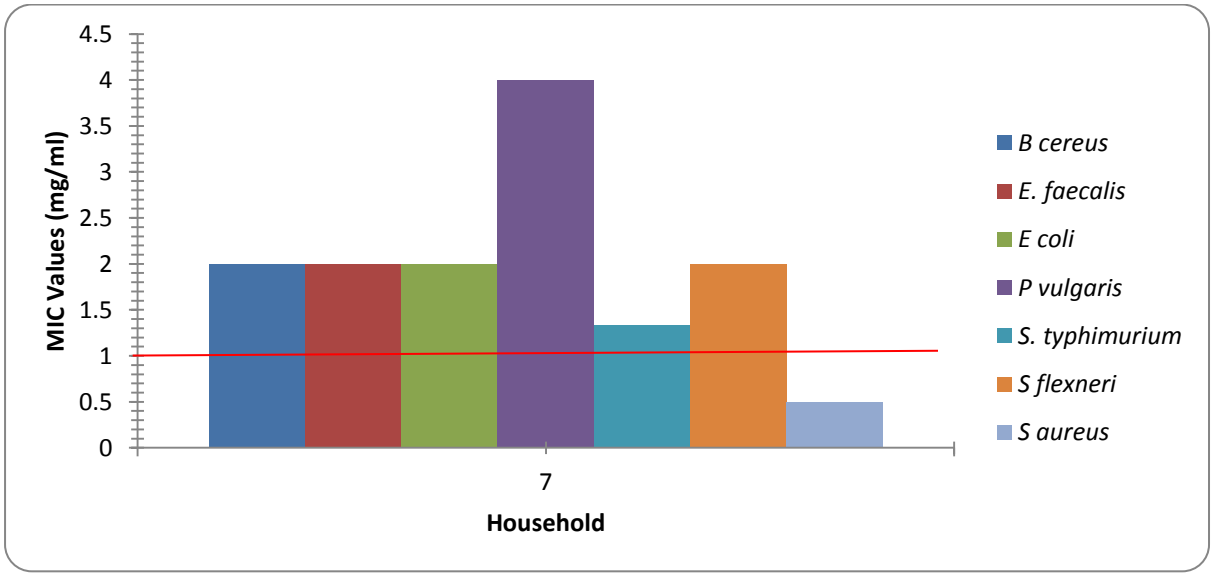

Figure 4.32 The mean MIC values of *Sclerocarya birrea* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.17 *Senna occidentalis*

Only one household (household 1, Mseleni) uses *Senna occidentalis* to treat diarrhoea. Dichloromethane:methanol crude extracts for household 1 showed some antibacterial activity for all diarrhoeal pathogens (Figure 4.33). The MIC range was between 1.00 mg/ml for *Escherichia coli* and 4.00 mg/ml for *Shigella flexneri.*

The aqueous extract of *Senna occidentalis* (household 1) was active against all diarrhoeal pathogens but *Enterococcus faecalis* and *Shigella flexneri* (Figure 4.34). The highest antibacterial activity was observed at the MIC value of 2.00 mg/ml against *Proteus vulgaris.*

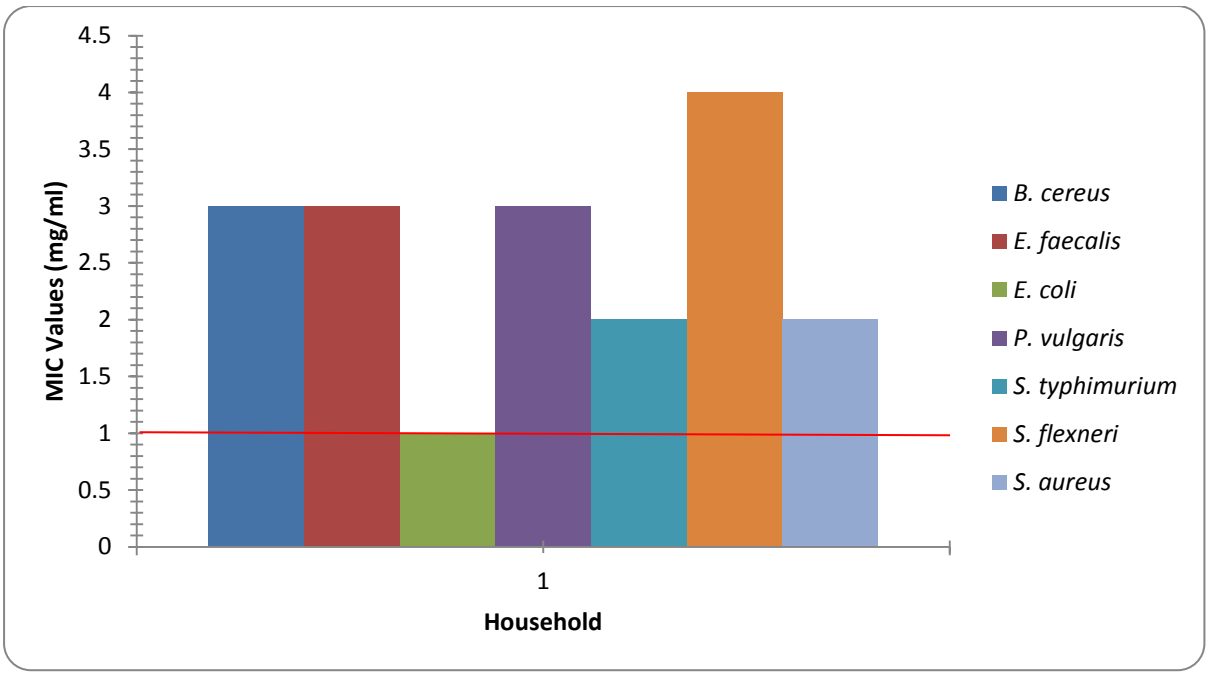

Figure 4.33 The mean MIC values of *Senna occidentalis* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

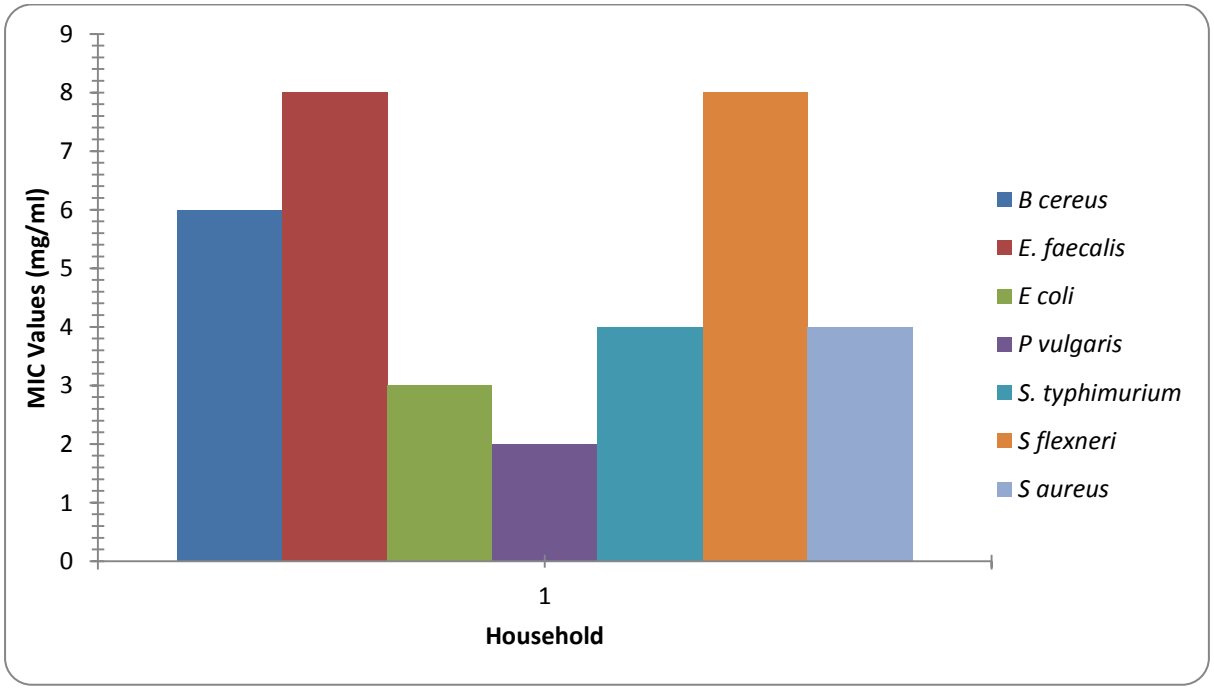

Figure 4.34 The mean MIC values of *Senna occidentalis* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

In the study conducted by Chomnawang *et al*. (2005), antibacterial activity of *Senna occidentalis* was observed at the MIC value of 2.50 mg/ml against *Propionibacterium acnes*. A compound Emodin isolated from *Senna occidentalis* showed antibacterial activity against *Staphylococcus aureus* (MIC value 0.0039 mg/ml), *Bacillus subtilis* (MIC value 0.0078 mg/ml), and MIC values of > 0.50 mg/ml were observed for *Klebsiella pneumoniae* and *Escherichia coli* (Chukwujekwu *et al.,* 2006). In the recent study conducted by Van Vuuren and Naidoo (2010), it was revealed that the leaf and seed extracts of this species possess antimicrobial activity against sexual transmitted infections pathogens.

4.2.18 *Strychnos madagascariensis*

Four households (12 from Mseleni; 28, 32 from Tshongwe and 66 from Olakeni) use *Strychnos madagascariensis* to treat diarrhoea. The extracts showed antibacterial activity for all diarrhoeal pathogens (Figure 4.35). The MIC range was between 1.00 mg/ml for *Shigella flexneri* to 8.00 mg/ml for *Salmonella typhimurium*. There was no major difference in the activity of samples collected from different households, with the exception of *Salmonella typhimurium* where slightly poorer activity was observed in household 32.

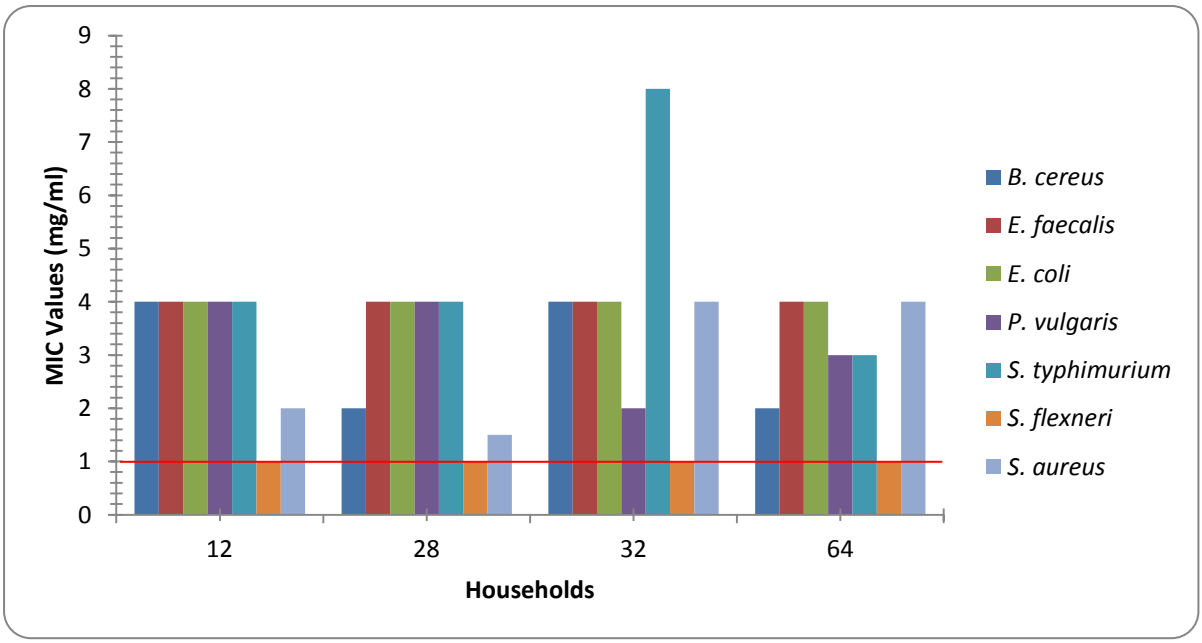

Figure 4.35 The mean MIC values of *Strychnos madagascariensis* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The aqueous extract of *Strychnos madagascariensis* from household 12 was also screened against all diarrhoeal pathogens (Figure 4.36) and displayed poor activity.

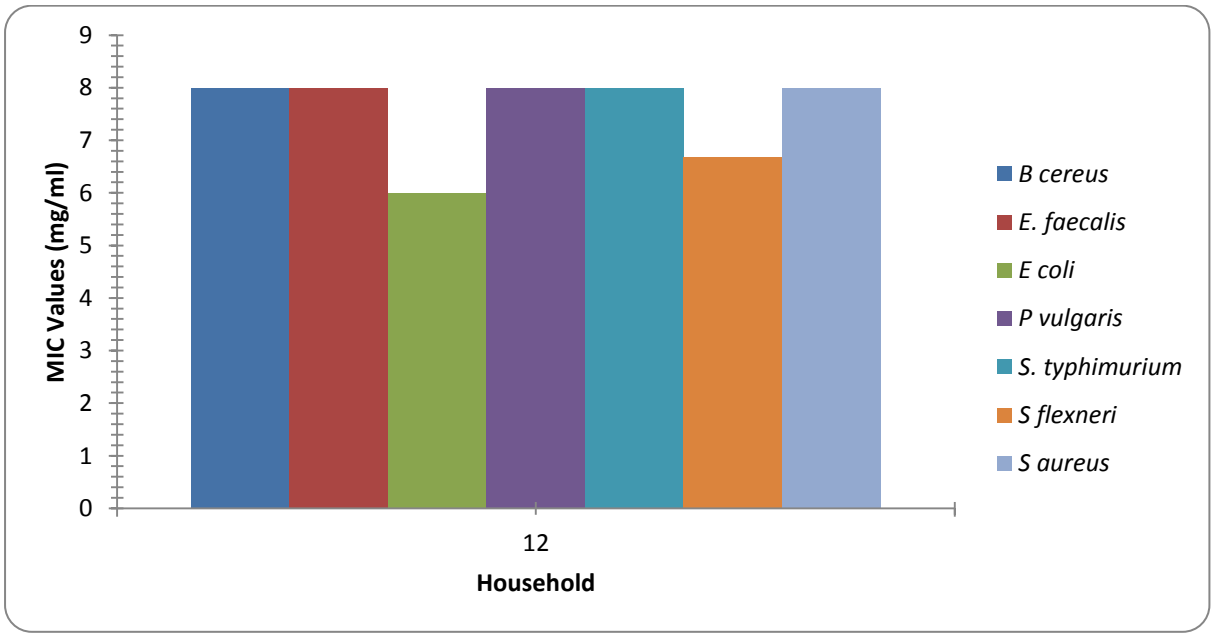

Figure 4.36 The mean MIC values of *Strychnos madagascariensis* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Slightly better activity was observed against two pathogens namely, *Escherichia coli* at the mean MIC value of 6.00 mg/ml and *Shigella flexneri* at the mean MIC value of 6.67 mg/ml. To the best of my knowledge there are no published research papers on the antibacterial activity of *Strychnos madagascariensis.*

4.2.19 *Syzygium cordatum*

Syzygium cordatum dichloromethane:methanol extracts showed antibacterial activity for all diarrhoeal pathogens (Figure 4.37). Four households' samples (4 Mseleni; 50, 60 Mabibi and 65 Olakeni) were tested. The MIC range was between 0.13 mg/ml (*Enterococcus faecalis* household 4, 50) and 6.00 mg/ml (*Enterococcus faecalis* household 60). *Enterococcus faecalis* (household 4 and 50) was the most susceptible pathogen at the MIC value of 0.13 mg/ml. There was no major difference in the activity of samples collected from different households except for samples from household 60 (Mabibi region) which showed poorer antimicrobial activity against all but one (*Staphylococcus aureus*) diarrhoeal pathogens.

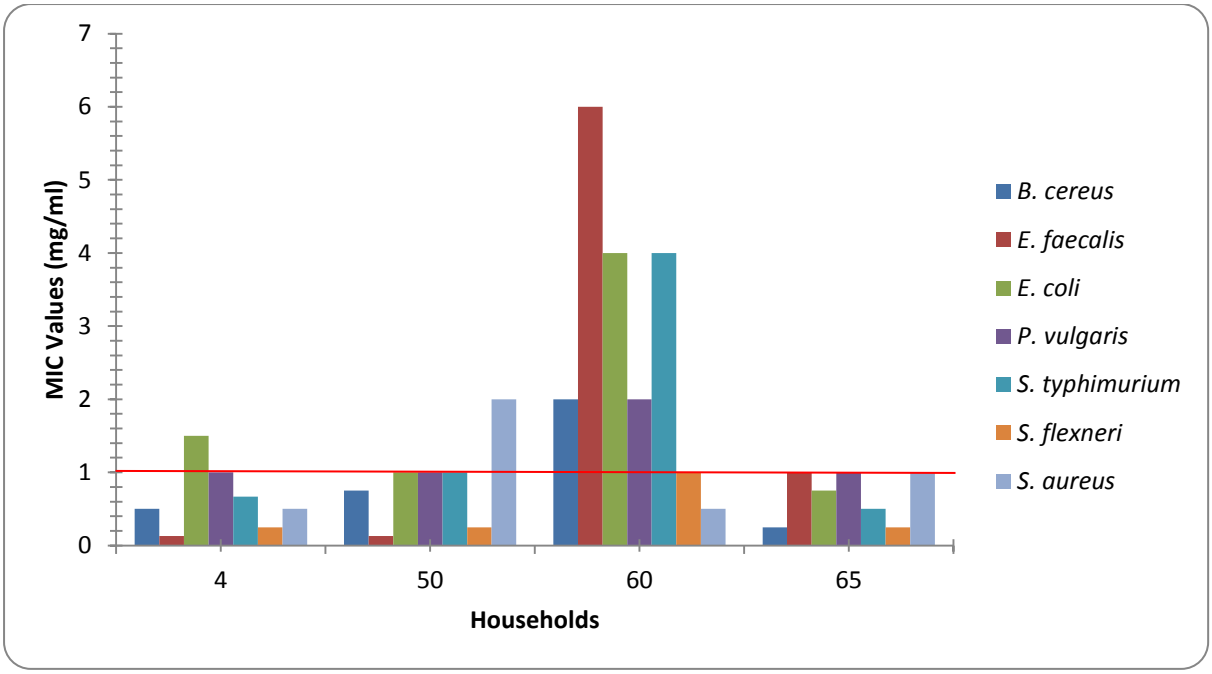

Figure 4.37 The mean MIC values of *Syzygium cordatum* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The aqueous extract from (household 60) of *Syzygium cordatum* was moderately active against two diarrhoeal pathogens namely *Shigella flexneri* at the mean MIC value of 4.00 mg/ml and *Enterococcus faecalis* at the mean MIC value of 6.00 mg/ml (Figure 4.38).

The activity of this plant species, collected in Swaziland, against diarrhoeal pathogens was also observed in the study conducted by Sibandze *et al.* (2010). The MIC value against *Escherichia coli* was found to be 1.44 mg/ml. This latter MIC value is within the MIC range of the present study for all households with the exclusion of household 60. In another earlier study, methanolic (MIC value 3.75 mg/ml) and aqueous (MIC value 2.50 mg/ml) extracts of *Syzygium cordatum* showed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively (Steenkamp *et al.,* 2007). The organic solvent MIC values for *Staphylococcus aureus* inhibition from this study (0.50 mg/ml to 2.00 mg/ml) are better compared to the results from the latter study but on the contrary, the aqueous from latter study showed enhanced activity.

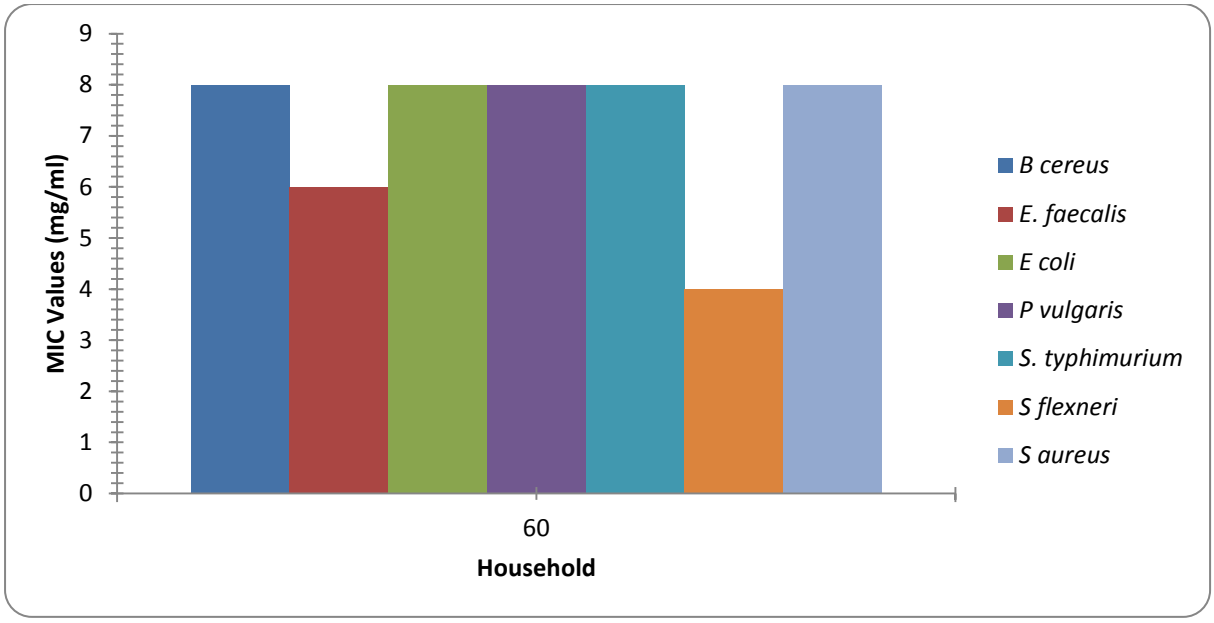

Figure 4.38 The mean MIC values of *Syzygium cordatum* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

In previous studies, the antidiarrhoeal activity for aqueous, acetone, ethanol and methanol crude extracts of *Syzygium cordatum* was at the range of 0.08 mg/ml - 0.31 mg/ml against *Staphylococcus aureus;* 0.16 mg/ml - 0.63 mg/ml against *Escherichia coli;* 0.16 mg/ml - 0.31 mg/ml against *Salmonella typhi* and 0.16 mg/ml - 0.31 mg/ml against *Shigella flexneri* (Mathabe *et al.,* 2006). The MIC values from the present study are slightly poorer than the results obtained by Mathabe *et al*. (2006). This may be caused by various factors including different extractions methods, geographical variations and different plant parts used.

Mulaudzi *et al*. (2012), demonstrated that crude leaves extracts of this plant species extracted with petroleum, ethanol, dichloromethane and aqueous possess antibacterial activity against *Bacillus subtilis* (MIC value range 0.195 mg/ml to 3.13 mg/ml), *Escherichia coli* (MIC value range 0.10 mg/ml to 1.56 mg/ml), *Klebsiella pneumoniae* (MIC value range 0.39 mg/ml 6.25 mg/ml) and *Staphylococcus aureus* (MIC value range 0.01 mg/ml to 6.25 mg/ml). Results obtained from the latter study are better when compared to this study for *Escherichia coli* (MIC value range 0.75 mg/ml to 8.00 mg/ml) and for *Staphylococcus aureus* (MIC value range 0.50 mg/ml to 8.00 mg/ml). These minor differences may be due to different extractants used.

The comparison of these activities indicated that indeed *Syzygium cordatum* does contain antidiarrhoeal properties. In another recent study by York *et al*. (2012), it was demonstrated that dichloromethane:methanol and aqueous extracts from mature bark of this plant species possess noteworthy antibacterial activity against *Staphylococcus aureus* at the MIC range values of 0.38 mg/ml to 0.25 mg/ml. This observation correlates with the results obtained in this study only for organic extracts (MIC value range 0.50 mg/ml to 4.00 mg/ml).

Mathabe *et al*. (2006) also showed that this medicinal plant has antidiarrhoeal activity against other pathogens namely: *Vibrio cholera*, *Shigella dysentery* and *Shigella boydii.* Dichloromethane:methanol and aqueous extracts were also screened by York *et al*. (2012) for *Cryptococcus neoformans* (MIC value range 0.42 mg/ml to 0.17 mg/ml), *Klebsiella pneumoniae* (MIC value range 1.00 mg/ml to 0.50 mg/ml), *Moraxella catarrhalis* (MIC value range 0.83 mg/ml to 0.50 mg/ml) and *Mycobacterium smegmatis* (MIC value range 1.33 mg/ml to 2.00 mg/ml). Steenkamp *et al.* (2007), also revealed that *Syzygium cordatum* have antifungal activity against different strains of *Candida albicans* at the MIC values of up to 0.04 mg/ml. Van Vuuren and Naidoo (2010), found that this species is active against sexual transmitted infections pathogens. All these activities prove that this plant species contains antibacterial properties.

4.2.20 *Terminalia sericea*

Terminalia sericea for household 22 (Tshongwe) was the only household that used this plant species for treating diarrhoea (Figure 4.39). This tree showed noteworthy activity for six pathogens. The mean MIC range was between 0.04 mg/ml (*Shigella flexneri*) and 1.00 mg/ml (*Escherichia coli*).

The aqueous extract of *Terminalia sericea* was active against six diarrhoeal pathogens except *Escherichia coli* at the mean MIC value of 8.00 mg/ml (Figure 4.40).

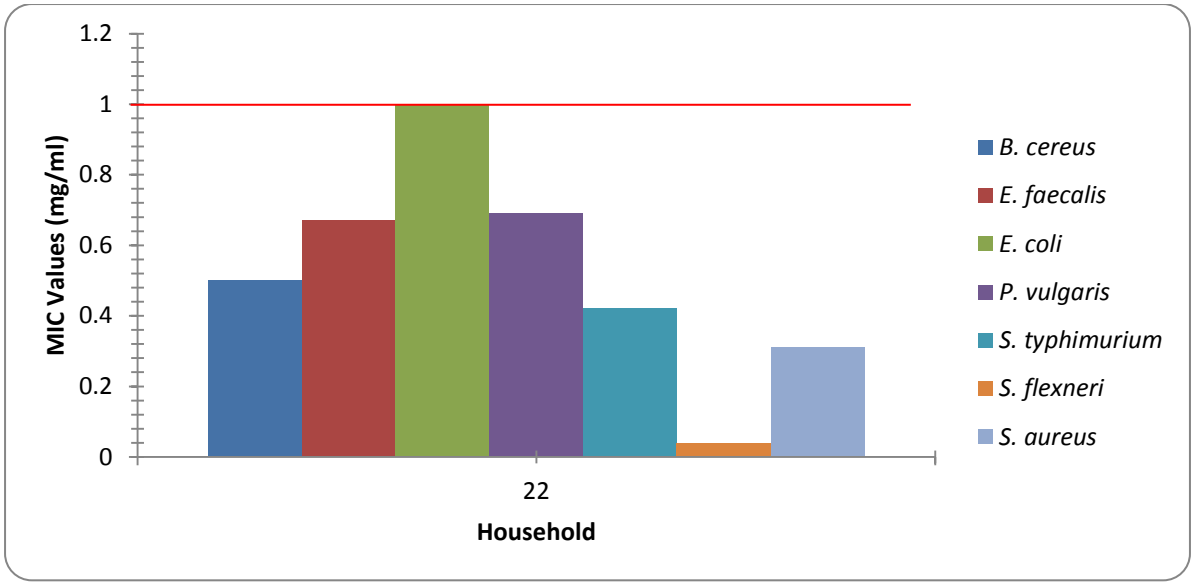

Figure 4.39 The mean MIC values of *Terminalia sericea* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

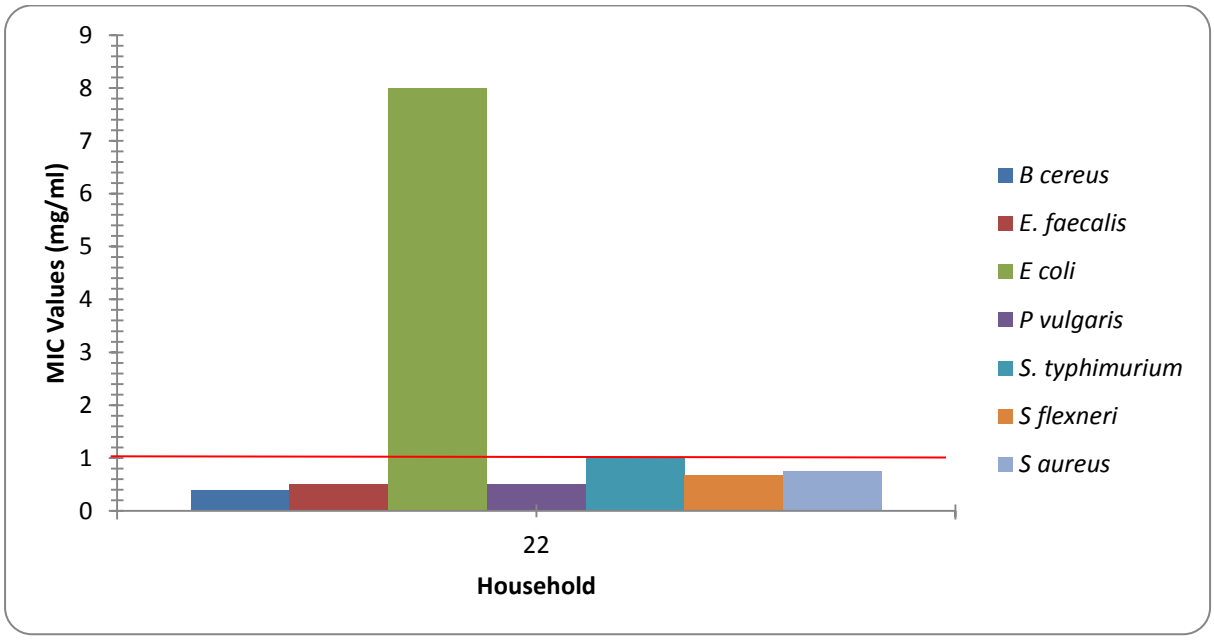

Figure 4.40 The mean MIC values of *Terminalia sericea* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

The highest antibacterial activity was observed at the mean MIC value of 0.38 mg/ml against *Bacillus cereus.* This is the highest antimicrobial activity found for aqueous extracts observed in this study. In a previous study, methanolic and aqueous root

extracts of this plant species studied by Steenkamp *et al.* (2007), showed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Methanol: MIC value 5.00 mg/ml and 2.50 mg/ml and aqueous: MIC value 2.00 mg/ml and 1.00 mg/ml) respectively. Another study done by Fyhrquist *et al.* (2002), on *Terminalia sericea* against *Staphylococcus aureus* demonstrated that it possess antibacterial activity at the MIC value of 5.3 mg/ml. These MIC values from the previous two studies show lower activity compared to the MIC values obtained from the present study (MIC value 0.31 - 0.75 mg/ml). York *et al*. (2012), demonstrated that dichloromethane:methanol and aqueous extracts from mature bark of this plant species possess noteworthy antibacterial activity against *Staphylococcus aureus* at the MIC value of 0.50 mg/ml for both extractants.The results from the latter study correlate with the results from this study (MIC value range 0.31 mg/ml to 0.75 mg/ml) for both extractants but although at the same range, organic results from this study proved to be better results (MIC value 0.31 mg/ml). Moshi and Mbambwo (2005), confirmed antimicrobial efficacy of *Terminalia sericea* using the disc diffusion method. Solvent extracts (petroleum ether, aqueous, ethanol, ethyl acetate, butanol, dichloromethane:methanol (1:1), methanol and dichloromethane) demonstrated activity against *Staphylococcus aureus* (1-14 mm), *Escherichia coli* (1-2 mm), *Bacillus anthracis* (1- 7 mm), *Pseudomonas aeruginosa* (4-8 mm), *Candida albicans* (14-18 mm) and *Aspergillus niger* (11-14 mm).

Another study revealed that acetone extracts of *Terminalia sericea* possess antimicrobial activity against two strains of *Mycobacterium tuberculosis* at the MIC value of 25 µg/ml (Green *et al.,* 2010). Van Vuuren and Naidoo (2010) had in their recent study reported that this species shows antimicrobial activity against sexual transmitted infections microbes. Antifungal properties had also been confirmed on this medicinal plant by Masoko *et al.* (2005) and Steenkamp *et al.* (2007). York *et al*. (2012), also showed that dichloromethane:methanol and aqueous extracts of this plant has antibacterial activity against *Cryptococcus neoformans* (MIC value range 0.33 mg/ml to 1.00 mg/ml), *Klebsiella pneumoniae* (MIC value range 0.67 mg/ml to 0.83 mg/ml), *Moraxella catarrhalis* (MIC value 2.00 mg/ml for both extractants) and *Mycobacterium smegmatis* (MIC value range 0.67 mg/ml to 2.67 mg/ml).

4.2.21 *Trichillia emetica*

Only one household (58, Mabibi) use *Trichillia emetica* for diarrhoea. Dichloromethane:methanol extracts showed activity against six pathogens at the MIC range between 0.16 mg/ml (*Enterococcus faecalis*) to 8.00 mg/ml (*Salmonella typhimurium*) (Figure 4.41).

The aqueous extract of *Trichillia emetica* was moderately active against five of the seven pathogens studied (Figure 4.42). The best antibacterial activity was observed at the mean MIC value of 4.00 mg/ml against *Escherichia coli, Proteus vulgaris, Shigella flexneri and Staphylococcus aureus.* The poorest activity was observed at the mean MIC value of 12.00 mg/ml against *Salmonella typhimurium*.

York *et al*. (2012), demonstrated that dichloromethane:methanol and aqueous leaf extracts of this plant possess noteworthy to moderate to poor antibacterial activity against *Staphylococcus aureus* at the MIC values of 0.83 mg/ml and 8.00 mg/ml, respectively. In this study better efficacy (dichloromethane:methanol 0.25 mg/ml and aqueous 4.00 mg/ml) was observed.

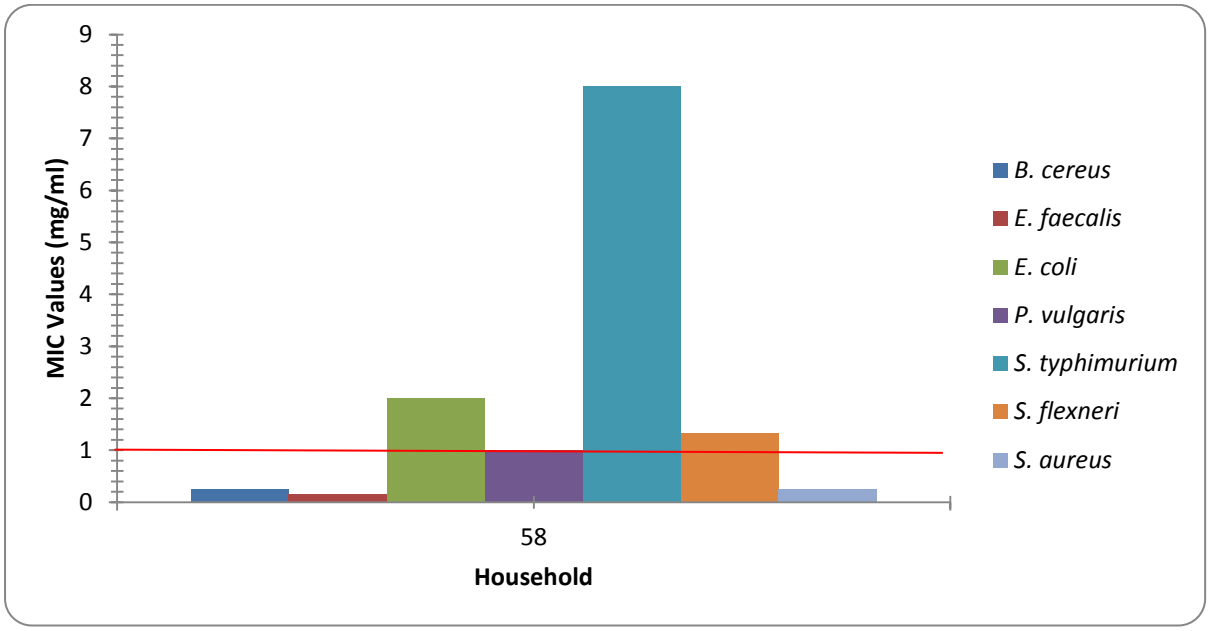

Figure 4.41 The mean MIC values of *Trichillia emetica* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Germano *et al.* (2005) demonstrated higher activity in the aqueous and ethyl ether extracts of this plant species against eight *Staphylococcus aureus* strains at the MIC range of 0.02 mg/ml to 0.03 mg/ml (ethyl ether) and > 0.50 mg/ml (aqueous). These variations may be due to geographical differences and types of extractants used. This was also confirmed by the disc diffusion method by Germano *et al.* (2005). Bioautography studies demonstrated that this medicinal plant possesses antibacterial activity against *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Shai *et al.,* 2008). Germano *et al.* (2005) also screened other microbes namely *Streptococcus pyogenes, Streptococcus pneumoniae, Moraxella catarrhalis* and *Haemophilus influenzae*. *Trichillia emetica* has demonstrated antifungal activity against *Candida albicans* and other pathogenic fungi (Motsei *et al.,* 2003; Shai *et al.,* 2008; Mahlo *et al.,* 2010). York *et al*. (2012), also showed that dichloromethane:methanol and aqueous extracts of this plant has antibacterial activity against *Cryptococcus neoformans* (MIC value range 0.27 mg/ml to 6.67 mg/ml), *Klebsiella pneumoniae* (MIC value range 1.67 mg/ml to 8.00 mg/ml), *Moraxella catarrhalis* (MIC value 1.40 mg/ml to 8.00 mg/ml) and *Mycobacterium smegmatis* (MIC value range 2.67 mg/ml to 8.00 mg/ml).

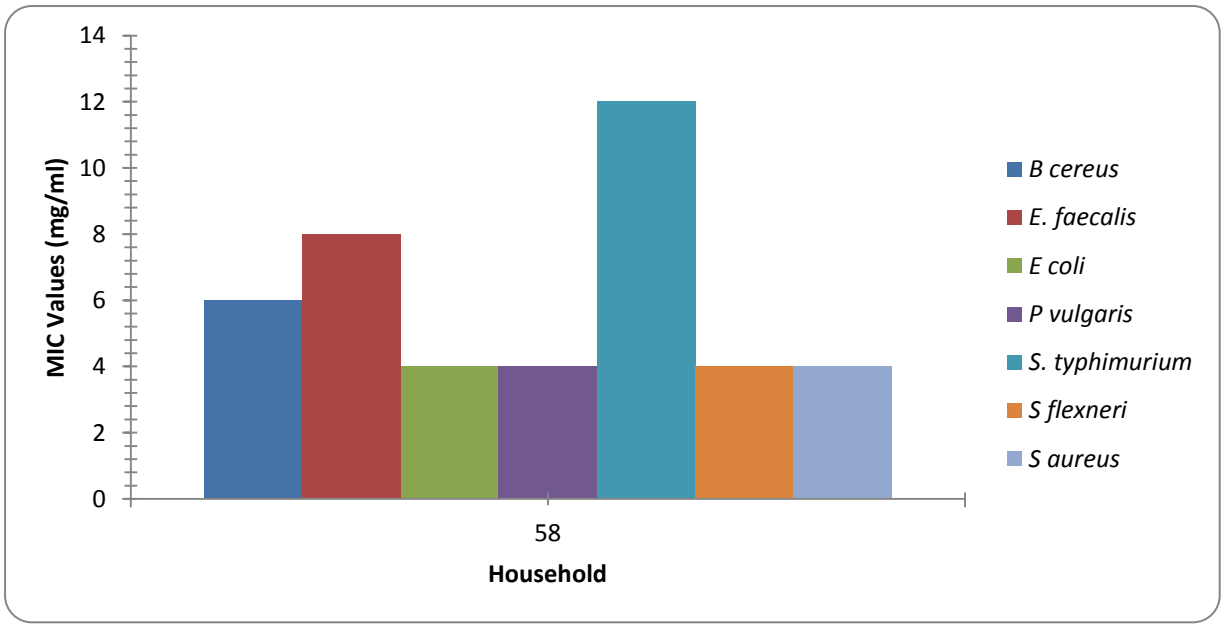

Figure 4.42 The mean MIC values of *Trichillia emetica* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

4.2.22 *Vangueria infausta*

Vangueria infausta dichloromethane:methanol extracts for household 62 (Mabibi) and 67 (Olakeni) showed activity against four pathogens (Figure 4.43). The MIC range was between 0.50 mg/ml and 8.00 mg/ml. The lowest MIC value was observed at the 0.50 mg/ml against *Enterococcus faecalis* (household 67).

The aqueous extract (household 67) of *Vangueria infausta* was active against one diarrhoeal pathogen, *Proteus vulgaris* (MIC value 4.00 mg/ml) (Figure 4.44).

The antibacterial activity of *Vangueria infausta*, especially for *Staphylococcus aureus* has been observed at the MIC value of 1.80 mg/ml (De Boer *et al.,* 2005). This previous study correlates somewhat with the results obtained from this study.

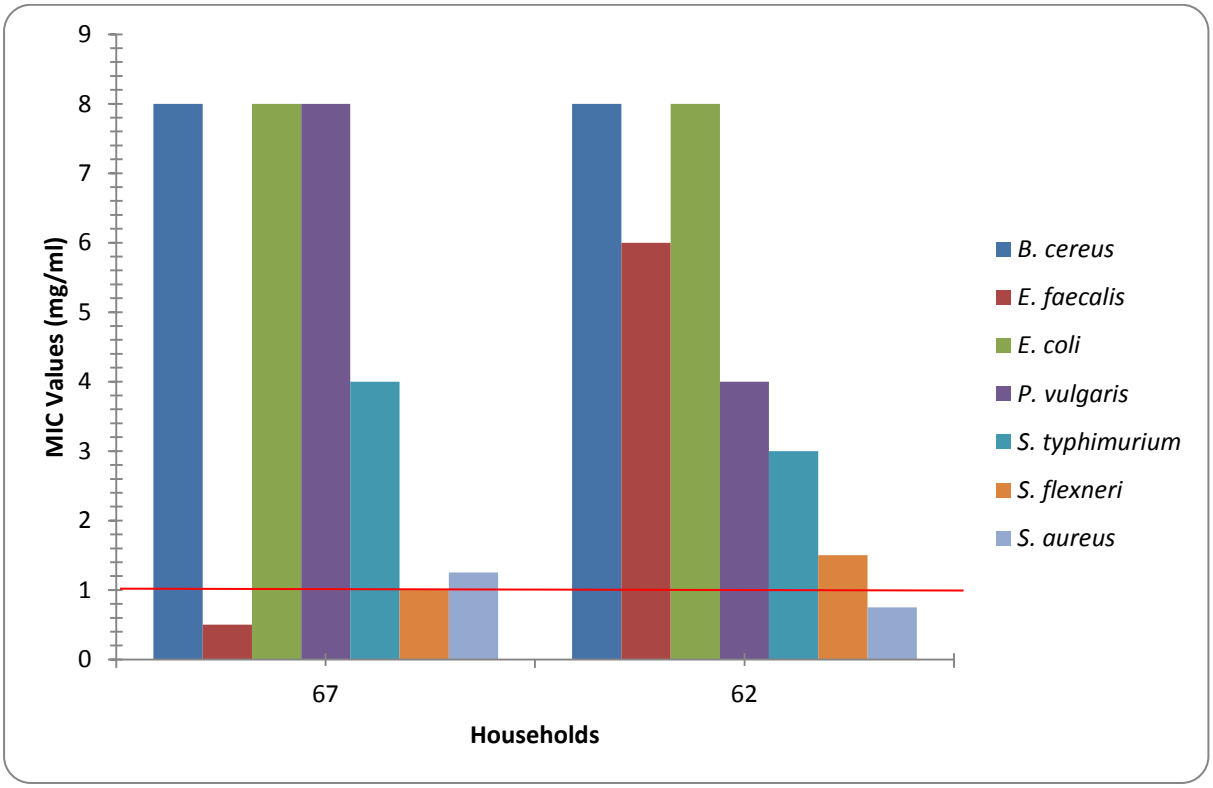

Figure 4.43 The mean MIC values of *Vangueria infausta* (dichloromethane:methanol extracts) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

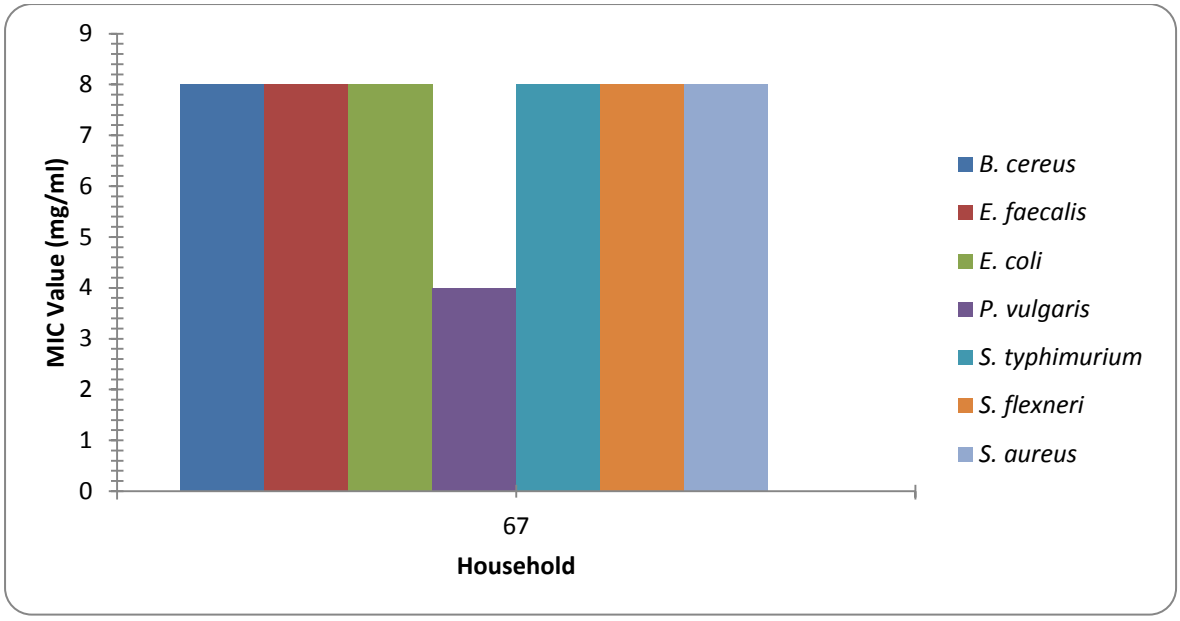

Figure 4.44 The mean MIC values of *Vangueria infausta* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and Acetone (MIC value ≥ 16 mg/ml).

4.2.23 *Vernonia natalensis*

Dichloromethane:methanol extracts for household 49 (Mabibi) showed positive antibacterial activity for all diarrhoeal pathogens tested except *Shigella flexneri* (Figure 4.45). The range for the MIC values was between 0.25 mg/ml (*Proteus vulgaris*) and 8.00 mg/ml (*Shigella flexneri*).

The aqueous extract of *Vernonia natalensis* was active against five diarrhoeal pathogens (Figure 4.46). The highest antibacterial activity was observed at the mean MIC value of 1.50 mg/ml against *Proteus vulgaris.* The poorest activity was observed at the mean MIC value of 8.00 mg/ml against *Salmonella typhimurium* and *Staphylococcus aureus.*

In the previous studies, the leaves of *Vernonia natalensis* crude extracts has showed antibacterial activity against *Escherichia coli* 3.13 mg/ml, *Bacillus subtilis* 0.59 mg/ml and *Staphylococcus aureus* 0.78 mg/ml (Fawole *et al.,* 2009). This activity of like pathogens corroborates with the findings observed in this study.

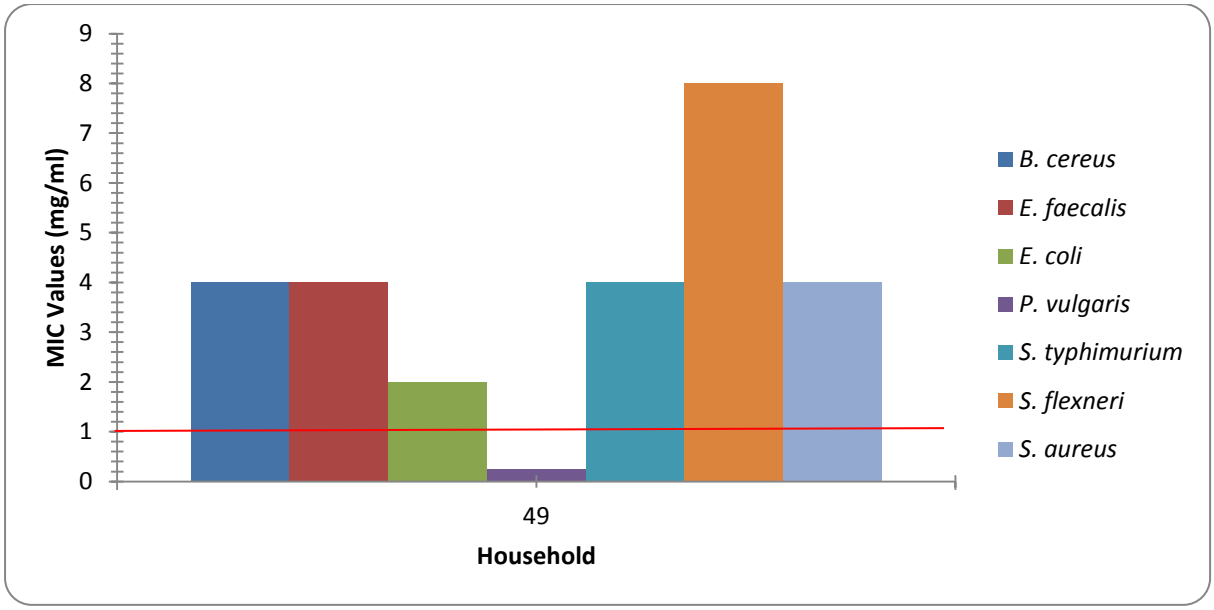

Figure 4.45 The mean MIC values of *Vernonia natalensis* (dichloromethane:methanol extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

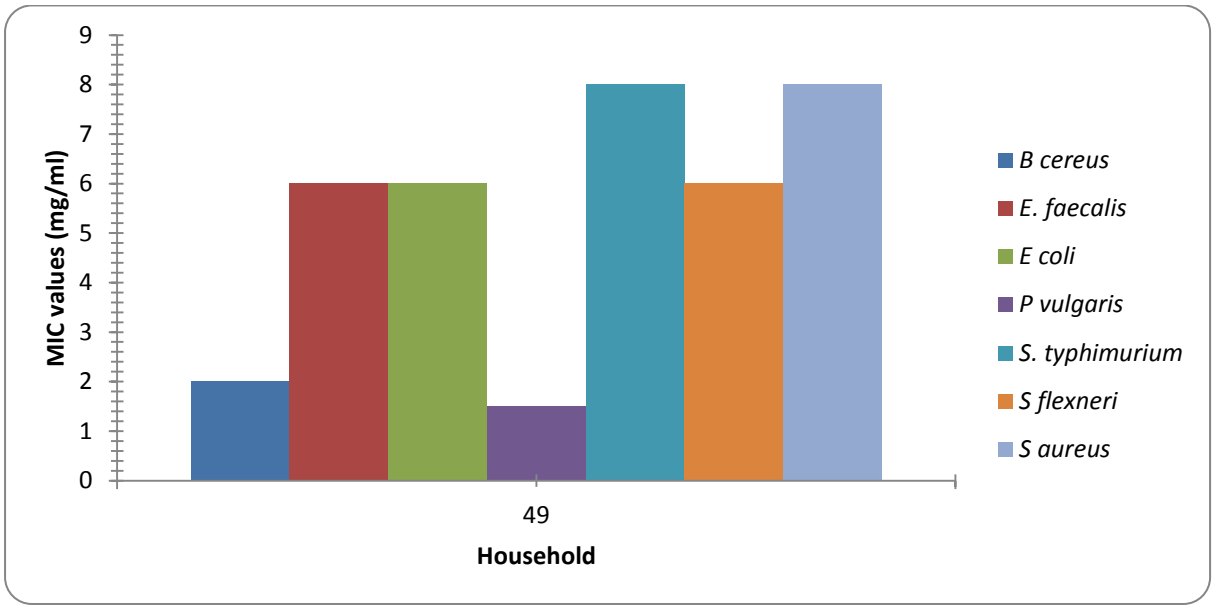

Figure 4.46 The mean MIC values of *Vernonia natalensis* (aqueous extract) against seven diarrhoeal pathogens. Controls: Ciprofloxacin (MIC value = 0.08 µg/ml) and acetone (MIC value ≥ 16.00 mg/ml).

Table 4.1 and 4.2 shows the mean MIC values for dichloromethane:methanol and aqueous extracts of *Sarcophyte sanguinea, Sclerocarya birrea, Strychnos henningsii*

and *Ximenia caffra* collected from muthi markets when screened against seven pathogens.

Table 4.1 Dichloromethane:methanol organic extracts mean MIC values (mg/ml).

MIC Values (Organic)								
Antimicrobial activity (mg/ ml)								
Plant species	**Locality**	*B. cereus (ATCC 11778)*	*E. faecalis (ATCC 29212)*	*E. coli (ATCC 8739)*	*P. vulgaris* (*ATCC* 33420)	*S. typhimurium (ATCC 14028)*	*S. flexneri (ATCC 25875)*	*S. aureus (ATCC 12600)*
S. sanguinea	Mtuba	8.00	4.00	6.00	1.00	2.00	**0.75**	1.00
S. sanguinea	Mona	8.00	4.00	5.00	1.50	4.00	1.00	1.50
S. birrea	Mona	1.00	1.00	2.00	1.00	1.50	1.50	1.00
S. henningsii	Mtuba	1.00	1.50	8.00	2.00	1.00	**0.75**	3.00
X. caffra	Mona	4.00	4.00	1.50	1.00	2.00	**0.50**	**0.38**

Bold = noteworthy activity (MIC value < 1.00 mg/ml)

Table 4.2 Aqueous extracts mean MIC values (mg/ml).

MIC Values (Aqueous)								
Antimicrobial activity (mg/ ml)								
Plant species	**Locality**	*B. cereus (ATCC 11778)*	*E. faecalis (ATCC 29212)*	*E. coli (ATCC 8739)*	*P. vulgaris* (*ATCC* 33420)	*S. typhimurium (ATCC 14028)*	*S. flexneri (ATCC 25875)*	*S. aureus (ATCC 12600)*
S. sanguinea	Mtuba	8.00	8.00	12.00	3.00	4.00	2.00	4.00
S. sanguinea	Mona	12.00	8.00	6.00	4.00	4.00	4.00	6.00
S. birrea	Mona	3.00	2.00	3.00	2.00	2.00	2.00	2.00
S. henningsii	Mtuba	2.00	2.00	8.00	2.00	3.00	2.00	8.00
X. caffra	Mona	6.00	6.00	4.00	3.00	3.00	2.00	4.00

Two samples of dichloromethane:methanol extracts of *Sarcophyte sanguinea* (Mona and Mtuba muthi market) were screened against diarrhoeal pathogens. The MIC range was around 0.75 mg/ml to 8.00 mg/ml (Table 4.1). The noteworthy was observed against *Shigella flexineri* at the mean MIC value of 0.75 mg/ml (Mtuba). Aqueous extracts demonstrated moderate to poor antibacterial activity at the MIC range of 2.00 mg/ml to 12.00 mg/ml (Table 4.2). The best activity was observed at 2.00 mg/ml against *Shigella flexneri* (Mtuba). To the best of my knowledge there is no documented antibacterial activity of this plant species.

One sample of dichloromethane:methanol extract of *Sclerocarya birrea* from Mona muthi market) was screened against diarrhoeal pathogens. This species showed good activity at the MIC range was around 1.00 mg/ml to 2.00 mg/ml (Table 4.1). The highest activity was observed against *Bacillus cereus, Enterococcus faecalis,*

Proteus vulgaris and *Staphylococcus aureus* at the mean MIC value of 1.00 mg/ml. Aqueous extracts demonstrated moderate antibacterial activity at the MIC range of 2.00 mg/ml to 3.00 mg/ml (Table 4.2). Highest activity was observed at 2.00 mg/ml against *Enterococcus faecalis, Proteus vulgaris, Salmonella typhimurium, Shigella flexneri* and *Staphylococcus aureus*. The antibacterial activity of this plant has been extensively described on the antimicrobial activity of plants obtained from the homesteads. It can be found in Chapter 5, Section 4.2.16.

One sample of dichloromethane:methanol extract of *Strychnos henningsii* from Mtuba muthi market) was screened against diarrhoeal pathogens. This species demonstrated good to poor activity at the MIC range was around 0.75 mg/ml to 8.00 mg/ml (Table 4.1). Noteworthy activity was observed against *Shigella flexneri* at the mean MIC value of 0.75 mg/ml. Aqueous extracts demonstrated moderate antibacterial activity at the MIC range of 2.00 mg/ml to 8.00 mg/ml (Table 4.2). Highest activity was observed at 2.00 mg/ml against *Bacillus cereus*, *Enterococcus faecalis, Proteus vulgaris* and *Shigella flexneri*. To the best of my knowledge there is no documented antibacterial activity of this plant species.

Ximenia caffra dichloromethane:methanol extract from Mona muthi market was screened against diarrhoeal pathogens. This species demonstrated noteworthy to poor activity at the MIC range was around 0.38 mg/ml to 4.00 mg/ml (Figure 4.53). Noteworthy activity was observed against *Shigella flexneri* and *Staphylococcus aureus* at the mean MIC value of 0.50 mg/ml and 0.38 mg/ml respectively. Aqueous extracts demonstrated moderate antibacterial activity at the MIC range of 2.00 mg/ml to 6.00 mg/ml (Figure 4.54). Highest activity was observed at 2.00 mg/ml against *Shigella flexneri*.

Methanol and ethanol extracts of this plant species had previously shown antidiarrhoeal activity against diarrhoeal pathogens such as *Staphylococcus aureus, Vibrio cholerae, Shigella dysentery, Shigella flexineri* and *Shigella boydii* at the MIC range of 0.156 mg/ml to 0.312 mg/ml (Mathabe *et al.,* 2006). From the present study (dichloromethane:methanol), the MIC range for the antidiarrhoeal activity against *Staphylococcus aureus* and *Shigella flexineri* is 0.38 mg/ml and 0.50 mg/ml respectively. The results obtained from Mathabe *et al*. (2006), proved to be slightly

better compared to the present study but they are in the same range. The reason for this variety will possibly be geographical variety and different type of extractions methods. Previous studies by Steenkamp *et al.* (2007), revealed that this species water and methanol root extracts possess some antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* at the MIC value range of 1.29 mg/ml and 10.30 mg/ml respectively. When comparing the results from the previously mentioned studies to the present study, it is clear that the present study demonstrated better results for *Staphylococcus aureus* at the range of 0.38 mg/ml to 4.00 mg/ml. Mulaudzi *et al*. (2012), demonstrated that crude roots and leaves' extracts of this plant species extracted with petroleum, ethanol, dichloromethane and aqueous possess antibacterial activity against *Bacillus subtilis* (MIC value range 0.195 mg/ml to 1.56 mg/ml)*, Escherichia coli* (MIC value range 0.39 mg/ml to 3.125 mg/ml), *Klebsiella pneumoniae* MIC value range 0.78 mg/ml 6.25 mg/ml) and *Staphylococcus aureus* (MIC value range 0.025 mg/ml to 6.25 mg/ml). Results obtained from the latter study are better for compared to this study for *Escherichia coli* (MIC value range 1.50 mg/ml to 4.00 mg/ml) and for *Staphylococcus aureus* (MIC value range 0.38 mg/ml to 4.00 mg/ml). These minor differences may be due to different extractants used. Acetone extracts of *Ximenia caffra* demonstrated some degree of antibacterial activity against *Mycobacterium tuberculosis* strains at a mean MIC range of 2.60 mg/ml to 7.20 mg/ml.

4.3 General overview

Tables 4.3 and 4.4 show the mean MIC values of the dichloromethane:methanol extracts and aqueous extracts respectively for all plant species against the seven diarrhoeal pathogens studied.

Nineteen of the 23 plant species (72 dichloromethane:methanol extracts and 23 aqueous extracts) were active (MIC value < 8.00 mg/ml) against *Bacillus cereus*. Only three were not active (*Chenopodium ambrosioides, Schotia brachypetala* and *Vangueria infausta*) (Table 4.3). *Psidium guajava* and *Trichillia emetica* showed the best antibacterial activity as they showed MIC values as low as 0.12 mg/ml. This supports the use of the majority of these plants to treat infections related to *Bacillus cereus,* a pathogen responsible for food poisoning.

Of the twenty-three plants studied against *Enterococcus faecalis*, twenty-two samples showed some antibacterial activity (Table 4.3). The best activity was observed for *Trichillia emetica* with a mean MIC value of 0.16 mg/ml.

Table 4.3 Organic extracts mean MIC values (mg/ml).

Plant species	*B. cereus (ATCC 11778)*	*E. faecalis (ATCC 29212)*	*E. coli (ATCC 8739)*	*P. vulgaris* (*ATCC* 33420)	*S. typhimurium (ATCC 14028)*	*S. flexneri (ATCC 25875)*	*S. aureus (ATCC 12600)*
A. burkei	3.00	2.00	1.00	**0.50**	3.00	**0.25**	1.00
A. glabratum	**0.88**	4.00	6.00	8.00	2.25	**0.44**	4.13
B.transvaalensis	**0.25**	1.07	2.00	1.75	1.25	**0.50**	1.25
C. roseus	**0.64**	1.93	3.71	4.07	4.71	**0.41**	3.50
C. ambrosioides	12.00	6.00	3.00	**0.25**	4.00	**0.50**	**0.25**
C. hirta	2.00	**0.42**	8.00	2.00	1.33	**0.38**	1.50
G. livingstonei	**0.12**	**0.34**	3.38	**0.75**	**0.19**	**0.38**	**0.26**
G. senegalensis	**0.94**	**0.56**	6.00	11.34	**0.79**	**0.63**	2.17
K. mosambicina	4.80	7.20	7.20	8.60	5.50	1.40	6.80
L. javanica	1.00	6.00	4.00	12.00	4.00	**0.50**	4.00
M. indica	**0.50**	**0.50**	1.00	**0.50**	2.00	**0.25**	1.00
M. azedarach	2.89	1.16	4.28	1.81	2.64	**0.57**	1.70
P. guajava	**0.34**	**0.63**	1.63	1.51	**0.65**	**0.33**	**0.93**
S. viminale	1.00	8.00	16.00	16.00	2.00	**0.50**	4.00
S. brachypetala	8.00	**0.63**	8.00	1.50	8.00	**0.58**	2.00
S. birrea	**0.29**	**0.29**	**0.95**	**0.75**	**0.20**	**0.34**	**0.35**
S. occidentalis	3.00	3.00	1.00	3.00	2.00	**4.00**	2.00
S. madagascariensis	3.00	4.00	4.00	3.25	4.75	1.00	2.88
S. cordatum	**0.88**	1.82	1.81	1.25	1.54	**0.43**	1.00
T. sericea	**0.50**	**0.67**	1.00	**0.69**	**0.42**	**0.04**	**0.31**
T. emetica	**0.25**	**0.16**	2.00	1.00	8.00	1.33	**0.25**
V. infausta	8.00	3.25	8.00	6.00	3.50	1.25	1.00
V. natalensis	4.00	4.00	2.00	**0.25**	4.00	8.00	4.00
Ciprofloxacin (µg/ml)	**0.50**	**0.25**	**0.02**	**0.40**	**0.20**	**0.05**	**0.80**
Acetone	16 .00	NS	NS	13.30	NS	8.00	NS
DMSO	8.00	NS	8.00	9.60	NS	8.00	NS
Overall mean activity	2.43	2.50	4.17	3.78	2.90	1.45	2.01

Bold = noteworthy activity (MIC value < 1.00 mg/ml); NS = Not susceptible at highest concentration tested (64 mg/ml).

Twenty plant species (87%) demonstrated antibacterial activity against *Escherichia coli*. *Sclerocarya birrea* showed noteworthy activity (mean MIC value 0.95 mg/ml).

This pathogen is the least susceptible pathogen as it shows the overall susceptibility at the mean MIC value of 4.17 mg/ml.

Eighty three percent of plants species tested against *Proteus vulgaris* showed antibacterial activity. Thirty percent of the plants species showed noteworthy activity with the highest MIC value of 0.25 mg/ml for *Chenopodium ambrosioides* and *Vernonia natalensis*. Most plants showed good to poor activity (0.25-16.00 mg/ml) against *Proteus vulgaris*.

When plant species were tested against *Salmonella typhimurium*, most of the plants species showed antibacterial activity and noteworthy activity was observed with *Garcinia livingstonei, Gymnosporia senegalensis, Psidium guajava, Sclerocarya birrea* and *Terminalia sericea.* Highest noteworthy activity was observed for *Garcinia livingstonei* and *Sclerocarya birrea* at the MIC value of 0.19 mg/ml.

Twenty two out of twenty three plant species showed antibacterial activity against *Shigella flexneri*. Seventeen plant species showed noteworthy antimicrobial activity with the best MIC value of 0.04 mg/ml for *Terminalia sericea*. *Shigella flexneri* was the most susceptible pathogen with the overall susceptibility at the mean MIC value of 1.45 mg/ml.

All plant species tested demonstrated high to moderate (mean MIC value 0.25 - 6.80 mg/ml) activity against *Staphylococcus aureus.* At the MIC value of 0.14 mg/ml, *Garcinia livingstonei* and *Sclerocarya birrea* showed the highest noteworthy antibacterial activity against *Staphylococcus aureus*. Other plant species that yielded noteworthy antimicrobial activity against this pathogen were *Chenopodium ambrosioides, Melia azedarach, Psidium guajava, Sclerocarya birrea, Terminalia sericea* and *Trichillia emetica*.

There was some difference in the antimicrobial activity of samples from different localities. Samples from Mseleni showed the most noteworthy activities while most households from Tshongwe showed the least activity compared to other regions. Viljoen *et al*. (2005) observed that there is variation in plant chemotypes of plants of the same species, irrespective where the plants are in the same or different

geographical regions. The differences in antimicrobial activity noted in this study may be due to chemotypic viariations.

Aqueous extracts showed good to poor antibacterial activity (Table 4.4). Only five aqueous extracts showed noteworthy antidiarrhoeal activity namely: *Acacia burkei* against *Bacillus cereus; Garcinia livingstonei against Escherichia coli* and *Staphylococcus aureus; Psidium guajava against Staphylococcus aureus; Sclerocarya birrea* against *Staphylococcus aureus* and *Terminalia sericea* against *Bacillus cereus, Enterococcus faecalis*, *Proteus vulgaris*, *Shigella flexneri* and *Staphylococcus aureus*. *Terminalia sericea* showed the highest antibacterial activity for the aqueous extracts and the best overall activity for the aqueous extracts.

Plant species that possess tannins showed better activity than those without tannins. The tannins are inhibitory to microbial growth, respiration and metabolism (Mahadevan and Muthukumar, 1980). *Acacia burkei, Catharanthus roseus, Mangifera indica, Psidium guajava* and *Sclerocarya birrea* are reported to contain tannins and their higher activities supports the previous studies (Aganga and Mosase, 2001; Elekwa *et al*., 2009; Engels *et al*., 2009; Islam *et al*., 2009; Kaur *et al*., 2010), where higher antimicrobial activity is linked to the presence of tannins.

The organic extracts from these observations possess better antibacterial activity than the aqueous extracts. This observation has been reported in a number of previous studies (Steenkamp *et al*., 2007; Fawole *et al*., 2009 and Bussmann *et al*., 2010). Most of the aqueous extracts screened showed antibacterial activity against at least one diarrhoeal pathogen compared to four by the organic extracts. A study undertaken by Jäger (2003) highlighted the poor activities of aqueous extracts in comparison with organic-derived extracts and raised concern in terms of antimicrobial efficacy when the traditional method is applied. It is a future challenge to translate the applied knowledge gained from intricate assays and make it meaningful to the ethnic people who rely on traditional medicine.

Table 4.4 Aqueous extracts mean MIC values (mg/ml).

Plant species	Locality	Household	*B. cereus (ATCC 11778)*	*E. faecalis (ATCC 29212)*	*E. coli (ATCC 8739)*	*P. vulgaris* (ATCC 33420)	*S. typhimurium (ATCC 14028)*	*S. flexneri (ATCC 25875)*	*S. aureus (ATCC 12600)*
A. burkei	Mseleni	2	**0.75**	1.00	1.50	3.00	3.00	1.00	1.50
A. glabratum	Mabibi	55	5.33	NS	NS	NS	6.00	4.00	6.67
B. transvaalensis	Mabibi	49	NS	NS	NS	NS	NS	4.00	NS
C. roseus	Olakeni	68	6.00	6.00	4 .00	4.00	4.00	4.00	8.00
C. ambrosioides	Tshongwe	28	3.00	8.00	8.00	3.00	8.00	8.00	4.00
C. hirta	Mabibi	65	6.00	4.00	8.00	8.00	8.00	8.00	6.00
G. livingstonei	Mabibi	42	1.50	2.00	**0.75**	1.50	2.00	2.00	**0.75**
G. senegalensis	Tshongwe	12	8.00	6.00	12.00	8.00	8.00	NS	8.00
K. mosambicina	Mseleni	17	8.00	5.33	8.00	8.00	6.67	6.67	8.00
L. javanica	Mseleni	16	8.00	4.00	8.00	4.00	6 .00	8.00	6.00
M. indica	Mabibi	51	NS	6.67	NS	**0.50**	NS	4.00	2.67
M. azedarach	Tshongwe	36	8.00	8.00	4.00	4.00	4.00	12.00	12.00
P. guajava	Mabibi	58	1.00	6.00	6.00	8.00	8.00	3.00	**0.50**
S. viminale	Mseleni	12	8.00	4.00	8.00	8.00	8.00	8.00	8.00
S. brachypetala	Tshongwe	40	1.50	2.00	8.00	2.00	2.00	4.00	1.00
S. birrea	Mseleni	7	2.00	2.00	2.00	4.00	1.33	2.00	**0.50**
S. occidentalis	Mseleni	1	6.00	8.00	3.00	2.00	4.00	8.00	4.00
S.madagascariensis	Tshongwe	12	8.00	8.00	6.00	8.00	8.00	6.67	8.00
S. cordatum	Mabibi	60	8.00	6.00	8.00	8.00	8 .00	4.00	8.00
T. sericea	Tshongwe	22	**0.38**	**0.50**	8.00	**0.50**	1.00	**0.67**	**0.75**
T. emetica	Mabibi	58	6.00	8.00	4.00	4.00	12 .00	4.00	4.00
V. infausta	Mabibi	67	8.00	NS	8.00	4.00	8.00	8.00	8.00
V. natalensis	Mabibi	49	2.00	6.00	6.00	1.50	8.00	6.00	8.00
Ciprofloxacin (µg/ml)			0.50	0.25	0.02	0.40	0.20	0.05	0.80
Acetone			16 .00	NS	NS	13.30	NS	8.00	NS
DMSO			8.00	NS	8.00	9.60	NS	8.00	NS

Bold = noteworthy activity (MIC value < 1.00 mg/ml); NS = Not susceptible at highest concentration tested (64 mg/ml).

The plants obtained from muthi market showed mostly moderate to poor antidiarrhoeal activity. At least one pathogen demonstrated noteworthy activity for the organic extracts of plant species *Sarcophyte sanguinea, Strychnos henningsii* and *Ximenia caffra* against *Shigella flexneri* (MIC value 0.50 - 0.75 mg/ml) and *Staphylococcus aureus* (MIC value 0.38 mg/ml). When compared to the plants from households, the antibacterial activity of plant species from muthi markets demonstrated poorer antibacterial activity. The sample of *Sclerocarya birrea* from muthi market showed reduced antidiarrhoeal activity compared to the samples collected from households. Geographical variations might also add to reduce the final antidiarrhoeal activity.

In correlation with the traditional use of these plants to treat diarrhoea (Chapter 3), it is evident that most of the plants used by homestead dwellers do possess antidiarrhoeal properties against the diarrhoeal pathogens tested. The plant species *Psidium guajava, Melia azedarach, Sclerocarya birrea* and *Syzygium cordatum* are known to be antimicrobially active, however, new plant species that showed antidiarrhoeal potential are *Terminalia sericea* and *Trichillia emetica*.The findings in this study also concur with the findings from other research articles.

4.5 Conclusions

- Organic extracts (MIC range 0.04 mg/ml to 16.00 mg/ml) showed better antidiarrhoeal activity when compared to the aqueous extracts (MIC range 0.38 mg/ml to 16.00 mg/ml).
- *Acacia burkei* (MIC value range 0.25 mg/ml 3.00 mg/ml), *Garcinia livingstonei* (MIC value range 0.12 mg/ml to 3.38 mg/ml), *Psidium guajava* (MIC value range 0.33 mg/ml to 1.63 mg/ml), *Sclerocarya birrea* (MIC value range 0.20 mg/ml to 0.95 mg/ml) and *Terminalia sericea* (MIC value range 0.04 mg/ml to 1.00 mg/ml) showed the overall best activities.
- *Shigella flexineri* was the most susceptible pathogen at the lowest MIC value of 0.04 mg/ml (tested against *Terminalia sericea*).
- Each of the 23 plant species showed antidiarrhoeal activity against at least four pathogens.

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Chapter 5

Antimicrobial Combination Studies

5.1 Introduction

In a recent review by Van Vuuren and Viljoen (2011), it has been stipulated that the therapeutic value of synergistic interactions has been known since ancient times and many different cultural systems rely on this principle in the belief that combination therapy may enhance efficacy. Combination therapy is often used to treat infectious diseases. In many westernised medicines, combined antimicrobials are used, such as the use of beta-lactam and aminoglycoside antibiotics. These have proved to be efficient in the treatment of diseases where pathogens are responsible for neonatal infections, sepsis or meningitis, intestinal perforation and pneumonitis (Chadwick *et al.*, 1986). Combination therapy may be advantageous because it may cover infections from a number of pathogens, can result in synergistic interactions, dosage reductions, decrease toxicity and may contribute towards the prevention of bacterial resistance (Chadwick *et al*., 1986; Van Vuuren and Viljoen, 2011).

Hutchings *et al*. (1996) mentioned a number of plants that are used in combination to enhance their healing power. For example, the combination of *Chenopodium ambrosioides* with *Chenopodium album* for anaemia and the combination of *Lippia javanica* with *Artemisia afra* to treat fevers and measles. In a review on the antimicrobial activity of South African medicinal plants, it has been stated that there have been a number of publications on the antibacterial activity of plants in South Africa, but very few studies have been done on plants that are used in combination (Van Vuuren, 2008). Up until recently, very little has been done to scientifically validate the efficiency of ethnopharmacological combinations (Van Vuuren and Viljoen, 2011). Most of the combination studies documented in the literature are combinations of plants or plant derivatives with conventional antimicrobials (Bapela *et al*., 2006; Hemaiswarya *et al*., 2008; Van Vuuren *et al*., 2009; Van Vuuren and Viljoen, 2011). Other plant to plant interactions include the study by Al-Bayati (2008), where research was conducted on the synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. The combinations were tested against pathogens *Staphylococcus aureus, Bacillus*

cereus, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Salmonella typhimurium, Salmonella typhi, Klebsiella pneumoniae and *Pseudomonas aeruginosa*. The essential oils and extracts both showed enhanced activity in combination. Some studies have been undertaken on South African medicinal plant combinations and these include investigations by Kamatou *et al.* (2006), on the antimicrobial interaction of *Salvia chamelaeagnea* and *Leonotis leonorus* where synergistic interactions were observed for selected plant ratios against pathogens *Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Staphylococcus aureus*. When the essential oils of *Artemisia afra* was combined with *Agathosma betulina, Eucalyptus globulus* and *Osmitopsis asteriscoides*, the combinations showed predominantly additive interactions (Suliman *et al.*, 2010). These studies show that plants used in combination have potential in improving the antimicrobial activity.

In the ethnobotanical survey (Chapter 3), four plant combinations (plant: plant) were mentioned as a combined treatment for diarrhoeal symptoms. Five plants were used in the combinations namely:

- *Acanthospermum glabratum* (Ag) with *Krauseola mosambicina* (Km)
- *Acanthospermum glabratum* (Ag) with *Psidium guajava* (Pg)
- *Brachylaena transvaalensis* (Bt) with *Psidium guajava* (Pg)
- *Brachylaena transvaalensis* (Bt) with *Sclerocarya birrea* (Sb)

MIC assays were performed to determine the antimicrobial activity of these plants independently (Chapter 4). The combination assays were performed to determine if there is increased susceptibility when used in combination. To the best of my knowledge, there has been no previous research undertaken on these combinations to validate their combined use to treat infections related to diarrhoeal diseases. This Chapter aims to identify whether these plants in combination show possible synergistic activity. Results may provide further evidence as to the choice of combining plants for increased efficacy.

5.2 Fractional inhibitory concentration (FIC) (1:1 combinations)

5.2.1 Dichloromethane:methanol extracts combinations

Table 5.1 shows the mean MIC values of plants used independently (shaded area is a summary of results attained and presented in Chapter 4) and in combination. The ΣFIC values of the plant combinations are given in Table 5.2.

Table 5.1 The mean MIC values (mg/ml) of crude dichloromethane:methanol extracts used independently and in combination (1:1) against seven diarrhoeal pathogens.

Plant species and combinations	Pathogens							
	B. cereus 11778*	*E. faecalis* 29212	*E. coli* 8739	*P. vulgaris* 33420	*S. typhimurium* 14028	*S. flexneri* 25875	*S. aureus* 12600	Average
A. glabratum	2.00	4.00	12.00	NS	3.00	**0.26**	8.00	4.88
B. transvaalensis	**0.25**	**0.13**	2.00	1.50	1.00	**0.50**	**0.50**	**0.84**
K. mosambicina	4.00	8.00	8.00	16.00	2.00	1.00	8.00	5.86
P. guajava	**0.18**	**0.13**	4.00	4.00	**0.50**	**0.38**	**0.50**	1.38
S. birrea	**0.25**	**0.33**	1.67	1.00	**0.17**	**0.63**	**0.38**	**0.63**
A. glabratum and *K. mosambicina*	**0.03**	6.00	NS	**0.16**	**0.63**	**0.03**	**0.04**	1.15
A. glabratum and *P. guajava*	**0.03**	**0.31**	**0.16**	**0.31**	**0.31**	**0.23**	**0.16**	**0.22**
B. transvaalensis and *P. guajava*	**0.02**	**0.16**	**0.16**	**0.23**	**0.31**	**0.02**	**0.31**	**0.17**
B. transvaalensis and *S. birrea*	**0.03**	**0.63**	**0.63**	**0.16**	**0.31**	**0.02**	**0.48**	**0.32**
Acetone control	16.00	NS	NS	13.30	NS	8.00	NS	12.43
DMSO control	8.00	NS	8.00	9.60	NS	8.00	NS	8.40
Ciprofloxacin control (µg/ml)	**0.50**	**0.25**	**0.02**	**0.40**	**0.20**	**0.05**	**0.80**	**0.32**

*= ATCC numbers; NS = Not susceptible at the highest concentration tested; **Bold** = noteworthy activity (MIC value < 1.00 mg/ml); Shaded area = summary of results obtained in Chapter 4.

The antimicrobial activity of *Acanthospermum glabratum* and *Krauseola mosambicina* was generally enhanced (average ΣFIC value of 0.30) when the two plants were combined compared to the independent activities of the plants. Synergy was detected for five out of seven pathogens studied. The most synergistic interactions (ΣFIC value 0.01) were noted for *Staphylococcus aureus* with a combined MIC value 0.04 mg/ml and *Bacillus cereus* (ΣFIC value of 0.02) and the respective MIC value of 0.03 mg/ml. An antagonistic interaction was only observed against *Escherichia coli*. The ΣFIC values for *Escherichia coli* and *Proteus vulgaris* could not be determined as there was no end point MIC value during the 1:1 combination study and MIC studies respectively (Tables 5.1 and 5.2). *Proteus*

vulgaris at 1:1 concentrations showed a synergistic interaction (MIC value of 0.16 mg/ml) compared to the MIC value of 16.00 mg/ml for *Krauseola mosambicina* independently.

When combined, *Acanthospermum glabratum* and *Psidium guajava* showed enhanced antibacterial activity against diarrhoea pathogens compared to the independent activity (Table 5.1). This combination showed synergistic interactions with an average ΣFIC value of 0.46 (Table 5.2). The ΣFIC value of 0.03 was noted for *Escherichia coli*. An additive interaction was noted for *Shigella flexneri* (MIC value 0.23 mg/ml; ΣFIC value 0.78). There were no antagonistic interactions observed. *Proteus vulgaris* demonstrated noteworthy antimicrobial activity in a 1:1 combination (MIC value of 0.31 mg/ml) but no ΣFIC value was determined due to the lack of endpoint MIC values observed for *Acanthospermum glabratum*.

The antimicrobial activity of *Brachylaena transvaalensis* and *Psidium guajava,* in combinationwas mostly enhanced with an average ΣFIC value of 0.39. The best activity was observed against *Bacillus cereus* and *Shigella flexneri* at the MIC value of 0.02 mg/ml. This combination showed synergistic interactions for four pathogens namely *Bacillus cereus, Escherichia coli, Proteus vulgaris*, *Salmonella typhimurium* and *Shigella flexneri* at the ΣFIC value range of 0.05 to 0.48. The most interesting synergistic interaction at the ΣFIC value of 0.05 and 0.06 was detected for *Shigella flexneri* and *Escherichia coli* respectively. An additive interaction was observed for *Staphylococcus aureus*. No antagonistic interactions were observed for the combination of *Brachylaena transvaalensis* with *Psidium guajava*.

When screened against diarrhoeal pathogens, the 1:1 combination of *Brachylaena transvaalensis* with *Sclerocarya birrea* (Tables 5.1 and 5.2) showed enhanced overall antibacterial activity with an average additive interaction at the ΣFIC value of 0.88. The combination exhibited synergistic interactions (Table 5.2) for *Bacillus cereus* (MIC value 0.03 mg/ml; ΣFIC value 0.16), *Escherichia coli* (MIC value 0.63 mg/ml; ΣFIC value 0.34), *Proteus vulgaris* (MIC value 0.16 mg/ml; ΣFIC value 0.13) and *Shigella flexneri* (MIC value 0.02 mg/ml; FIC value 0.04). Antagonism was not observed with this combination.

Table 5.2 The sum fractional inhibitory concentration (ΣFIC) values of plants (dichloromethane:methanol extracts) used in combination.

Plant combinations	Pathogens							
	B. cereus 11778*	*E. faecalis* 29212	*E. coli* 8739	*P. vulgaris* 33420	*S. typhimurium* 14028	*S. flexneri* 25875	*S. aureus* 12600	ΣFIC averages
A. glabratum and *K. mosambicina*	**0.02**	1.13	ND*	ND*	**0.26**	**0.07**	**0.01**	**0.30**
Interpretation	**Synergism**	Non-interactive	Antagonism	**Synergism**	**Synergism**	**Synergism**	**Synergism**	**Synergism**
A. glabratum and *P. guajava*	**0.13**	1.27	**0.03**	ND*	**0.37**	0.78	**0.17**	**0.46**
Interpretation	**Synergism**	Non-interactive	**Synergism**	**Synergism**	**Synergism**	Additive	**Synergism**	**Synergism**
B. transvaalensis and *P. guajava*	**0.10**	1.23	**0.06**	**0.11**	**0.48**	**0.05**	0.62	**0.39**
Interpretation	**Synergism**	Non-interactive	**Synergism**	**Synergism**	**Synergism**	**Synergism**	Additive	**Synergism**
B. transvaalensis and *S. birrea*	**0.16**	3.32	**0.34**	**0.13**	1.10	**0.04**	1.11	0.88
Interpretation	**Synergism**	Non-interactive	**Synergism**	**Synergism**	Non-interactive	**Synergism**	Non-interactive	Additive

ND* = Not determined as no end point was obtained for MIC determination, however, tentative interpretation is given based on MIC values (Table 5.1): FIC < 0.5 = synergy (indicated in bold); FIC 0.5-1.0 = additive; FIC > 1-4 = indifferent or non- interactive; FIC > 4.0 = antagonism; * = ATCC numbers.

5.2.2 Aqueous extracts

Table 5.3 shows the mean MIC values of plants used independently (shaded area is a summary of results attained and presented in Chapter 4) and in combination. The ΣFIC values of the plant combinations are given in Table 5.4.

Table 5.3 The mean MIC values (mg/ml) of aqueous crude extracts used independently and in combination (1:1) against seven diarrhoeal pathogens.

Plant species and combinations	Pathogens							
	B. cereus 11778*	*E. faecalis* 29212	*E. coli* 8739	*P. vulgaris* 33420	*S. typhimurium* 14028	*S. flexneri* 25875	*S. aureus* 12600	Average
A. glabratum	5.33	NS	NS	NS	6.00	4.00	6.67	5.50
B. transvaalensis	NS	NS	NS	NS	NS	4.00	NS	4.00
K. mosambicina	8.00	5.33	8.00	8.00	6.67	6.67	8.00	7.24
P. guajava	1.00	6.00	6.00	8.00	8.00	3.00	**0.50**	4.64
S. birrea	2.00	2.00	2.00	4.00	1.33	2.00	**0.50**	1.98
A. glabratum and *K. mosambicina*	12.00	8.00	16.00	4.00	4.00	16.00	8.00	9.71
A. glabratum and *P. guajava*	4.00	4.00	NS	2.00	8.00	8.00	2.00	4.67
B. transvaalensis and *P. guajava*	8.00	NS	16.00	2.00	2.00	8.00	4.00	6.67
B. transvaalensis and *S. birrea*	8.00	8.00	NS	3.00	12.00	1.50	3.00	5.92
Acetone control	16 .00	NS	NS	13.30	NS	8.00	NS	12.43
DMSO control	8.00	NS	8.00	9.60	NS	8.00	NS	8.40
Ciprofloxacin control (µg/ml)	0.50	0.25	0.02	0.40	0.20	0.05	0.80	0.32

***** = ATCC numbers; NS = Not susceptible at the highest concentration tested; **Bold** = noteworthy activity (MIC value < 1.00 mg/ml); Shaded area = summary of results obtained in Chapter 4.

The combination of *Acanthospermum glabratum* with *Krauseola mosambicina* showed no major improvement in antibacterial activity of when screened against diarrhoeal pathogens. The overall interaction of the combination demonstrated non-interaction at the FIC average of 1.70. The combination of the plants demonstrated the MIC range of 4.00 mg/ml to 16.00 mg/ml. An additive interaction was noted for *Salmonella typhimurium* at the ΣFIC value of 0.63. A non-interactive interaction was observed for *Bacillus cereus* (ΣFIC value 1.88), *Shigella flexneri* (ΣFIC value 3.20) and *Staphylococcus aureus* (ΣFIC value 1.10). There were no ΣFIC values determined for other pathogens because there was no end point MIC value of *Acanthospermum glabratum* against these pathogens but the combined MIC values were used to demonstrate interactions.

Tables 5.3 and 5.4 shows the 1:1 combination of *Acanthospermum glabratum* and *Psidium guajava* when screened against the diarrhoeal test pathogens. The combination showed moderate to poor activity against *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Shigella flexineri* and *Staphylococcus aureus*. The MIC values ranged from 2.00 mg/ml to 16.00 mg/ml. Even though the overall interaction of this combination showed non-interactive interactions at the average ΣFIC of 2.00, tentative synergistic interactions were observed for two pathogens namely *Enterococcus faecalis* and *Proteus vulgaris*. An antagonistic interaction was observed against *Escherichia coli*. The ΣFIC values of *Enterococcus faecalis, Escherichia coli* and *Proteus vulgaris* were not determined as there were no end point MIC values for *Acanthospermum glabratum* against these pathogens. The combined efficacy against *Proteus vulgaris* was enhanced at the MIC value of 2.00 mg/ml. Other pathogens showed non-interactive interactions.

Improved efficacy was observed in the 1:1 combination of *Brachylaena transvaalensis* and *Psidium guajava* (Table 5.3) against *Proteus vulgaris* and *Salmonella typhimurium* with the MIC value of 2.00 mg/ml respectively. Synergy was also observed against the previously mentioned pathogens (Table 5.4). Non-interactive interactions were observed for pathogens *Bacillus cereus, Enterococcus faecalis*, *Escherichia coli* and *Shigella flexneri*. An antagonistic interaction was observed for *Staphylococcus aureus*. This combination demonstrated an overall broad-spectrum interaction of 2.33 (non-interactive).

The 1:1 combination and the ΣFIC values of *Brachylaena transvaalensis* and *Sclerocarya birrea* (Tables 5.3 and 5.4) exhibited mostly improved antibacterial activity. The overall average ΣFIC value was 0.57 (additive). Improved antibacterial activity was shown for *Proteus vulgaris* (MIC value 3.00 mg/ml) and *Shigella flexneri* (MIC value 1.50 mg/ml). Synergy was observed for *Proteus vulgaris* but no ΣFIC value could be determined as *Sclerocarya birrea* had no MIC end point value against this pathogen. An additive interaction was demonstrated for *Shigella flexneri* with the ΣFIC value of 0.57. For the other pathogens a non-interactive interaction was displayed.

Table 5.4 The sum fractional inhibitory concentration (ΣFIC) values of plants used in combination.

Plant combinations	Pathogens							
	B. cereus 11778*	*E. faecalis* 29212	*E. coli* 8739	*P. vulgaris* 33420	*S. typhimurium* 14028	*S. flexneri* 25875	*S. aureus* 12600	ΣFIC averages
A. glabratum and *K. mosambicina*	1.88	ND*	ND*	**ND***	0.63	3.20	1.09	1.70
Interpretation	Non-interactive	Non-interactive	Non-interactive	**Synergism**	Additive	Non-interactive	Antagonism	Non-interactive
A. glabratum and *P. guajava*	2.38	**ND***	ND*	**ND***	1.17	2.33	2.14	2.00
Interpretation	Non-interactive	**Synergism**	Antagonism	**Synergism**	Non-interactive	Non-interactive	Non-interactive	Non-interactive
B. transvaalensis and *P. guajava*	ND*	ND*	ND*	**ND***	**ND***	2.33	ND*	2.33
Interpretation	Non-interactive	Non-interactive	Non-interactive	**Synergism**	**Synergism**	Non-interactive	Antagonism	Non-interactive
B. transvaalensis and *S. birrea*	ND*	ND*	ND*	**ND***	ND*	0.57	ND*	0.57
Interpretation	Non-interactive	Non-interactive	Antagonism	**Synergism**	Non-interactive	Additive	Non-interactive	Additive

ND* = Not determined as no end point was obtained for MIC determination, however tentative interpretation is given based on MIC values (Table 5.3) FIC < 0.5 = synergy (indicated in bold); FIC 0.5-1.0 = additive; FIC > 1-4 = indifferent or non-reactive; FIC > 4.0 = antagonism; * = ATCC numbers.

5.3. Isobologram studies

Isobologram studies were selectively performed for dichloromethane:methanol extracts because these extracts predominantly demonstrated higher levels of synergy. Selection was also based on the availability of plant material (*Acanthospermum glabratum* with *Krauseola mosambicina*) and also on the most mentioned combination (*Brachylaena transvaalensis* and *Psidium guajava*). The pathogens used were selected randomly as limited plant material was available i.e. *Escherichia coli* was not tested in the *Acanthospermum glabratum: Krauseola mosambicina* combination and *Enterococcus faecalis* and *Staphylococcus aureus* not tested for the *Brachylaena transvaalensis: Psidium guajava* combination. Isobolograms were used to interpret interactions at various ratios of the two selected plant species. Isobolograms give more detailed account of interaction, not just the 1:1 combinations but with the different ratios as well varied concentrations of the two plants in the study. Figure 5.1 and Figure 5.2 show the Isobologram plots from the raw data (Appendix 3) for the mean of each experiment which was undertaken in duplicate. Points on the isobologram show synergy, if plotted at the 0.5 point or lower. Additive interactions are observed between values >0.5-1.0. Indifferent/non-interactive values are between > 1-4 and antagonistic interactions are made where values > 4.0 are observed (Suliman *et al.*, 2010).

5.3.1 *Acanthospermum glabratum* in combination with *Krauseola mosambicina*

The interaction of the combination of *Acanthospermum glabratum* and *Krauseola mosambicina* at various ratios against the pathogens *Bacillus cereus, Staphylococcus aureus, Shigella flexneri, Enterococcus faecalis* and *Salmonella typhimurium* is indicated in Figure 5.1 and summarised in Table 5.5. The combination of *Acanthospermum glabratum* and *Krauseola mosambicina* showed synergistic interactions in all ratios tested against *Bacillus cereus* and *Staphylococcus aureus*.

Eight synergistic interactions were observed when the combination of *Acanthospermum glabratum* with *Krauseola mosambicina* was screened against *Proteus vulgaris*. The interaction of the combination of *Acanthospermum glabratum* with *Krauseola mosambicina* at various ratios against *Salmonella typhimurium* showed synergistic

interactions for eight ratios. One additive interaction was observed at 4:6 ratio where *Acanthospermum glabratum* was found in higher concentration. When *Acanthospermum glabratum* was combined with *Krauseola mosambicina* against *Shigella flexneri,* only the 1:1 interaction showed a synergistic interaction. Varied interactions were observed for other combinations ranging from additive to antagonistic.

Additive interactions were observed for six ratios and a non-interaction was noted for three ratios with studies against *Enterococcus faecalis*. No antagonism was observed for any of the ratios for all pathogens studied.

Table 5.5 shows the summary of all ratios of the combination of *Acanthospermum glabratum* and *Krauseola mosambicina* demonstrating synergy and the interpretation.

Table 5.5 Summary of ratios demonstrating synergy and the interpretation.

Ratios displaying synergy	**Pathogens**	**Interpretation**
9	*B. cereus*	All ratios synergistic
9	*S. aureus*	All ratios synergistic
8	*P. vulgaris*	1:1 combination showed additive interaction
8	*S. typhimurium*	4:6 ratio displayed additive interaction
0	*S. flexneri*	1:1 combinations displayed additive interaction. Two other combinations where *K. mosambicina* was in majority also displayed additive interaction.
0	*E. faecalis*	Six additive interactions were observed where *A. glabratum* was combined with *K. mosambicina*.
Total synergistic interactions = 34/45 = 76%		

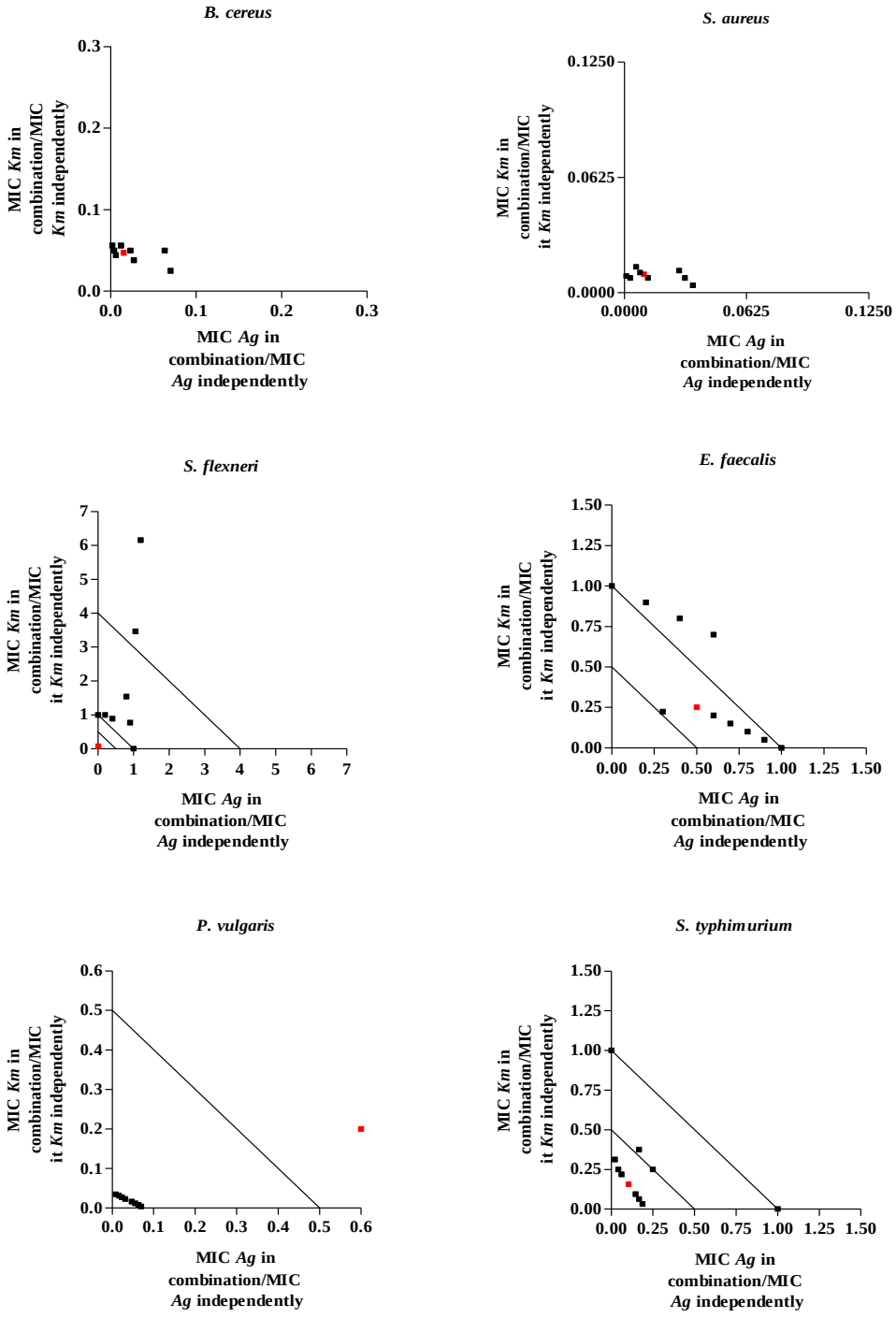

Figure 5.1 The Isobolograms for *Bacillus cereus, Staphylococcus aureus, Shigella flexneri, Enterococcus faecalis, Proteus vulgaris,* and *Salmonella typhimurium* when exposed to the combination of *Acanthospermum glabratum* (Ag) and *Krauseola mosambicina* (Km) at various ratios.

5.3.2 *Brachylaena transvaalensis* in combination with *Psidium guajava*

Figure 5.2 and Table 5.6 (summary) shows the antimicrobial interaction of the combination of *Brachylaena transvaalensis* with *Psidium guajava* at various ratios against five pathogens namely *Bacillus cereus, Shigella flexneri, Escherichia coli, Proteus vulgaris* and *Salmonella typhimurium*.

Table 5.6 Summary of ratios demonstrating synergy and the interpretation.

Ratios displaying synergy	Pathogens	Interpretation
0	*E. coli*	One additive interaction was observed where *Psidium guajava* was in high concentrations and two other additive interactions where the concentrations of *Brachylaena transvaalensis* were higher.
0	*S. flexneri*	The 1:1 combination displayed an additive interaction. Four other ratios (7:3, 4:6, 2:8 and 1:9) showed additive interactions, three of which were found where *Psidium guajava* was found in higher concentrations.
5	*P. vulgaris*	1:1 combination displayed a synergistic interaction. Four other ratios where *Psidium guajava* was in high concentration demonstrated synergistic interactions (4:6, 3:7, 2:8 and 1:9). Additive interaction was observed for one ratio (9:1) where the concentration of *Brachylaena transvaalensis* was high.
1	*B. cereus*	Synergy observed where *Psidium guajava* was presented in a higher ratio (1:9). Additive interactions were observed mainly where *Psidium guajava* was in high concentration.
2	*S. typhimurium*	Two ratios (1:9 and 2:8) where *Psidium guajava* was in higher concentration displayed synergistic interaction and six other ratios displayed additive interactions.
Total synergistic interactions = 8/45 = 17.7%		

The combination against *Escherichia coli* showed additive interactions in three ratios. Two additive interactions were observed where *Brachylaena transvaalensis* was found in higher concentrations (7:3 and 6:4) and one was where the concentration of *Psidium guajava* was higher (1:9 ratio).

When *Brachylaena transvaalensis* was combined with *Psidium guajava* at various ratios and tested against *Shigella flexneri* five ratiosdisplayed additive interactions. Most additive interactions were shown when the concentrations were higher or equal for *Psidium guajava* while non-interactive interaction was noted when *Brachylaena transvaalensis* was present in higher concentrations.

The interactions of the combination of *Brachylaena transvaalensis* with *Psidium guajava* against *Proteus vulgaris* presented five synergistic interactions. The interactions were observed where the concentrations were high or equal for *Psidium guajava* at the ratios 5:5, 3:7, 2:8 and 1:9. Non-interactive interactions were observed mainly where the concentrations were higher for *Brachylaena transvaalensis*.

When *Brachylaena transvaalensis was* combinedwith *Psidium guajava* and tested against *Bacillus cereus,* synergy was observed for one ratio (1:9) where the concentration of *Psidium guajava* was higher. Additive interactions were noted for five ratios, most of which were observed where there were higher concentrations of *Psidium guajava*. Non-interactive reactions were observed mainly where the concentration was equal to or higher for *Brachylaena transvaalensis* (ratios 9:1, 8:2 and 5:5). Synergistic interaction was observed in two ratios (1:9 and 2:8) when *Brachylaena transvaalensis* with *Psidium guajava* combination at various ratios against *Salmonella typhimurium*. Synergy was noted where *Psidium guajava* was in high concentrations. Additive interactions were observed when the concentration of *Brachylaena transvaalensis* was mostly higher (ratios 8:2, 7:3, 6:4, 5:5, 4:6 and 3:7). No antagonistic interactions were observed against any of the pathogens tested.

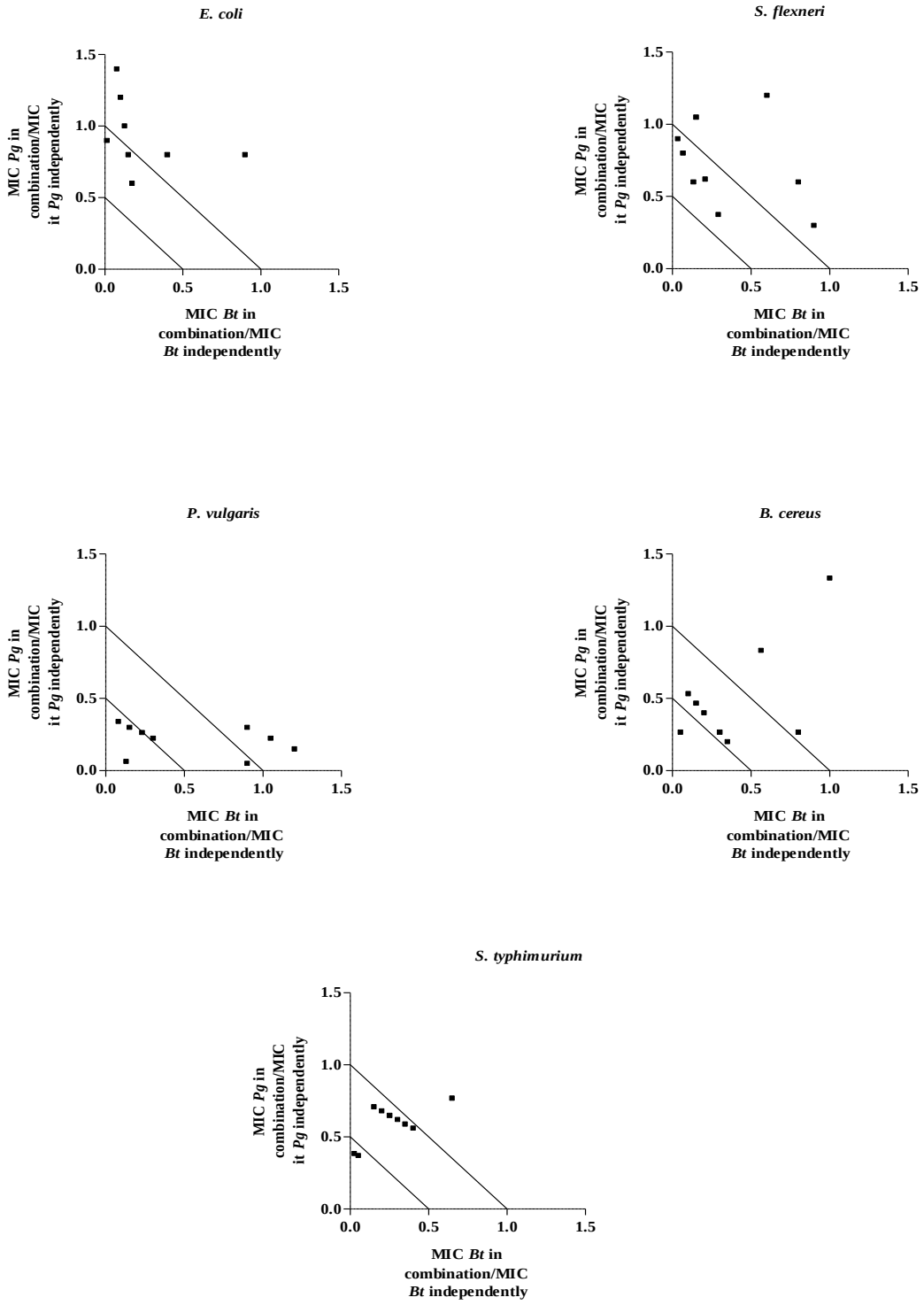

Figure 5.2 The Isobolograms for *Escherichia coli, Shigella flexneri, Proteus vulgaris, Bacillus cereus* and *Salmonella typhimurium* when exposed to the combination of *Brachylaena transvaalensis* (Bt) and *Psidium guajava* (Pg) at various ratios.

5.4 Discussion

The combinations ΣFIC of *Acanthospermum glabratum* with *Krauseola mosambicina, Acanthospermum glabratum* with *Psidium guajava*, *Brachylaena transvaalensis* with *Psidium guajava* and *Brachylaena transvaalensis* with *Sclerocarya birrea* in dichloromethane: methanol solutions showed enhanced antibacterial activity against at least five diarrhoeal pathogens. This was an enhanced activity, compared to the use of these plants individually. The highest antibacterial activities (MIC values up to 0.02 mg/ml) were observed mostly against *Bacillus cereus* and *Shigella flexineri* (Table 5.1 and ΣFIC in Table 5.2).

The combination of *Acanthospermum glabratum* with *Krauseola mosambicina* is the most used combination by the residents of the homesteads.

Acanthospermum glabratum with *Psidium guajava* and *Brachylaena transvaalensis* with *Psidium guajava* showed the most synergistic and additive interactions. Both these combinations have the common factor which is *Psidium guajava. Psidium guajava* has demonstrated high potency in inhibiting the growth of other pathogens (Abdelrahim *et al*., 2002; Goncalves *et al.,* 2008; Barbalho *et al*., 2012.), and this was observed in this study (Chapter 4). Thus it was interesting to note that when used in combination efficacy is enhanced.

All combinations demonstrated synergy against *Bacillus cereus* and *Proteus vulgaris* therefore these proved to be the most susceptible pathogens.Both these pathogens are strongly linked to diarrhoeal diseases and thus demonstrate some validity to the selection of these plants to treat diarrhoeal diseases.

The aqueous extracts in 1:1 combinations (Table 5.3) showed little or no improvement when the combinations were screened against seven pathogens. *Acanthospermum glabratum* and *Psidium guajava* and *Brachylaena transvaalensis* with *Psidium guajava* combinations showed the best interactions (synergistic interactions against two pathogens) (Table 5.4). Just like the organic extracts combinations, the latter

combinations have the same common factor which is *Psidium guajava.* Surprisingly, the homestead residents use the combinations in aqueous form but the tests showed mainly non-interactive interactions. Possibly the *in vivo* screening might yield different outcomes to the *in vitro,* testing observed here. Furthermore the practitioners may be using the plants for relief of other symptoms (e.g. antispasmodic, anti-inflammatory effects) and not merely as an antimicrobial. No isobolograms were done for aqueous extracts.

Where isobologram studies were performed on the combination *Acanthospermum glabratum* with *Krauseola mosambicina,* the varied ratio combinations showed synergistic interactions (76%). Eight (17.7%) synergistic interactions were observed in different ratios when the combination of *Brachylaena transvaalensis* and *Psidium guajava* were screened against diarrhoeal pathogens. At least one additive interaction was also observed mostly where *Psidium guajava* was in majority. *Psidium guajava* demonstrated better MIC values than *Brachylaena transvaalensis* when these plant species were tested individually. From these observations, it is apparent that *Psidium guajava* was responsible for the synergistic and additive interactions. This correlates with the belief that homesteads residents have on the antidiarrhoeal properties of *Psidium guajava,* keeping in mind the frequency of use (43% homestead users), (Chapter 3).

From this study, it was observed that the combination of *Acanthospermum glabratum* with *Krauseola mosambicina* (which was the residents' most frequently selected combination) was the most antimicrobially active and synergistic. This combination needs to be studied further with respect on possible toxicity and interaction of active molecular compounds responsible for the antidiarrhoeal activity.

It was also observed that not always did the isobologram analysis correlate with the FIC data from Table 5.2. Some combination of *Acanthospermum glabratum* with *Krauseola mosambicina* correlated for interactions with *Bacillus cereus, Salmonella typhimurium* and *Staphylococcus aureus* while other pathogens didn't correlate. The same trend was

observed for the combination of *Brachylaena transvaalensis* and *Psidium guajava*against *Bacillus cereus* and *Proteus vulgaris* showed correlation. This kind of observation has been previously reported (Van Vuuren and Viljoen, 2008).

5.5 Conclusions

- The 1:1 organic extract combinations demonstrated mostly enhanced antimicrobial activity when compared to the activity of the plants when examined on their own.
- All 1:1 combinations for organic extracts showed noteworthy activity (MIC value < 0.50 mg/ml) against *Bacillus cereus, Proteus vulgaris, Shigella flexneri* and *Staphylococcus aureus*.
- The aqueous extracts in combination didn't show much enhanced antimicrobial activity.
- *Acanthospermum glabratum* with *Psidium guajava* and *Brachylaena transvaalensis* with *Psidium guajava* combinations were the most favourable aqueous combinations showing two synergistic interactions against the tested pathogens.
- Isobolograms of *Acanthospermum glabratum* with *Krauseola mosambicina* showed numerous (76%) synergistic interactions compared to 17.7% shown by the combination of *Brachylaena transvaalensis* with *Psidium guajava*.
- The traditional favoured uses of *Psidium guajava* correlates with the enhanced activity of this plant both independently and in combination.

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Chapter 6

Summary and Conclusions

6.1 Ethnobotanical survey

The first objective was to conduct an ethnobotanical survey on plants used traditionally to treat diarrhoea by a rural community in northern Maputaland. Interviews were conducted among 72 homestead inhabitants using structured questionnaires. Four localities in this region were visited namely: Mabibi, Mseleni, Mbazwana/ Olakeni and Tshongwe. The survey revealed 23 plant species (15 families) are being used to treat diarrhoeal diseases. A muthi market survey was included where three plant species were sold to treat diarrhoea (*Sarcophyte sanguinea*, *Ximenia caffra* and *Strychnos henningsii*). Four plants namely: *Acacia burkei*, *Brachylaena transvaalensis, Cissampelos hirta* and *Sarcostemma viminale* were recorded for the first time globally to treat diarrhoea. The three antidiarrhoeal plants most frequently used in the study area are *Psidium guajava*, *Catharanthus roseus* and *Melia azedarach* (all three exotic to South Africa) followed by *Sclerocarya birrea* and *Strychnos madagascariensis* which are indigenous. Seven plants are used in five different combinations for enhanced antidiarrhoeal efficacy. These are *Brachylaena transvaalensis* with *Psidium guajava*; *Sclerocarya birrea*, *Acanthospermum glabratum* in combination with *Krauseola mosambicina*; *Psidium guajava* and *Mangifera indica* in combination with *Sarcophyte sanguinea*. Most plants were used as leaf or bark decoctions. Not many roots were used. The interviewees obtained their medicinal plant knowledge mostly from their grandmothers (33%) and elders (20%). Regardless of the accessibility to 13 clinics and two hospitals in this Municipality district, all the interviewees prefer the use of medicinal plants above western medicine. The reason given was that traditional medicine was part of their culture. The study also revealed that the choice of plants used was based on the availability of the plant in and around their homesteads. None of the plants being used are on the endangered list and the sustainable use of the plants is well practiced in the homesteads. The results of this survey strengthens the evidence of Dahlberg and Trygger (2009), that medicinal plants play an important role in the primary health care system of the rural people in northern Maputaland, KwaZulu-Natal.

6.2 Antimicrobial validation

The second objective of this study was to investigate the *in vitro* antimicrobial activity of the antidiarrhoeal plants collected from the different homesteads as well as from the two muthi markets. Thirdly to compare the antibacterial activity between the same plant species collected from different homestead in the four areas and between the plants samples collected from the muthi market.

More than 80% of plant species screened were active against each pathogen. Organic extracts of *Terminalia sericea* showed noteworthy antibacterial activity of up to the mean MIC value of 0.04 mg/ml against *Shigella flexneri* (Chapter 4, Table 4.3). The plants that had no documented reports (*Acacia burkei, Brachylaena transvaalensis* and *Cissampelos hirta*) relating them to antidiarrhoeal activity also proved to possess some antidiarrhoeal activity. *Garcinia livingstonei, Mangifera indica, Psidium guajava*, *Sclerocarya birrea* and *Terminalia sericea* showed the most noteworthy activity (MIC value 0.50 mg/ml). Aqueous extracts showed less antimicrobial activity compared to dichloromethane:methanol extracts but *Acacia burkei, Garcinia livingstonei, Sclerocarya birrea* and *Terminalia sericea* though showed better activity compared to the rest of the collected plant samples. *Proteus vulgaris* showed overall the least susceptibility while *Shigella flexneri* proved to be the most susceptible pathogen.

The values of solvent extract combinations demonstrated mostly synergy (ΣFIC < 0.50) against at least four or more pathogens (Chapter 5, Table 5.2). Combinations of *Acanthospermum glabratum* with *Psidium guajava* and *Brachylaena transvaalensis* with *Psidium guajava*, proved to be the best combinations during the FIC screening. Both the previously mentioned combinations have a single common plant (*Psidium guajava*). Aqueous combinations showed synergy for few pathogens. *Krauseola mosambicina* proved to be responsible for the synergistic effect in the combination of *Acanthospermum glabratum* with *Krauseola mosambicina* while *Psidium guajava* demonstrated synergistic interactions in the combination of *Brachylaena transvaalensis* and *Psidium guajava*. The combination of *Acanthospermum glabratum* with *Krauseola*

mosambicina showed (76%) synergistic interactions in different ratios while (17.7%) synergistic interactions were observed in different ratios with the combination of *Brachylaena transvaalensis* and *Psidium guajava*

Samples from Tshongwe region showed overall less antibacterial activity. The antibacterial activity of plant species collected from household's demonstrated better activity compared to those collected from the two muthi markets.

6.3 Correlation between traditional use and antimicrobial screening

Psidium guajava was the most mentioned plant species (31 times) followed by *Catharanthus roseus* (22 times) and *Melia azedarach* with *Sclerocarya birrea* (20 times). When investigating the antimicrobial activity, *Psidium guajava*, *Melia azedarach* and *Sclerocarya birrea* had noteworthy activity for four to seven pathogens, but not *Catharanthus roseus*. Although not as frequently mentioned as the above specimens *Garcinia livingstonei* (4 times) and *Terminalia sericea* (3 times) proved to possess high efficacy against six pathogens. The aqueous extracts of *Garcinia livingstonei* and *Terminalia sericea* as prepared by the interviewees demonstrated once again the highest efficacy against selected pathogens.

When looking at the combined plant remedies, the most frequently used combination (*Psidium guajava* and *Brachylaena transvaalensis*) showed less synergy in ratios (three pathogens) compared to the rarely used combination (*Krauseola mosambicina* and *Acanthospermum glabratum*) which showed synergy against four pathogens. Additive interactions were also observed with very few antagonistic interactions. The aqueous combinations displayed once again lower efficacy. In general the combinations displayed some level of activity and thus concurred with the traditional use to treat diarrhoea.

6.4 Recommendations for future studies

It was observed in this study that most of the plant species possessed some antimicrobial activity, but it's clear that there is a long way to go until most of the plants

in this study could be used as novel drugs. During the study some medicinal plants used for diarrhoea showed little or no activity against the diarrhoeal pathogens screened. One can ask this question “What can be the reason for this?” One could speculate that the plants used in this study might be active against other causes (pathogens i.e. bacteria, parasites and viruses) of diarrhoea. It is well known that there are many causes of diarrhoea including food poisoning, poor hygiene, poor sanitation etc. In this study easily culturable strains were used. These plant species might be active against other diarrhoeal bacteria (e.g. *Vibrio cholerae* or *Campylobacter jejuni*), viral (e.g. Norovirus or Rotavirus) or parasitic (e.g. *Giardia lamblia, Entamoeba histolytica*)

For the researchers and public in general to understand the basis of activity, further studies such as time-kill assays to determine how quickly the most active plant species will work should be carried out. *In vivo* (clinical) studies can also pave the way in validating the *in vitro* activity. Toxicity tests should be used to evaluate the safety of the plant extracts. Plants like *Melia azedarach* are said to be toxic when taken in large volumes, therefore toxicity needs to be studied further to prevent mortalities while using at an *in vivo* level. Appendix 4 shows the plants with documented toxicity and those without documented toxicity.

6.4 Conclusion

Dahlberg and Trygger (2009) stated that medicinal plants play an important role in the primary health care system of the rural people in northern Maputaland. The care-givers in the rural homesteads generally treat the same ailment by using a diverse range of plants which is primarily dependent upon plant availability in their area. The concept of treatment is based on the principle that the wider the choice of plant, the better the chance of a cure (Howard, 2003). Indigenous medicinal plant knowledge is slowly disappearing from our rural community. In this study, follow-up visits to the study area revealed that some of the older members interviewed had deceased. Had the botanical knowledge not been recorded earlier, it might have been lost. The results of the present study strengthen the evidence and authenticity of the traditional plant knowledge used

to treat diarrhoea and most likely other infectious diseases. This therefore, concludes that investigation, validation and documentation of such knowledge are essential.

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Appendix 1

RESEARCH QUESTIONNAIRES

Date: Questionnaire No.

Name of the Interviewer:

Particulars of the area

GPS reading:

Name of the Area:

Name of the Sub-location/Sub-Area:

Name of the Village (Precise place):

Sociodemographic data

Gender: Age:

Male	
Female	

15-24	
25-34	
35-44	
45-54	
55-64	
Over 65	

Plant Species Particulars

Zulu names:

Plant 1:_____

Plant 2:_____

Plant 3:_____

Plant 4:_____

Scientific name:

Plant 1:_____

Plant 2:_____

Plant 3:_____

Plant 4:_____

English name:

Plant 1:_____

Plant 2:_____

Plant 3:_____

Plant 4:_____

Source of plant material:

Collected from the wild	
Cultivated (home-garden)	

What are the other uses of the plant?

_____

_____

_____

_____

_____

Plant usage and Collection

Question	Usage
Which part(s) used?	
Are the plants sold?	
In which state are the plants sold? (Fresh or Dry)	
If collected from the wild, when? (season)	
Any specific time for collection during the day?	
What places does the plant prefer to grow in? (wetland, dry land, grassland, forests, old fields, as weeds among the plants	

Preparation Method:

a) How is the medicine taken (e.g. by mouth or as enema)?

b) How is the medicine prepared?

Storage Method:

Dosage:

a) What is the dosage (e.g. one cup three times a day?)----------

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b) For how many days are the medicine taken? ----------

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c) Are there any known side effects? ----------

Where did the knowledge come from (e.g. grandmother, relative)?

FORM OF CONSENT FOR USING ETHNOBOTANICAL INFORMATION

Researchers: Mduduzi Nkwanyana (20030214)
(Supervisors - Dr. H de Wet and Prof. S van Vuuren).

Institution:
University of Zululand - Department of Botany.

Research Project:
This project aims to document Ethnobotanical knowledge on the usage of home grown plants for diarrhoea and other uses in the Maputaland area. The data will be conducted using structured questionnaires. We will also collect plant material for identification and antibacterial screening. This project is for academic purposes only and is of no commercial value. Results from this study will be presented at conferences and published in academic journals. The data will also be used towards the completion of a Masters Degree by the above mentioned students.

Please take note of the following:

You are under no obligation to share any secrets or personal information which you do not feel comfortable in sharing with us.

Follow up visit:

We undertake to reveal the main results of this study to every homestead visited on completion of this project.

Signature of interviewee:

Appendix 2

Raw MIC data for isobolograms

Dichloromethane:methanol extracts

1. *Enterococcus faecalis*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	1.25	0	0.31	0.00	1.000	0.000
9:1	1.125	0.125	0.28	0.03	0.898	0.099
8:2	1	0.25	0.25	0.06	0.799	0.200
7:3	0.875	0.375	0.16	0.09	0.524	0.300
6:4	0.75	0.5	0.09	0.06	0.300	0.200
5:5	0.625	0.625	0.08	0.08	0.249	0.249
4:6	0.5	0.75	0.09	0.14	0.288	0.447
3:7	0.375	0.875	0.07	0.16	0.224	0.524
2:8	0.25	1	0.07	0.25	0.216	0.799
1:9	0.125	1.125	0.03	0.28	0.100	0.899
0:10	0	1.25	0.00	0.31	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *S. birrea* (Sb) (Y axis)

2. Bacillus cereus

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	1.25	0	4.00	0.00	1.000	0.000
9:1	1.125	0.125	0.28	0.03	0.070	0.025
8:2	1	0.25	0.25	0.06	0.063	0.050
7:3	0.875	0.375	0.11	0.05	0.027	0.038
6:4	0.75	0.5	0.09	0.06	0.023	0.050
5:5	0.625	0.625	0.06	0.06	0.015	0.047
4:6	0.5	0.75	0.05	0.07	0.012	0.056
3:7	0.375	0.875	0.02	0.06	0.006	0.044
2:8	0.25	1	0.02	0.06	0.004	0.050
1:9	0.125	1.125	0.01	0.07	0.002	0.056
0:10	0	1.25	0.00	1.25	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

3. Enterococcus faecalis

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	4.00	0.00	1.000	0.000
9:1	14.4	1.6	3.60	0.40	0.900	0.400
8:2	12.8	3.2	3.20	0.80	0.800	0.800
7:3	11.2	4.8	2.80	1.20	0.700	1.200
6:4	9.6	6.4	1.20	0.80	0.300	0.800
5:5	8	8	1.00	1.00	0.250	1.000
4:6	6.4	9.6	0.80	1.20	0.200	1.200
3:7	4.8	11.2	0.60	1.40	0.150	1.400
2:8	3.2	12.8	0.40	1.60	0.100	1.600
1:9	1.6	14.4	0.10	0.90	0.025	0.900
0:10	0	16	0.00	1.00	0.000	1.000

A. glabratum (AG) (X axis) vs. *P. guajava* (PG) (Y axis)

4. *Staphylococcus aureus*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	1.25	0	0.31	0.00	1.000	0.000
9:1	1.125	0.125	0.28	0.03	0.898	0.388
8:2	1	0.25	0.25	0.06	0.799	0.781
7:3	0.875	0.375	0.22	0.09	0.703	1.175
6:4	0.75	0.5	0.09	0.06	0.300	0.781
5:5	0.625	0.625	0.08	0.08	0.249	0.975
4:6	0.5	0.75	0.06	0.09	0.192	1.125
3:7	0.375	0.875	0.05	0.11	0.160	1.375
2:8	0.25	1	0.02	0.06	0.064	0.750
1:9	0.125	1.125	0.01	0.07	0.032	0.875
0:10	0	1.25	0.00	0.08	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

5. *Enterococcus faecalis*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	8.00	0.00	1.000	0.000
9:1	14.4	1.6	7.20	0.80	0.900	0.050
8:2	12.8	3.2	6.40	1.60	0.800	0.100
7:3	11.2	4.8	5.60	2.40	0.700	0.150
6:4	9.6	6.4	4.80	3.20	0.600	0.200
5:5	8	8	4.00	4.00	0.500	0.250
4:6	6.4	9.6	2.40	3.60	0.300	0.225
3:7	4.8	11.2	4.80	11.20	0.600	0.700
2:8	3.2	12.8	3.20	12.80	0.400	0.800
1:9	1.6	14.4	1.60	14.40	0.200	0.900
0:10	0	16	0.00	16.00	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

6. Proteus vulgaris

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	8.00	0.00	1.000	0.000
9:1	14.4	1.6	0.56	0.06	0.070	0.004
8:2	12.8	3.2	0.50	0.13	0.063	0.008
7:3	11.2	4.8	0.44	0.19	0.055	0.012
6:4	9.6	6.4	0.38	0.25	0.047	0.016
5:5	8	8	4.80	3.20	0.600	0.200
4:6	6.4	9.6	0.25	0.38	0.031	0.023
3:7	4.8	11.2	0.19	0.44	0.023	0.027
2:8	3.2	12.8	0.13	0.50	0.016	0.031
1:9	1.6	14.4	0.06	0.56	0.008	0.035
0:10	0	16	0.00	16.00	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

7. Salmonella typhimurium

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	1.25	0	3.00	0.00	1.000	0.000
9:1	1.125	0.125	0.56	0.06	0.187	0.031
8:2	1	0.25	0.50	0.13	0.167	0.063
7:3	0.875	0.375	0.44	0.19	0.146	0.094
6:4	0.75	0.5	0.75	0.50	0.250	0.250
5:5	0.625	0.625	0.31	0.31	0.104	0.157
4:6	0.5	0.75	0.50	0.75	0.167	0.375
3:7	0.375	0.875	0.19	0.44	0.062	0.219
2:8	0.25	1	0.13	0.50	0.042	0.250
1:9	0.125	1.125	0.06	0.63	0.021	0.313
0:10	0	1.25	0.00	2.00	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

8. Shigella flexneri

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	8	0	2.00	0.00	1.000	0.000
9:1	7.2	0.8	1.80	0.20	0.900	0.769
8:2	6.4	1.6	1.60	0.40	0.800	1.538
7:3	5.6	2.4	2.10	0.90	1.050	3.462
6:4	4.8	3.2	2.40	1.60	1.200	6.154
5:5	4	4	0.02	0.02	0.010	0.077
4:6	3.2	4.8	1.60	2.40	0.800	9.231
3:7	2.4	5.6	1.20	2.80	0.600	0.778
2:8	1.6	6.4	0.80	3.20	0.400	0.889
1:9	0.8	7.2	0.40	3.60	0.200	1.000
0:10	0	8	0.00	0.26	0.000	1.000

K. mosambicina (Km) (X axis) vs. *A. glabratum* (Ag) (Y axis)

9. Escherichia coli

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	8	0	0.50	0.00	1.000	0.000
9:1	7.2	0.8	0.45	0.05	0.900	0.100
8:2	6.4	1.6	0.40	0.10	0.800	0.200
7:3	5.6	2.4	1.05	0.45	2.100	0.900
6:4	4.8	3.2	0.90	0.60	1.800	1.200
5:5	4	4	1.50	1.50	3.000	3.000
4:6	3.2	4.8	0.80	1.20	1.600	2.400
3:7	2.4	5.6	0.60	1.40	1.200	2.800
2:8	1.6	6.4	0.30	2.40	0.600	4.800
1:9	0.8	7.2	0.40	3.60	0.800	7.200
0:10	0	8	0.00	0.50	0.000	1.000

P. guajava (Pg) (X axis) vs. *A. glabratum* (Ag) (Y axis)

10. Bacillus cereus

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	0.25	0.00	1.000	0.000
9:1	14.4	1.6	0.56	0.63	0.563	0.833
8:2	12.8	3.2	0.80	0.20	0.800	0.267
7:3	11.2	4.8	0.35	0.15	0.350	0.200
6:4	9.6	6.4	0.30	0.20	0.300	0.267
5:5	8	8	1.00	1.00	1.000	1.333
4:6	6.4	9.6	0.20	0.30	0.200	0.400
3:7	4.8	11.2	0.15	0.35	0.150	0.467
2:8	3.2	12.8	0.10	0.40	0.100	0.533
1:9	1.6	14.4	0.05	0.20	0.050	0.267
0:10	0	16	0.00	0.18	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *P. guajava* (Pg) (Y axis)

11. Escherichia coli

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	4.00	0.00	1.000	0.000
9:1	14.4	1.6	3.60	0.40	0.900	0.800
8:2	12.8	3.2	1.60	0.40	0.400	0.800
7:3	11.2	4.8	0.70	0.30	0.175	0.600
6:4	9.6	6.4	0.60	0.40	0.150	0.800
5:5	8	8	0.50	0.50	0.125	1.000
4:6	6.4	9.6	0.40	0.60	0.100	1.200
3:7	4.8	11.2	0.30	0.70	0.075	1.400
2:8	3.2	12.8	0.20	0.80	0.050	1.600
1:9	1.6	14.4	0.05	0.45	0.013	0.900
0:10	0	16	0.00	0.50	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *P. guajava* (Pg) (Y axis)

12. *Proteus vulgaris*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	1.00	0.00	1.000	0.000
9:1	14.4	1.6	0.90	0.10	0.900	0.050
8:2	12.8	3.2	1.20	0.30	1.200	0.150
7:3	11.2	4.8	1.05	0.45	1.050	0.225
6:4	9.6	6.4	0.90	0.60	0.900	0.300
5:5	8	8	0.13	0.13	0.130	0.065
4:6	6.4	9.6	0.30	0.45	0.300	0.225
3:7	4.8	11.2	0.23	0.53	0.230	0.265
2:8	3.2	12.8	0.15	0.60	0.150	0.300
1:9	1.6	14.4	0.08	0.68	0.080	0.340
0:10	0	16	0.00	2.00	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *P. guajava* (Pg) (Y axis)

13. Salmonella typhimurium

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	4.00	0.00	1.000	0.000
9:1	14.4	1.6	2.60	0.30	0.650	0.770
8:2	12.8	3.2	1.60	0.40	0.400	0.560
7:3	11.2	4.8	1.40	0.60	0.350	0.590
6:4	9.6	6.4	1.20	0.80	0.300	0.620
5:5	8	8	1.00	1.00	0.250	0.650
4:6	6.4	9.6	0.80	1.20	0.200	0.680
3:7	4.8	11.2	0.60	1.40	0.150	0.710
2:8	3.2	12.8	0.20	0.80	0.050	0.370
1:9	1.6	14.4	0.10	0.90	0.0250	0.385
0:10	0	16	0.00	2.50	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *P. guajava* (Pg) (Y axis)

14. *Shigella flexineri*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	1.50	0.00	1.000	0.000
9:1	14.4	1.6	1.35	0.15	0.900	0.300
8:2	12.8	3.2	1.20	0.30	0.800	0.600
7:3	11.2	4.8	0.44	0.19	0.293	0.380
6:4	9.6	6.4	0.90	0.60	0.600	1.200
5:5	8	8	0.31	0.31	0.207	0.620
4:6	6.4	9.6	0.20	0.30	0.133	0.600
3:7	4.8	11.2	0.23	0.53	0.153	1.060
2:8	3.2	12.8	0.10	0.40	0.067	0.800
1:9	1.6	14.4	0.05	0.45	0.033	0.900
0:10	0	16	0.00	0.50	0.000	1.000

B. transvaalensis (Bt) (X axis) vs. *P. guajava* (Pg) (Y axis)

Aqueous Extracts

1. *Proteus vulgaris*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	8	0	8.00	0.00	1.000	0.000
9:1	7.2	0.8	7.20	0.80	0.900	0.200
8:2	6.4	1.6	3.20	0.80	0.400	0.200
7:3	5.6	2.4	5.60	2.40	0.700	0.600
6:4	4.8	3.2	1.80	1.20	0.225	0.300
5:5	4	4	2.00	2.00	0.250	0.500
4:6	3.2	4.8	1.60	2.40	0.200	0.600
3:7	2.4	5.6	1.20	2.80	0.150	0.700
2:8	1.6	6.4	0.80	3.20	0.100	0.800
1:9	0.8	7.2	0.40	3.60	0.050	0.900
0:10	0	8	0.00	4.00	0.000	1.000

P. guajava (Pg) (X axis) vs. *B. transvaalensis* (Bt) (Y axis)

2. *Enterococcus faecalis*

PLATE	CONCENTRATIONS		MIC VALUES		RATIO VALUES	
	X	Y	X	Y	X	Y
10:0	16	0	2.00	0.00	1.000	0.000
9:1	14.4	1.6	1.80	0.20	0.900	0.025
8:2	12.8	3.2	3.20	0.80	1.600	0.100
7:3	11.2	4.8	2.80	1.20	1.400	0.150
6:4	9.6	6.4	2.40	1.60	1.200	0.200
5:5	8	8	3.00	3.00	1.500	0.375
4:6	6.4	9.6	3.20	4.80	1.600	0.600
3:7	4.8	11.2	2.40	5.60	1.200	0.700
2:8	3.2	12.8	1.60	6.40	0.800	0.800
1:9	1.6	14.4	0.80	7.20	0.400	0.900
0:10	0	16	0.00	8.00	0.000	1.000

P. guajava (Pg) (X axis) vs. *B. transvaalensis* (Bt) (Y axis)

Appendix 3

List of equations

Equation 1:

$$FIC_a = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

Equation 2:

$$FIC_b = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}}$$

Equation 3:

$$FIC_{index} = FIC^{(a)} + FIC^{(b)}$$

Appendix 4

Previous reports on the toxicity of the plants used to treat diarrhoea in rural Maputaland.

Plant species	Previous reports on toxicity	References
Acacia burkei	None found	
Acanthospermum glabratum	None found	
Brachylaena transvaalensis	None found	
Catharanthus roseus	Catharine an indole alkaloid of this herb leads to hypoglycaemia and neurotoxicity.	Steenkamp, 2003
	Causes DNA damage and chromosomal aberrations (mutagenic).	Fennell *et al*., 2004
Chenopodium ambroisioides	Essential oils administered orally to mice led to toxicity.	Monzote *et al*., 2007
	50-500 mg/kg administration led to small alterations but was not lethal.	Pereira *et al*., 2011
	α Ascaridole isolated from this herb and fed to mice, showed toxicity by blocking the ETC in the mitochondria.	Monzote *et al*., 2009
Cissampelos hirta	None found	
Garcinia livingstonei	None found	
Gymnosporia senegalensis	None found	
Krauseola mosambicina	None found	
Lippia javanica	The leaf aqueous extracts have been tested for toxicity in BALB/c mice. It was concluded that it might be detrimental to human health if taken at very high doses.	Madzimure *et al*., 2011
	It has mutagenic effects.	Elgorashi *et al*., 2003
Mangifera indica	Mango stems and bark extracts did not show embryo toxicity or organotoxicity when fed to Sprague Dawley rats.	González *et al*., 2007
	Extracts showed light toxicity but did not induce mutagenic or genotoxic effects when fed on mice.	Rodeiro *et al*., 2006
Melia azedarach	Ingestion of whole fruit may be toxic as it contains tetranorterpenes, meliatoxin A1, A2, B1 and B2.	Oelrichs *et al*.,1983
	Fruits (chinaberries) were found to cause poisoning in dogs.	Ferreiro *et al*.,2010
	Melia azedarach poisoning in humans may lead to gastrointestinal, cardiovascular, respiratory or neurological effects and death in severe cases.	Phua *et al*., 2008

Plant species	Previous reports on toxicity	References
Psidium guajava	Acute toxicology tests in rats have found *P. guajava* leaf extracts to be non-toxic, while *in vitro* genotoxicity and mutagenicity tests on human peripheral blood lymphocytes found no disturbances in cell division.	Gutiérrez *et al*., 2008
Sarcostemma viminale	Pregnane glycosides is said to cause hypersensitivity, Inco- ordination, convulsions and paralysis in humans animals.	Botha and Penrith, 2008
Senna occidentalis	Four percent of seeds concentration fed to pregnant goats may be toxic to kids.	Barbosa-Ferreira *et al*., 2011
	It was found to be toxic in rats fed with the seeds.	Barbosa-Ferreira *et al*., 2005
Schotia brachypetala	Tested positive on the brine shrimp toxicity assay.	McGaw *et al*., 2007
Sclerocarya birrea	It was negative on the Ames and VITOTOX toxicity tests.	Elgorashi *et al*., 2003
	It was also negative on the brine shrimp toxicity assay	McGaw *et al*., 2007
	Genotoxicity was exhibited on the micronucleus test.	Fennell *et al*., 2004
	Sclerocarya birrea stem bark aqueous and methanolic extracts were tested on mice, and LD50 values suggested these extracts to be relatively safe. In a brine shrimp test, no toxic effects were evident for hexane, methanol and aqueous extracts.	Ojewole *et al*., 2010
	Dichloromethane:methanol (1:1) extracts showed *in vitro* toxicity with tests done in Chang liver, 3T3-L1 adipose and C2C12 muscle cells.	Van de Venter *et al*., 2008
Strychnos madagascariensis	None found	
Syzygium cordatum	It has antigenotoxic effects. A review revealed that it lowers mutagenic effects of mytomycin C.	Fennell *et al*., 2004
	Tested negative on the Ames and VITOTOX toxicity tests.	Elgorashi *et al*., 2003
Terminalia sericea	Cytotoxicity was observed on the velvet monkey kidney cells with ID value of 24 µg/ml.	Tshikalange *et al*., 2005
	Showed no mutagenic activity when tested.	Eldeen *et al*., 2006
	In the brine shrimp assay *T. sericea* acetone extracts were relatively non-toxic.	Eloff *et al*., 2008

Plant species	Previous reports on toxicity	References
Trichilia emetica	Tested negative on the Ames and VITOTOX toxicity tests.	Elgorashi *et al*., 2003
	The toxicity of leaf and stem bark extracts were studied on lymphocytes using the lymphoproliferation assay and results showed that the methanolic leaf extracts are very toxic. The dichloromethane extract of the stem bark was found to be highly toxic to monkey kidney cells. Leaf aqueous and diethyl ether extracts did not show cytotoxicity in the brine shrimp assay	Komane *et al*., 2011
	Micronucleus test also revealed that it was toxic.	Taylor *et al*., 2003
Vanngueria infausta	None found	
Vernonia natalensis	None found	

Appendix 5

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Ethnopharmacological communication

Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa

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ABSTRACT

Aim of the study: To document the ethnobotanical knowledge on antidiarrhoeal plant use by lay people in a rural community in northern Maputaland.
Materials and methods: Interviews were conducted amongst homestead inhabitants, using structured questionnaires. The focus was on the medicinal plants which are growing in and around the immediate vicinity of the homesteads.
Results: Twenty-three plant species are used in the study area to treat diarrhoea. Four plants (*Acacia burkei*, *Brachylaena transvaalensis*, *Cissampelos hirta* and *Sarcostemma viminale*) are recorded for the first time globally as an antidiarrhoel. The three antidiarrhoeal plants most frequently used in the study area are *Psidium guajava*, *Catharanthus roseus* and *Melia azedarach* (all three are exotic to South Africa), followed by *Sclerocarya birrea* and *Strychnos madagascariensis* which are indigenous. Seven of the 23 plant species are used in five different plant combinations for increased antidiarrhoeal efficacy.
Conclusion: The wide variety of plants that are used to treat diarrhoea in this area supports the traditional value that medicinal plants have in the primary health care system of the rural people in northern Maputaland, KwaZulu-Natal.

1. Introduction

World-wide, nearly nine million children (under the age of five years) die every year as a result of diarrhoea (WHO, 2009). An estimated 88% of diarrhoeal-related deaths are caused by inadequate sanitation and poor hygiene. Chronic diarrhoea is also related to HIV/AIDS conditions, as immunocompromised infected people are susceptible to opportunistic infections. South Africa is one of the highest HIV infection rates in the world, with KwaZulu-Natal being the worst affected province (Thurlow et al., 2009). Furthermore, northern Maputaland is one of the most poverty stricken areas in South Africa, where the availability of clean drinking water, lack of education and sanitary ablutions are particularly problematic (Municipal Demarcation Board South Africa, 2001). Under these conditions diarrhoea is a major concern to resident rural communities.

A study on traditional remedies around the Eastern Cape region of South Africa demonstrated that diarrhoea was one of the most prominent diseases treated with traditional medicines (Dambisya and Tindimwebwa, 2003). It is known that antidiarrhoeal plant extracts have antispasmodic properties, delay gastrointestinal transit, suppress gut motility, stimulate water adsorption and/or reduce electrolyte secretion (Palombo, 2006). Studies done on the ethnobotanical use of antidiarrhoeal plants in South Africa have mostly focused on information obtained from traditional healers and that found in the literature (Lin et al., 2002; Mathabe et al., 2006; Appidi et al., 2008; Fawole et al., 2009). Presently, only two studies have focused on rural dwellers in South Africa. One such study was conducted in the Eastern Cape Province (Appidi et al., 2008) and a case study was restricted to the Mbazwana area (northern Maputaland) where ten homesteads were approached (De Wet et al., 2008). The latter study revealed 12 plants species that are used to treat diarrhoea. Both studies emphasised that medicinal plants play an important role in the primary health care of rural people, that there was a need to continue with these types of studies and that there is still a wealth of undiscovered ethnobotanical information to be documented in this remote area of KwaZulu-Natal (De Wet et al., 2008).

As many of the rural people in the study area are illiterate or semi-illiterate, the knowledge on plant use is often restricted to verbal communications only. A study by Zobolo and Mkabela (2006) in northern KwaZulu-Natal has found that the younger generation regards indigenous knowledge as primitive. While having the writing skills, the youth have shown little interest in the documentation

Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; WHO, World Health Organization.

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