Antimicrobial activity testing of traditionally used plants for treating wounds and sores at Ongoye area KwaZulu-Natal, South Africa.

By

Ntombeziningi Shirley Mthethwa

200021480

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In the Department of Biochemistry and Microbiology

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Supervisor: Dr H de Wet

Co-Promoter: Dr AK Basson

Mrs NR Ntuli

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Abbreviations

2n	diploid
ATCC	American Type Culture Collection
CFU	Colony Forming Units
DMSO	Dimethylsulfoxide
EC	*Escherichia coli*
INT	Iodonitro-tetrazolium
KP	*Klebsiella pneumonia*
MDR	Multi drug resistant
MH	Meuller-Hinton
MIC	Minimum inhibitory concentration
SA	*Staphylococcus aureus*
TLC	Thin Layer Chromatography

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Dedication

My sincere thanks and gratitude are to the following people without their motivation and support I was going to fail to walk that extra mile.

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Abstract

This study focused on the investigation of plants used for the treatment of wounds and sores by local people living around the Ongoye forest, KwaZulu-Natal. An ethnobotanical survey was conducted in eighty homesteads in this area. The ethnobotanical data revealed that 33 plant species were used in treating sores and wounds, but only 15 plant species were collected from the wild and homesteads and three plant species were bought from a muthi market. According to the ethnobotanical information *Hypericum aethiopicum* (unsukumbili) was the most used plant for treating sores and wounds in this area. The survey showed that women (62.5%) possessed more knowledge than the men (37.5%) who were interviewed at the homesteads regarding the medicinal uses of plants. Acetone, methanol, cold and hot water extracts from the different plant parts (bark, leaves, stems and the whole plant) were done on 18 species. These plants species are: *Acanthospermum australe*, *Acorus calamus*, *Albizia adianthifolia*, *Baccharoides adoensis*, *Clerodendrum hirsutum*, *Combretum erythrophyllum*, *Faurea saligna*, *Gerbera ambigua*, *Gunnera perpensa*, *Hypericum aethiopicum*, *Hypoxis hemerocallidea*, *Lippia javanica*, *Pentanisia prunelloides*, *Sclerocarya birrea*, *Solanum aculeastrum*, *Trichilia dregeana*, *Warburgia salutaris*, *Ziziphus mucronata*. The above-mentioned plants were screened for antibacterial activity against the following bacteria strains: *Bacillus subtilis* (6051), *Escherichia coli* (7751, U1405s, U16406, U16403), *Klebsiella pneumoniae* (13883), *Staphylococcus aureus* (12600, P5020, P4790, T1266), '*Salmonella* spp., *Shigella flexneri* and *Shigella sonnei*'. The antibacterial activities were determined by disk-diffusion, agar-well diffusion, minimum inhibitory concentrations (MIC) and bio-autographic methods. The plant extracts were also screened for the following phytochemicals: alkaloids, flavonoids, saponins, anthraquinones, cardiac glycosides and tannins. The following plants were the most effective against the micro-organisms tested: *Gunnera perpensa*, *Hypericum aethiopicum*, *Hypoxis hemerocallidea*, *Lippia javanica*, *Pentanisia prunelloides*, *Trichilia dregeana* and *Warburgia salutaris*. The bio-autographic results showed several compounds separated on the TLC with activity against the test organism, *Staphylococcus aureus* (ATCC2600). This study thus lends some support to traditional knowledge and may serve as a basis for selecting the most active medicinal plants to use in traditional medicine practices in the future.

CHAPTER 1

INTRODUCTION

Plants have generally been used by human beings in the treatment of common, infectious diseases. Some of these traditional medicines are still used in regular treatments for various diseases (Rios & Reico, 2005). For example, the use of bearberry and cranberry juice to treat urinary infections is reported in the literature on phytotherapy, while plant species such as lemon balm, garlic and the tea tree are described as broad-spectrum antimicrobial agents (Rios & Reico, 2005).

Infectious diseases are aggravated by factors such as inadequate sanitation, poor hygiene and overcrowded living conditions (Kerr & Lacey, 1995). The control of infectious diseases has come as a result of a comprehensive understanding of disease processes, improved sanitary practices, and the discovery and use of antimicrobial agents. Human dependence on plants as a source of medicine is common in developing countries where traditional medicine plays a major role in healthcare (Farnsworth, 1994; Srivastava *et al*., 1996).

Medicinal plants have been a source of medicine in virtually all cultures (Banquar, 1995). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. Traditional medicine has continued to be used not only in the primary healthcare of poor people in developing countries, but also in countries where conventional medicine is predominant in the national healthcare system (Lafranco, 1999). According to the World Health Organisation (WHO, 2001), herbal medicines serve the health needs of about 80% of the world's population, especially the millions of people in the vast rural areas of developing countries. The Traditional Medicine Program of the WHO defines traditional medicine as the sum total of all the knowledge and practices used in the diagnosis, prevention and elimination of physical, mental or social imbalances (Rukangira, 2001).

According to Van Wyk *et al.* (1997) many people on earth still rely on medicinal plants and other material for everyday healthcare needs. Rural populations are more involved in traditional ways of treatment because of their beliefs, and the easy availability and low cost of traditional medicines (Banquar, 1993). It is more likely that

the profound knowledge of herbal remedies in traditional cultures, developed over many years, were carefully passed on verbally from generation to generation. The passage of the knowledge of traditional medicines from one generation to another relies mostly on practical experience and observation. The variation between communities gives traditional medicine its diverse plurality. In the history of traditional systems, every region had its own traditional medicines. In different parts of the world, different tribes have their own traditional medicines which vary from one another. For instance, Chinese, Arabic and African medicines are all rooted in their cultural contexts (Van Wyk *et al.*, 1997).

The attention given by governments to the healthcare application of traditional medicines has given a new direction to research, investment and the design of programmes in this field in several developing countries (Rukangira, 2001). Of all medicine systems, African traditional medicine is the oldest and the most diverse (Van Wyk *et al*., 1997). Remedies made from plants and traditional healers play an important role in the health of millions of African people. The increase in Africa's population increases the demand for traditional medicines since most Africans rely on them. Reflected in the marked regional differences, is the biological and cultural diversity of Africa, which constitutes the cradle of mankind in healing practices (Van Wyk *et al*., 1997). Medicinal drugs derived from plants are of strategic and economic value for the African continent. Southern Africa contains about 10% of the world's diverse plants, but relatively little chemical work has been done on medicinal plants from this region (Eloff, 1997).

Although people now live in a world where many pathogenic microorganisms are able to be controlled, the emergence of multiple drug resistant strains is becoming a reason for serious concern (Madigan *et al.,* 2003*).* In South Africa, many people still use plants as alternative medicines, or as a supplement to visiting a western healthcare practitioner (Van Wyk *et al*., 1997). Besides, South Africa is home to over 30,000 species of higher plants, 3000 of which species have been found to be used in traditional medicine across the country (Van Wyk *et al*., 1997).

There are over 27 million users of indigenous medicine (Mander, 1998) and an estimated 200,000 indigenous traditional healers, whom up to 60% of the population in South Africa consult (Van Wyk *et al.,* 1997). The current nature conservation

legislation in South Africa, which was implemented in April 2008, allows researchers to have an unlimited access to biological resources, provided the researcher is in possession of a permit and is following the terms and conditions of the permit. This allows the utilization of biological resources to be under control to avoid their overexploitation. For example, an integrated export and bioprospecting permit may only be issued if the minister responsible is satisfied that the export of the indigenous biological resources for bioprospecting will be for a purpose that is in the public interest, including the conservation of biodiversity in South Africa, the economic development of South Africa, or enhancing the scientific knowledge and technical capacity of South African people and institutions (Van Schalkwyk, 2008).

Knowledge of these plants is very important because not only does it have the potential to lead to the discovery of new alternatives for the treatments of illnesses, it also has potential from a conservation point of view. A national council consisting of 150 smaller associations was formed by traditional healers in 1986. This has led to numerous attempts at forming umbrella bodies of traditional healers. These bodies have experienced successes in terms of representativeness and sustainability over the years. Through co-operation, collaboration and incorporation, the current government has shown an intention to build bridges between modern and traditional medical systems. This is why there are a large number of organizations that regulate and register traditional healers in South Africa (LeClerc-Madlala, 2002).

If certain plant species are found to be under threat due to a high demand for plant medicines, measures can then be implemented to try and ensure the sustainability of such plant species. This sustainability is also important from a cultural point of view because much of the knowledge is being lost due to not being passed on from one generation to the next. Thus, it is important to document this knowledge for future generations, who may one day need the information (Hutchings *et al*., 1996). Indigenous women have extensive knowledge of medicinal plants because of their role as caretakers of children at home (Kothari, 2003). The recognition and restoration of indigenous women's knowledge through institutional support, and these women's efforts to pass the knowledge onto their future generations, would enhance the sustainable use of natural resources (Mikkelsen, 2005). According to Zobolo and Mkhabela (2005), different types of medicinal plants were grown by

women in their homesteads in the rural areas of Eshowe. Even though Western medicinal facilities are accessible, women still depend on indigenous medicinal plants. A sick person is taken to a western doctor only when, at first, an indigenous intervention has failed (Zobolo and Mkhabela, 2005).

Research by Zobolo and Mkhabela (2005) revealed that all the women involved in their study had greater knowledge of the Zulu names of plants and of their uses in traditional healthcare practice, especially in the treatment of coughs, headache, stomachache, toothaches, diarrhoea, wounds, asthma and diabetes. Modern allopathic medicine has its roots in ancient medicine; and it is still likely that many important new remedies will be discovered and commercialized in future by following the leads provided by traditional knowledge and experience (Van Wyk *et al*., 1997).

In South Africa, as in most developing parts of the world, traditional herbal medicine still forms the backbone of rural healthcare. Although the majority of the South African population consults traditional healers for some or all of their healthcare needs, South African government healthcare services provide only western medical care (Light *et al.,* 2005). It is, however, largely due to the cultural importance of traditional medicine that the demand for these herbal remedies remains so high (McGaw *et al.*, 2000). According to Light *et al.* (2005), very little work has been published in the *Journal of Ethnopharmacology* on the wound-healing properties of South African plants over the past years. For instance, only one article was published between 2000 and 2004, which shows that there is a great need to evaluate wound-healing remedies.

1.1 OBJECTIVES

The objectives of this study were:

- To document the ethno-knowledge on the usage of indigenous plants for wound- and sore-healing through the use of questionnaires.
- To identify plants and prepare voucher specimens of the collected plants.
- To prepare plant extracts using different solvents.
- To screen for antibacterial activities of plant extracts against four different American Type Culture Collection (ATCC) strains and multi-drug-resistant strains.
- To determine the Minimum Inhibitory Concentration (MIC) of the active plant extracts.
- To compare the availability and activity of the plants sold on local markets for treating wounds and sores against the availability and activity of plants used in the homesteads.
- To analyze the plant extracts with bio-autographic assays.
- To investigate the phytochemical properties of these plants.

CHAPTER 2

MATERIALS AND METHODS

2.1 LOCALITIES AND VOUCHER SPECIMENS OF PLANTS COLLECTED

An ethnomedical survey was done through the use of questionnaires (see Appendix I). Approximately 80 homesteads were visited. The areas which were surveyed are in the Uthungulu District Municipality, in KwaZulu-Natal Province, South Africa, namely:
Makholokholo (S28º 45′ 434″, E31º 44′ 351″, Alt 118 m)
Mhlaleni (S28º 47′ 966″, E31º 45 ′ 890″, Alt 98 m)
Ntshidi (S28º 47′ 790″, E31º 43′ 191″, Alt 106 m)
Ekuphumuleni (S28º 47′ 966″ E31º, 45′ 890″, Alt 98 m)
Macekane (S28º 48′ 76″, E31º 46′ 990″, Alt 95 m)
Obisana (S28º 49′ 516″, E31º 48′ 574″, Alt 119m)
Gugushe (S28º 50′ 137″, E31º 45′ 169″, Alt 306 m)
Ophongola (S28º 49′ 630″, E31º 47′ 567″, Alt 240 m)
Sinjigwane (S28º 45′ 995″, E31º 44′ 340″, Alt 88 m).

2.2 ETHNOBOTANICAL DATA

2.2.1 Plant information collected from the survey.
The plants collected from homesteads were identified and stored as voucher specimens at the University of Zululand Herbarium.

Table 2.1 Plants that were collected and tested for antimicrobial activity.

Scientific Name	Zulu name	Parts used	Voucher no.	Interviewee
Acanthospermum australe (Loefl.) O. K Kuntze	Umgwaqeni	Leaves, stem	NSM2	Nkwanyana 2007, pers. comm
Acorus calamus L.	Kalumuzi	Stem	Specimen from muthi market	Zondo 2007, pers. comm
Albizia adianthifolia (Schumach.) W. F. Wight	Usolo	Bark	NSM26	Gumede 2007, pers. comm.
Baccharoides adoensis (Sch. Bip. Ex Walp.) Isawumi*, El-Ghazaly & B. Nord.*	Inyathelo	Leaves, stem	NSM28	Dindi 2007, pers. comm.
Clerodendrum hirsutum (Hchst.) H. Pearson	Umathanjana	Leaves, stem	Specimen from muthi market	
Combretum erythrophyllum (Burch.) Sond.	Umdubu	Stem	NSM22	Zondo 2007, pers. comm.
Faurea saligna Harv.	Isigqalaba	Bark	NSM29	Mhlongo 2007, pers. comm.
Gerbera ambigua (Cass.) Sch. Bip.	Uhlambihloshana	Leaves	NSM9	Mhlongo 2007, pers. comm.
Gunnera perpensa L.	Ugobho	Corm	NSM30	Ngema 2007, pers. comm.
Hypericum aethiopicum Thunb.	Unsukumbili	Leaves	NSM17	Dindi 2007, pers. comm.
Hypoxis hemerocallidea	Ilabatheka	Corm	NSM1	Ngema 2007, pers. comm.

Scientific Name	Zulu name	Parts used	Voucher no.	Interviewee
Lippia javanica (Burm. f.) Spreng	Umsuzwane	Leaves, stem	NSM10	Mhlongo 2007, pers. comm.
Pentanisia prunelloides (Klotzsch ex Eckl. & Zeyh.) Walp	Icishamlilo	Roots	Specimen from muthi market	Dindi 2007, pers. comm.
Sclerocarya birrea (A. Rich.) Hochst	Umganu	Bark	NSM18	Mhlongo 2007, pers. comm.
Solanum aculeastrum Dun	Intuma	Roots	NSM14	Mathenjwa 2007, pers. comm.
Trichilia dregeana Sond.	Umkhuhlu	Leaves	NSM13	Mhlongo 2007, pers. comm.
Warburgia salutaris (Bertol. f.) Chiov	Isibhaha	Leaves	NSM31	Lithuli 2007, pers. comm.
Ziziphus mucronata	Umlahlankosi	Leaves	NSM12	Gcabashe 2007, pers.comm.

Table 2.2 Plants that were not tested for antimicrobial activity.

Scientific names	Zulu names	Parts used	Interviewee
Achyropsis avicularis (E. Mey. Ex Moq.) Cooke & Wright	Ibohlololo	Leaves	Myeza 2007, pers. comm.
Aizoon canariense L.	Umadinsane	Leaves	Gumede 2007, pers. comm.
Bherkheya sp.	Ushwawu	Leaves	Dindi 2007, pers. comm.
Blumea alata (D. Don) DC	Ugodide	Roots	Msimang 2007, pers. comm.
Calilepis laureda DC.	Impila	Roots	Luthuli 2007, pers. comm.

Scientific names	Zulu names	Parts used	Interviewee
Crinum bulbispermus (Burm. f.) Milne-Redh. & Schweick	Umduze	Roots	Myeza 2007, pers. comm.
Croton gratissimus	Uyethi	Roots	Luthuli 2007, pers. comm.
Diospyros galpinii (Hiern) De Winter	Umpishimpishi	Leaves, roots	Myeza 2007, pers. comm.
Erythrina caffra Thunb. var.	Umnsinsi	Leaves	Mhlongo 2007, pers. comm.
Kalanchoe rotundifolia (Schltr.) Schltr.	Dambisa	Leaves	Mhlongo 2007, pers.comm.
Rauvolfia caffra	Umhlambamanzi	Barks	Luthuli 2007, pers. comm.
Ranunculus multifidus Forssk.	Xhaphozi	Leaves, stem	Ncube 2007, pers. comm.
Sansevieria hyacinthoides (L) Druce	Iskholokotho	Leaves	Dindi 2007, pers. comm.
Spilanthes mauritiana (Pers.) DC.	Isisinini	Leaves, stem	
Tulbaghia alliacea L. f.	Ishaladi lezinyoka	Leaves	Gumede 2007, pers. comm.
Vangueria infausta	Umviyo	Leaves, stem	Myeza 2007, pers. comm.

2.2.1 Participants participated in the survey.

Table 2.3 The profile of the participants.

Surname	Gender	Place	Age-range
A. Mhlongo	F	Ncekwane	25-34
E. Mhlongo	M	Ncekwane	25-34
K. Madonsela	F	Ncekwane	35-44
K. Masekane	F	Ncekwane	35-44
Z. Mhlongo	F	Ncekwane	35-44
T. Mkhize	F	Ncekwane	35-44
M. Mthembu	F	Ncekwane	35-44
G. Mhlongo	M	Ncekwane	45-54
B. Buthelezi	F	Ncekwane	45-54
M. Ngubane	F	Ncekwane	45-54

Surname	Gender	Place	Age-range
S. Gumede	M	Ncekwane	45-54
M. Mthembu	M	Ncekwane	45-54
S. Nkosi	F	Ncekwane	45-54
N. Mthiyane	F	Ncekwane	45-54
B. Masekane	M	Ncekwane	55-64
D. Ngema	M	Ncekwane	55-64
Z. Gumede	M	Ncekwane	55-64
V. Gumede	M	Ncekwane	55-64
Z. Dlamini	F	Ncekwane	55-64
J. Masekane	F	Ncekwane	55-64
L. Mhlongo	M	Ncekwane	Over 65
N. Luthuli	F	Obisana	55-64
T. Zungu	F	Ntshidi	15-24
M. Mtshali	M	Ntshidi	35-44
S. Mkhize	F	Ntshidi	35-44
C. Zungu	F	Ntshidi	45-54
W. Zungu	F	Ntshidi	45-54
N. Zungu	F	Ntshidi	55-64
L. Zungu	F	Ntshidi	55-64
L. Xulu	F	Ntshidi	Over 65
Z. Mhlongo	F	Ntshidi	Over 65
B. Zungu	F	Ntshidi	Over 65
M. Mathenjwa	M	Makholokholo	15-24
J. Thungo	F	Makholokholo	25-34
L. Miya	F	Makholokholo	35-44
L. Ndawonde	F	Makholokholo	35-44
H. Thusi	M	Makholokholo	45-54
M. Dlamini	M	Makholokholo	55-64
E. Mathebula	F	Makholokholo	55-64
P. Mhlongo	F	Makholokholo	55-64

Surname	Gender	Place	Age-range
M. Dlamini	M	Makholokholo	Over 65
F. Ngubane	M	Ekuphumuleni	15-24
E. Mhlongo	F	Ekuphumuleni	25-34
Z. Mhlongo	M	Ekuphumuleni	25-34
W. Mhlongo	F	Ekuphumuleni	35-44
T. Gcabashe	M	Ekuphumuleni	35-44
D. Mhlongo	M	Ekuphumuleni	45-54
Q. Mhlongo	F	Ekuphumuleni	55-64
X. Mkhize	F	Mhlaleni	15-24
S. Msimang	F	Mhlaleni	35-44
D. Msimang	M	Mhlaleni	35-44
S. Msimang	M	Mhlaleni	35-44
V. Mhlongo	F	Mhlaleni	45-54
F. Mhlongo	F	Mhlaleni	55-64
R. Mhlongo	M	Mhlaleni	55-64
S. Mhlongo	F	Mhlaleni	Over 65
Z. Mhlongo	F	Macekane	25-34
S. Myeza	M	Macekane	25-34
T. Gumede	M	Macekane	45-54
S. Dlamini	F	Macekane	45-54
T. Khoza	F	Macekane	55-64
S. Dladla	F	Macekane	55-64
S. Ncube	F	Macekane	55-64
T. Nkwanyana	M	Phongolo	15-24
P. Mlambo	F	Phongolo	15-24
S. Nkwanyana	F	Phongolo	15-24
J. Shobede	F	Phongolo	25-34
S. Mgenge	F	Phongolo	35-44
K. Khumalo	M	Phongolo	45-54
M. Nkwanyana	F	Phongolo	45-54

Surname	Gender	Place	Age-range
Z. Nkwanyana	F	Gugushe	25-34
B. Nsele	F	Gugushe	35-44
N. Nkwanyana	F	Gugushe	35-44
T. Mhlongo	F	Gugushe	35-44
M. Ngema	F	Gugushe	45-54
E. Dindi	F	Gugushe	45-54
H. Dindi	M	Gugushe	45-54
G. Mhlongo	F	Gugushe	55-64
T. Gumede	F	Gugushe	55-64
S. Dindi	M	Gugushe	55-64

2.3 BACTERIA USED IN THE STUDY

American Type Culture Collection (ATCC) cultures and strain numbers.

Bacillus subtilis (6051); ***Escherichia coli (7751)***; ***Klebsiella pneumonia (13883)*** ***Staphylococcus aureus (12600)***

Gram-negative, multi-drug-resistant (MDR) cultures from Lancet Laboratories (Durban) with their strain numbers.

Escherichia coli **(U1505s)*; Escherichia coli*** **(U16403)*; Escherichia coli*** **(U16406)**

Table 2.4 Sensitivity table of MDR Gram-negative bacteria against different antibiotics.

Antibiotics	***Escherichia coli***		
	U16403	U15055	U16406
Amikacin	S	S	S
Ampicillin	R	R	S
Augmentin	S	R	S
Cefpodoxime	S	R	S
Cefuroxime	S	R	S
Cephalothin	S	R	S

Antibiotics	***Escherichia coli***		
	U16403	U15055	U16406
Ciproflox	S	R	S
Cotrimoxazole	R	R	S
Fosfomycin	S	S	S
Gentamycin	S	R	S
Levoflox	S	R	S
Loracarbef	S	R	S
Nitrofurantoin	S	S	S

R = resistant, S = sensitive

Gram-positive, multi-drug-resistant (MDR) cultures from Lancet Laboratories (Durban) with their strain numbers.

***Staphylococcus aureus* (P4790)*; Staphylococcus aureus* (P5020)**
***Staphylococcus aureus* (T1266)**

Table 2.5 Sensitivity table of MDR Gram-positive bacteria against different antibiotics.

Antibiotics	***Staphylococcus aureus***		
	P5020	T1266	P4790
Ampicillin	S	NT	NT
Aug	S	NT	NT
Augmentin	S	S	S
Cefoxitin (FOX)	S	S	S
Ceftriox	S	NT	NT
Cefurrox	S	NT	NT
Cephaloth	S	S	S
Cip: GATI	S	S	S
Cip: LEVO	S	S	S
Cip: MOXI	S	S	S

Antibiotics	***Staphylococcus aureus***		
	P5020	T1266	P4790
Clinda	S	NT	NT
Clindamycin	S	R	R
Cotrimoxozole	S	S	R
Erythro	S	NT	NT
Erythromycin	R	R	R
Fucid. Acid	S	S	S
Gentamycin	R	R	R
Levo	S	NT	NT
Linezolid	S	S	S
Oxa	R	NT	NT
Oxa: Clox	S	S	S
Oxa: methi	S	S	S
Pen	S	NT	NT
Penicillin	R	R	R
Rifampicin	S	S	S
Telcoplanin (TEI)	S	S	S
Telithro	S	NT	NT
Tetra	R	NT	NT
Vancomycin	S	S	S
Venco	S	NT	NT

R = resistant, S = sensitive, NT = not tested

2.4 LIST OF CHEMICALS AND REAGENTS

Acetic acid (Ferene)
Acetic anhydride (Ferene)
Ammonium solution (Ferene)
Benzene (Ferene)
Chloroform (Ferene)
Dichloromethane (Adogene)
Dimethylsulfoxide (Adogene)
Dioxane (Adogene)

Dragendorff's reagent
Ethanol (Ferene)
$FeCl_3$ (Ferene)
Gelatine
Glacial acetic acid (Adogene)
HCL (Adogene)
Magnesium turnings
Mayer's reagent
Methanol (Ferene)
Mueller-Hinton (Oxoid)
NaCl (Oxoid)
Neomycin
Nutrient broth (Oxoid)
P-iodotetrazolium (Dow corning)
Sulphuric acid (Ferene)
Toluene (Ferene)

2.5 STANDARD EXPERIMENTAL PROCEDURES

2.5.1 Preparation of plant extracts

Different plant parts were dried at room temperature and ground to a fine powder with a mill (Scientific RSA Hammer Mill 430). Plant extractions were done using hot water, cold water, methanol, and acetone. Five grams of powder material were mixed separately with 50 ml of each solvent. The mixtures were left overnight, on a mechanical shaker at 150 rpm, at room temperature, and were covered with tin foil to avoid quick evaporation. The extracts were filtered through Whatman No. 1 filter paper, using the Bűchner funnel. The yields from all extracts used were weighed, recorded and redissolved in dimethylsulphoxide (DMSO) to a final concentration of 100 mg/ml. The samples were then stored at 4 ºC for further use.

2.5.2 Culture and media preparation

The ATCC microorganisms are the American type culture collections, which are standard cultures. They are the sources of medical research and the world premier biological culture respository. The cultures from Lancet Laboratories were isolated from different patients in different hospitals. The cultures were maintained on nutrient agar plates in a fridge and restreaked on fresh media every 3 weeks in order to keep them alive. Two litres of Mueller Hinton agar, for Gram-negative bacteria; two litres of Mueller Hinton agar plus 4% of sodium chloride (NaCl), for Gram-positive bacteria,

were prepared for testing the sensitivity of microorganisms against plant extracts. The disc diffusion and agar well methods were used. Mueller Hinton broth (200 ml) and 200 ml of Mueller Hinton broth plus 4% of NaCl were prepared for making the spread plates and for MIC purposes. 5 ml of the nutrient broth was poured in each test tube. The test tubes and the bottles of nutrient agar were autoclaved for 15 minutes at 104 kpa. The nutrient agar and the nutrient agar plus 4% of NaCl were aseptically poured into labeled sterile Petri dishes. The nutrient agar in the Petri dishes was allowed to solidify and kept at 4 ºC with nutrient broths until further use.

Bacterial cultures that were going to be used for agar well diffusion, disc diffusion and MIC were prepared by transferring one colony into a flask containing 200 ml sterile Mueller Hinton broth and incubated to a 0.5 McFarland standard (approximately $1x10^6$ CFU/ml). The spectrophotometer was set to 640 nm for measuring the McFarland standard. The plain nutrient broth was used as the blank to calibrate the spectrophotometer to zero. The nutrient broth with the microorganism was measured. The reading on the spectrophotometer had to be between 0.08 nm and 0.1 nm, which is referred to as the McFarland standard (Eloff, 1998).

2.5.3 Antibacterial assays

2.5.3.1 Agar well diffusion assay

The activity of the plant extracts was tested using agar well diffusion assay. Agar plates were prepared using sterile Mueller-Hinton (MH) agar. Spread plates were made to spread the cultures (30 µl) evenly on the surface of the agar, using a sterile glass spreader which was dipped in alcohol and flamed using a Bunsen burner to sterilize it. The culture was allowed to absorb into the agar. Wells were made in each plate with sterile Pasteur pipette tips. 30 µl of each plant extract (100 mg/ml) was added to each well. 30 µl of DMSO was used as a negative control and Neomycin (0.1 mg/ml) was used as a positive control. The agar plates were allowed to diffuse for one hour at room temperature and then incubated at 37 ºC overnight. (Mathabe *et al.*, 2006).

The plates were observed for the presence of inhibition of bacterial growth that was indicated by the clear zone around the wells. The size of the zone of inhibition was measured and the antibacterial activity was expressed in terms of the average diameter of zone inhibition in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Mathabe *et al.*, 2006)

2.5.3.2 Disc diffusion assay

Three methods were carried out for antibacterial testing for comparison of efficacy of each method and learning. The disc-diffusion technique was used for testing for antibacterial activity. Mueller-Hinton broth culture was prepared and the McFarland standard was maintained as described above. Fifty microliter (50 µl) of the respective bacterial strains were spread over the surface of the plate containing 10 ml Mueller Hinton agar in sterile 9 cm petri dishes. Different plant extracts (30 µl) were applied to a sterile filter paper disc (Whatman No.1). The plates were allowed to dry before the discs were placed on the agar. DMSO was used as the control and was prepared in the same manner as the plate samples. Each plate contained 4 paper discs with different plant extracts. A disc with 30 µl neomycin was placed on the agar and used as a positive control. The plates were incubated at 37 ºC overnight, and the zones of inhibition were measured in millimeters (Vlietinck *et al*., 1995).

2.5.3.3 Serial dilution assay for determination of the minimal inhibitory concentration (MIC).

A micro-dilution technique using a sterile 96 well micro-plate, as described by Eloff (1998) was used to obtain MIC values of the crude extracts against the bacteria. The microbial cultures were diluted in fresh Mueller Hinton broth to a 0.5 McFarland standard (approximate inoculum size of $1x10^6$ CFU/mL) and 25 µl of the Mueller Hinton broth was added to all wells. Each plant extract (50 µl), which was prepared in the Dimethyl Sulphoxide (DMSO) at a concentration of 100 mg/ml, was serially diluted from the first rows of the microtitre plate. The 96-well sterile plates were prepared by dispensing 25 µl of inoculated broth, 50 µl of the plant extract and 25 µl of the nutrient broth. Similar serial dilutions were performed for neomycin (0.1 mg/ml), a positive control, and DMSO, a negative control, instead of the plant

extract. The starting concentration in the first well after the dilution was 50 mg/ml. Micro plates were covered with lids and incubated at 37 ºC overnight. 40 µl of the 0.2% solution of *P*-Iodonitrotetrazolium violet (Sigma) (0.2 mg/ml) reagent was used to indicate the presence of uninhibited bacterial growth (a pink/purple colour) or the inhibition (colourless) of bacterial growth in each well. The lowest concentration of the crude plant extract that inhibited bacterial growth was taken as the MIC (Mathabe *et al.*, 2006).

2.6 PHYTOCHEMICAL SCREENING

2.6.1 Alkaloids

Dried plant material (1 g) was extracted with 5 ml ethanol and evaporated to dryness. The residue was heated in a boiling water bath with 5 ml HCl (2M). It was then cooled and filtered. The filtrate was divided into two equal portions, of which one was treated with a few drops of Mayer's reagent and the other portion treated with the same amount of Dragendorff's reagent. The samples were observed for turbidity or precipitation, which is an indication of the presence of alkaloids (Harborne, 1973).

2.6.2 Flavonoids

Dried plant material (1 g) was extracted with 5 ml ethanol and evaporated to dryness. The residue was treated with a few drops of concentrated HCl and 0.5 g magnesium turnings. The presence of flavonoids was indicated by the development of a pink or magenta-red colour within three minutes (Harborne, 1973).

2.6.3 Saponins

Plant material (2.5 g) was extracted with boiling water (5 ml) and then allowed to cool. The extract was then shaken vigorously to froth and allowed to stand for 20 minutes. The presence of saponins is determined by the presence of froth (Brain & Turner 1975).

2.6.4 Anthraquinones

Dried plant material (1 g) was extracted with 5 ml ethanol and evaporated to dryness. The residue was redissolved in 10 ml of benzene. The extracts were then shaken and filtered through Whatman's filter paper. An ammonia solution of 5 ml was added to the filtrate and the mixture was allowed to settle. The presence of pink, red or violet color in the ammonia solution (lower phase) was the indication of the presence of anthraquinones (Odebiyi & Sofowora, 1978).

2.6.5 Cardiac glycosides

Two portions of dried plant material (1 g) were extracted with 5 ml ethanol and evaporated to dryness. Acetic anhydride (2 ml), 2 ml of chloroform and 2 ml of glacial acetic acid containing one drop of 10% $FeCl_3$ was added to plant extracts one and two respectively. Extract one was mixed and cooled in ice and 1 ml of sulphuric acid was added carefully down the sides. A color change from violet to blue to green is indicative of cardiac glycosides. Sulphuric acid (1 ml) was added to extract two. A brown ring at the interface, or a violet ring below the interface or a greenish ring above the interface, which gradually spreads throughout the layer, is indicative of the presence of cardiac glycosides (Sofowora, 1993).

2.6.6 Tanins

Dried plant material (1 g) was extracted with 5 ml ethanol and evaporated to dryness. The residue was extracted with 10 ml of a hot 0.9% NaCl solution, filtered and divided into 3 equal portions. The NaCl solution was added to the first extract, 1% gelatine to the second exract and a gelatine-salt reagent to the third extract. Precipitation with the gelatine-salt solution or gelatine was indicative of the presence of tannins. Positive tests were confirmed with the addition of $FeCl_3$ to the extract. This should result in a characteristic blue, blue-black, green or blue-green colour and precipitate, depending on the concentration of tannins in the extract (Mojab *et al*., 2003).

2.7 BIO- AUTOGRAPHIC ASSAY METHOD

The extracts were prepared by dissolving 1 g of the plant powder in 10 ml of dichloromethane. The mixture was covered with foil, shaken overnight with a mechanical shaker and filtered. The filtrate was used for the bio-autographic assay. Thin-layer bio-autographic assay was carried out by placing 5 µl of the extract on the silica gel thin-layer chromatography Merck-manufactured TLC plates (Alugram R Sil G/UV_{254}, 0.2 mm). The plates were developed with acid mixture (72% toluene, 20% dioxane, 8% acetic acid). Two TLC plates were prepared for each extract under identical conditions: one to be incubated with the test microorganism *Staphylococcus aureus* (ATCC 2600) and Mueller-Hinton agar and a reference TLC plate without a test microorganism and agar. Both were kept at room temperature. The developed TLC plate was dried and sterilized for one hour under ultraviolet (UV) light. It was then placed on to the agar. The Mueller Hinton agar (15 ml) containing *Staphylococcus aureus*, (approximately 1 $x10^6$ inoculums) was overlaid on top of the TLC plates. The plates were allowed to pre-diffuse for one hour at 4 ºC; and, thereafter, the plates were incubated for 24 hours at 37 ºC. The plates were sprayed with iodonitro-tertrazolium (INT) (0.2 mg/ml) and compared to the reference TLC plate (Van Vuuren, 2007).

CHAPTER 3
ETHNOBOTANY

3.1 INTRODUCTION

Ethnobotany is the use of plants and their properties, including medicinal properties, in relation to a culture or a group of people. Its importance lies in the fact that, in addition to contributing to knowledge and conservation of ancestral popular culture, it opens up the possibility of finding new uses for medicinal plants and can serve to discover new medicines derived from plants (Akerreta *et al.*, 2007). According to Veilleux and King (1996), the earliest recorded uses of plants for medicinal purposes were found in Babylon in 1770 BC and in ancient Egypt in 1550 BC. The ancient Egyptians believed medicinal plants to have utility in the afterlife of their pharaohs; these plants have been recorded from the time of the Giza pyramids.

In Philadelphia in 1895, Dr John Harshberger described his field of study in one of his lectures as ethnobotany, which marked the origin of the term. This field of study was described as the study of plants used by primitive and aboriginal people (Robbins *et al.,* 1916).

Robbins (1916) used a more scientific approach which investigated the following questions:

- What are the primitive ideas and conceptions of plant life?
- What are the effects of a given plant environment on the lives, customs, religion, thoughts and everyday practical affairs of the people studied?
- What use do they make of the plants around them for food, medicine, material culture and ceremonial purposes?
- What is the extent of their knowledge of the parts, functions and activities of plants?
- Into which categories are plant names and words that deal with plants grouped in the language of the people studied; and what can be learned concerning the working of the folk mind by the study of these names?

These questions are still relevant for present-day ethnobotanical research (De Wet, 2005).

Indigenous women play a major role in the management of plant biodiversity. They make an effort to preserve this biodiversity by collecting, cultivating and managing different plants in their home gardens. They also exchange plant varieties that have been brought informally from outside their gardens and naturalised in their environments. Women show the importance of culinary traditions, as well as other domestic arts and skills for the preservation of their culture and of their plant biodiversity. There is also an interrelationship between the spiritual belief system, plant management and biodiversity conservation (Howard, 2003). There is a relationship between women's specialized knowledge and skills in relation to plants, in relation to their contribution to subsistence, and in relation to their social position and status within their communities. The gendered nature of knowledge and the importance of women's intergenerational knowledge transmission for the maintenance of biological and cultural diversity are revealed. The erosion of indigenous knowledge, cultures and biodiversity, as well as the drive to counter this erosion is illustrated by women. Outsiders who seek to conserve biodiversity are able to recognize women's knowledge and work to promote indigenous cultures, women's status and welfare, while at the same time, conserving the biodiversity that constitutes their wealth (Howard, 2003).

3.2. PLANTS USED TO TREAT WOUNDS

The following plants, which are used to treat sores and wounds were collected at the Ongoye area.

3.2.1 Scientific name : *Acanthospermum australe* (Loefl.) O. Kuntze

Zulu name : *Umgwaqeni*

Common name : Creeping star bur

Figure 3.1 *Acanthospermum australe* in its flowering stage.
(www.cricket.biol.sc.edu/acmoore)

Botanical description

It is an annual herb with prostrate branches. Fertile achenes are usually 6-9, fused an enlarged inner phyllary into a fruit which is 7 mm, long and covered with numerous hooked spines (Bromilow*,* 2001).

Distribution

In South Africa it is found mostly in KwaZulu-Natal (Bromilow*,* 2001).

Conservation status

It is a problem weed in KwaZulu-Natal (Bromilow*,* 2001).

Medicinal uses

Acanthospermum australe is used traditionally to treat skin diseases in Paraguay (Portillo *et al*., 2001).

Data from ethnobotanical survey

The plant is ground, mixed with cold water and then used as an enema once a day. The dose depends on the condition of the sores of the person (Nkwanyana 2007, pers. comm.).

Chemical content

Acanthospermum australe contains heliantheae, carboxyl group at C-10, geranylgeranoil derivatives, sesquiterpene, diterpene lactones (Bohlmann, 1980).

3.2.2. Scientific name : *Acorus calamus* L.

Zulu name : *Kalumuzi*

Common name : Sweet calomel

Figure 3.2 A flowering *Acorus calamus*. (www.hlasek.com/Acorus_calamus_a207.html)

Botanical description

Sweet flag is a reed-like, rhizome-forming, perennial that can grow up to 2 m high. It inhabits perpetually wet areas like the edges of streams, around ponds and lakes, and in ditches and seeps. The plant has long, creeping roots that spread just below the surface of the soil. This plant very rarely flowers or sets fruit, but when it does the flowers are 3-8 cm long, cylindrical in shape, greenish brown and covered in a multitude of rounded spikes. The fruits are small, berry-like, and contain few seeds. This plant flowers from early to late summer (Stafford, 1992).

Distribution

Acorus calamus has been cultivated since earley colonial times and has become naturalized along a stream bank in Noth West province (Van Wyk *et al*., 1997).

Conservation status

No information on its conservation status was found.

Medicinal uses

The *Acorus calamus* is used to treat diarrhoea (Shoba & Thomas, 2001). It is also used as an expectorant, carminative, antispasmodic and nervine sedative (Van Wyk *et al.,* 1997; Padmaja *et al.*, 2002).

Data from ethnobotanical survey

Its stem is ground, mixed with cold water, and the person with sores has to drink a quarter of a cup of the mixture three times a day (Zondo, 2007, pers. comm.).

Chemical content

Essential oils are found in the leaves, rhizomes and roots. Tannins are found in the rhizomes and roots; and ascorbic acid is found in the leaves and rhizomes. Known compounds from the plant include acorin, choline essence containing asarone, eugenol, pinene, cetylic acid, palmitic acid and vitamin B_1 (Hutchings *et al.* 1996). Sesquiterpenes (acorenone) were also detected in this plant (Padmaja *et al.* 2002). Essential oil contains numerous monoterpenoids e.g. Camphene, p-cymene, linalool. Toxicity is ascribed to β-asarone, a phenyl propanoid (Van Wyk *et al*., 1997).

3.2.3. Scientific name : *Albizia adianthifolia* (Schumach.) W. F. Wight

Zulu name : *Usolo*

Common name : Flat crown

Figure 3.3 An adult *Albizia adianthifolia* tree.
(www.plantzafrica.com/plantab/albiziadian.htm)

Botanical description

Albizia adianthifolia has a conspicuous flat crow. The bark is grey to reddish brown and rough. Its branchlets and peduncles have grey to brown hairs, with the young tips tinged pinkish red (Lewis *et al*., 2005).

Figure 3.4 Rectangular leaflets with midribs diagonally across them. (www.plantzafrica.com/plantab/albiziadian.htm)

Its leaves are very characteristic; the 4-8 pairs of pinnae bear 6-12 pairs of leaflets each. Every leaflet is obliquely rhombic-quadrate (rectangular), with the midrib diagonally across it. The petiole has a gland at the base. The flowers are striking, forming relatively large, half–spherical heads. Petals are white or greenish white, and are joined for at least two thirds of their length. Stamens are fused partly to form a tube and are reddish pink or green at the tips. In South Africa the tree flowers in spring during the months of September-November (Lewis *et al*. 2005).

Figure 3.5 The fruits of *Albizia adianthifolia.*
(www.plantzafrica.com/plantab/albiziadian.htm)

The fruit is a thin pod with a conspicuous margin and veins. As the pod dehisces and opens up, the margins often persist as the centre parts falls off. The seeds are flat and brown (Lewis *et al*., 2005).

Distribution

It usually occurs in moist tropical areas such as forests, as well as in areas that are transitional to woodland. Geographically, it is distributed from the northern parts of the Eastern Cape in South Africa, throughout the tropical countries up into Senegal in the west and Ethiopia in the east of Africa. It also occurs on Madagascar (Krige, 2007).

Conservation status

Although this species is not threatened at the moment, over-exploitation and the ring-barking of trees for the medicinally important bark are becoming more and more common (Krige, 2007).

Medicinal uses

Aqueous lotions made from a pounded bark and roots are used for eczema and other itchy skin complaints. Roots pounded in a small amount of water are used to make drops for inflammation of the eyes. The bark is used to make love charm emetics, and enemas are administered to pregnant women to clear their urine. It can

be powdered and taken as a snuff for treating headaches. The bark is, furthermore, used to treat bronchitis in Maputo. The Vhavenda use the leaves and roots for stomachache, toothache, dysentery, haemmorhoids and as purgatives; and roots for improving memory and for inflammation of the eyes (Watt & Breyer-Brandwijk, 1962).

Data from ethnobotanical survey

The bark is boiled and the resulting water is prepared as an enema to be taken for sores on the body (Gumede 2007, pers. comm.).

Chemical content

Its pods contain 3.5% of tannin. Large amounts of histamine (+2 mg/g dry tissue) were found in the bark, roots, trunk and branches. Acetylhistamine and imidazole acetic acid and other minor imidazole compounds were also detected in the bark (Hutchings *et.al.* 1996).

3.2.4 Scientific name : *Baccharoides adoensis* (= *Vernonia adoensis*) Isawumi, El-Ghazaly & B. Nord

Zulu name : *Inyathelo*

Common name : The English name could not be found.

Figure 3.6 A flowering *Baccharoides adoensis.*
(www.sntc.org.sz/flora/photo.asp?phid=z329)

Botanical description

Baccharoides adoensis is a shrub that grows up to 2.3 m high. It is thinly downy throughout. The leaves are about 120 x 30 mm, roughly hairy, grey, hairy beneath. The margins of the leaves are irregularly toothed, tapering to narrowly wing at the base. The flower heads are purple, mauve, and fading white; while the bracts are broad, loose and about 20 mm (Nergard *et al*., 2004).

Distribution

This plant is found in the open grassland of KwaZulu-Natal to Ethopia up to 800 m (Nergard *et al*., 2004).

Conservation status

No information on its conservation status was found.

Medicinal uses

A powder made from the roots of the plant is the first-line treatment for gastro-intestinal ailments, ulcers, gastritis and abdominal pain. The plant was also reported to be used for the treatment of malaria, asthma, headache, men's sexual deficiency, reflux and nausea in pregnancy, schistosomiasis, dysmenorrhea; and, furthermore, as an antihypertensivum, a wound-healing remedy and an aid to ameliorate digestion (Nergard *et al.* 2004).

Data from ethnobotanical survey

The leaves of this plant are mixed with other unknown plant parts, ground and then applied to the wound as much as is needed (Dindi 2007, pers. comm.).

Chemical content

Baccharoides adoensis contains arabinose, rhamnose, galactose, xylose, mannose, glucose, fucose, glucoronic acid and galacturonic (Nergard *et al.* 2004).

3.2.5. Scientific name : *Clerodendrum hirsutum* (Hchst.) H. Pearson

Zulu name : Umathanjana

Common name : Matabele violet

Figure 3.7 The stem of *Clerodendrum hirsutum* from muthi market (empangeni). (N. Mthethwa, 2009)

Botanical description

This herb grows up to a height of 450 mm and is found in grassland. It has a woody rootstock, and a hairy branched angled stem. Its leaves are opposite each other and have hairy reddish margins, while its flowers are bright purplish blue with two white markings (Pooley, 1998).

Distribution

It is found in the coastal region in Durban, Pietermaritzburg, Estcourt, and Newcastle (Pooley, 1998).

Conservation status

No information on its conservation status was found.

Medicinal uses

The plant is used traditionally to treat intestinal worms and scrofula swellings (Pooley, 1998).

Data from ethnobotanical survey

The stem is mixed with cold water and a quarter of a cup of the mixture is drunk three times a day for sores (Zondo 2007, pers. comm.).

Chemical content

No information on its chemical contents was found.

3.2.6 Scientific name : *Combretum erythrophyllum* (Burch.) Sond.
Zulu name : Umdubu
Common name : Bush willow

Figure 3.8 *Combretum erythrophyllum* in flower.
(www.metafro.be/prelude/prelude_pic/combretum_erythrophyllum1.jpg)

Botanical description

This is a medium to large deciduous tree with reddish autumn colors. Its flowers are cream to pale yellow. Its fruits are small, four-winged and a greenish-brown color ripening to yellowish brown and drying to a honey-brown. They remain on the tree for a long time and are reputed to be poisonous, causing hiccups. The bark is a pale brown, smooth but flaking with age to expose grey patches, which gives it a mottled appearance. Knob-like outgrowths commonly occur in older trees, giving them an old, gnarled look. The young leaves are yellowish and shiny, maturing to a fresh mid-green. The trees of the plant are often multi-stemmed and somewhat willow-like in habit (Le Roux, 2003).

Distribution

This species is found in the northeastern part of southern Africa, from Zimbabwe in the north, down to the Eastern Cape in the south, with a thin line following the Orange River westward. This is a riverine species occurring alongside rivers or away from rivers where sufficient groundwater is available. It is found at almost all altitudes and can, therefore, tolerate a fair amount of climatic variation and diverse soils, such as heavy black loam, sandy riverine alluvium and granite sand (Le Roux, 2003).

Conservation status

No information on its conservation status was found.

Medicinal uses

The roots, which are regarded as poisonous, are used as a purgative and to treat venereal diseases (Van Wyk *et al.* 2000).

Data from ethnobotanical survey

The bark is mixed with other herbs (unknown) to make a decoction that is drunk in the morning and evening, quarter of a cup for sores (Buthelezi 2007, pers. comm.).

Chemical content

The leaves yield seven flavonoids, four of these were identified as flavonols and three were identified as flavones. Six of these flavonoids were being reported for the first time (kaempferol, rhamnocotrin, rhamnazin, quiercetin-5, 3' dimethylether, genkwanin and 5- hydroxyl-4', 7- dimethoxyflavone) (Le Roux, 2003).

3.2.7 Scientific name : *Hypoxis hemerocallidea* L.

Zulu name : *Inkomfe*

Common name : African potato

Figure 3.9 *Hypoxis hemerocallidea* in its flowering stage.
(www.plantzafrica.com/planthij/hypoxis.htm)

Botanical description

Being a geophytic herb, *Hypoxis hemerocallidea* overcome winter conditions in the form of an underground rootstock called a corm. These corms are hard, fleshy, mucilaginous and white or yellow-orange within. Sliced corms turn black with oxidation when exposed to the atmosphere. In spring, a new set of leaves grows from the apex of the corm. In most species, the leaves are arranged one above the other in three rows that radiate outwards. In some species, leaf bases are enclosed in a sheath, forming a false stem. The leaves range from linear to broadly lance-shaped, are hairy in most species and die back over the winter months. Flowering stems appear with the leaves after the first rains in spring. They are unbranched, with 2-12 flowers per stalk. The flowers are symmetrical with 6 tepals, rarely 4 or 8 in number, are bright- yellow, giving the genus its common name "yellow stars". The fruit is a capsule that splits across its diameter to expose the small black seeds (Snijman & Singh, 2003).

Figure 3.10 Cross-section through the corm of the *Hypoxis hemerocallidea.*
(www.plantzafrica.com/planthij/hypoxis.htm)

Distribution

Hypoxis hemerocallidea are widely distributed in the grassland areas of the eastern parts of South Africa (Van Wyk *et al.,* 1997).

Conservation status

This species is under threat because of its commercial and cultural value (Snijman & Singh, 2003).

Medicinal uses

The rootstock of *Hypoxis hemerocallidea* is used in various ways in South Africa. It has been used by Zulu traditional healers for centuries in the treatment of urinary infections, heart weakness, internal tumours and nervous disorders. Corms are used as an emetic against fearful dreams. The Sotho people use *Hypoxis hemerocallidea* as a charm against lightning and storms. Corms are used to alleviate many immune related ailments such as the common cold, flu, arthritis, cancer. Preparations of hypoxoside are being used in primary healthcare in South Africa to boost immunity in HIV/AIDS patients (Snijman, 2000).

Data from ethnobotanical survey

The corm is diced, boiled and drunk. The dose depends on a person with sores (Ngema 2007, pers. comm.).

Chemical content

An alkaloid and an organic acid have been isolated from the tubers (Hutchings *et.al.* 1996). The value of this plant is in its content of a sterol called hypoxoside, which once in the human gut, readily converts to rooperol, a biologically active compound that balances the immune system (Snijman *et al*. 2003).

3.2.8 Scientific name : *Faurea saligna* Harv.

Zulu name : *Isigqalaba*

Common name : Transvaal Beech

Figure 3.11 The *Faurea saligna* plant.
(www.plantzafrica.com/efg/faureasal.htm)

Botanical description

Faurea saligna is a small to medium-sized tree. Its trunk is slender, gracefully twisted, and sometimes swollen at the base. A spreading, fairly sparse crown develops with age. It has a smooth, grey-brown bark resembling a drooping gum, at a distance, with alternate bright-green leaves. These leaves turn bright-red in autumn and have wavy margins and a short stalk. Tertiary reticulate venation is visible on the lower surface. It has greenish white flowers, and its fruits are round, green, fleshy nutlets, which are eaten by birds (Pooley, 1993).

Distribution

Faurea saligna has only been mapped 33 times in KwaZulu-Natal. The wide distribution of this species in the Midlands and the Lowlands is not recorded, as it is apparently common in the Tugela River valley, with outliers towards Vryheid. It has not been recorded on distribution maps for the Port Shepstone area either (Coates Palgrave, 2000).

Conservation status

No information on its conservation status was found.

Medicinal uses

The bark is boiled in broth and taken as a tonic, while the roots are boiled and the liquid drunk as a remedy for diarrhoea and indigestion (Hutchings *et al.,* 1996).

Data from ethnobotanical survey

The bark is boiled and the resulting liquid is drunk. The person with sores has to drink a spoon three times a day (Mhlongo 2007, pers. comm.).

Chemical content

Tannins are reported to be found in the bark (Hutchings *et.al.*, 1996).

3.2.9 Scientific name : *Gerbera ambigua* (Cass.) Sch. Bip.
Zulu name : *Uhlambihloshana*
Common name : Botter blom

Figure 3.12 *Gerbera ambigua* in its flowering stage.
(www.plantzafrica.com/plantefg/gerberambig.htm)

Botanical description

Gerbera ambigua is a stemless, perennial herb with a basal rosette of leaves emerging from a silky crown. Its cylindrical roots are thick and fleshy, while its leaves are very variable in shape, size, petiole length and covering hairs. Its flower stalks

emerge from the crown and bear a single flower which is up to 5 cm in diameter. The ray florets are commonly white, with a pinkish underside. Its pappous hair, which gives the daisy centre its color, may be deep-purple or cream. The flowers occur mainly from September to December, but may be found throughout the year (Ambrosius, 2003).

Distribution

Widespread throughout Africa, from the coast to about 1900 m inland, this species is found in grassland, open woodland and damp areas (Ambrosius, 2003).

Conservation status

No information on its conservation status was found.

Medicinal uses

Pounded leaf infusions are used in Zulu traditional medicine for tapeworm and stomachache; whereas root infusions are taken orally for coughs. In Zimbabwe, root infusions are taken for heart pain and abdominal pain in babies (Hutchings *et.al.,* 1996).

Data from ethnobotanical survey

The leaves are ground and mixed with water; and then the person with the sores has to bath twice a day in the mixture (Mhlongo 2007, pers. comm.).

Chemical content

A tricyclic sesquiterpenoid has been isolated (Hutchings *et.al.,* 1996).

3.2.10 Scientific name : *Gunnera perpensa* L.

Zulu name : *uGobho*

Common name : River pumpkin

3.13 The *Gunnera perpensa* plant.
(www.plantzafrica.com/plantefg/gunnerperp.htm)

Botanical description

Gunnera perpensa is a robust, erect, perennial herb which is up to 1 m tall and always grows near water. Its roots are 300 mm thick, creeping in black muddy soil. Its inside tissues are yellow-brown. All the leaves arise from a central tuft near the top of the apex, just above the soil level. They are large, dark bluish-green, kidney-shaped and covered with hairs on both surfaces especially along the veins, in young leaves. The margins of the leaves are irregularly toothed. The veins are very noticeable on the lower surface of the leaf, radiating from the point where the petiole joins the leaf, referred to as palmate radiation. The flowers are numerous, small and not very noticeable, pinkish, reddish brown, and borne on a long slender spike, which is taller than the leaves. There will be female flowers at the base, male flowers at the top and bisexual flowers in the middle of each spike, all flowering between September and February (Ngwenya *et al.,* 2003).

Distribution

Gunnera perpensa is widespread in tropical Africa, from Sudan, Ethopia, Zaire, Rwanda, Uganda, Kenya, Tanzania, Zimbabwe and Mozambique, extending along the central and eastern areas of southern Africa down to the Western Cape, including Swaziland and Lesotho (Bergman *et al.,* 1992; Mendes 1978).

Conservation status

The species is quite common, but has not yet been evaluated for the Red Data List (Von Ahlenfeldt *et al.,* 2003). However, the destruction of its habitat due to mismanagement of the wetlands, and uncontrolled harvesting for the medicinal trade market, are the main threats to the survival of *Gunnera perpensa*.

Medicinal uses

In South Africa, a decoction of the roots of *Gunnera perpensa* is used to induce labour, expel the placenta after birth or to relieve menstrual pains and as an antenatal medication to tone uterus, treat stomach trouble, rheumatic fever, swelling and stomach bleeding. It is applied externally for the dressing of wounds for psoriasis (Ngwenya *et al.* 2003; Van Wyk *et al.,* 1997; Von Ahlenfeldt *et al.* 2003).

Data from ethnobotanical survey

The roots are boiled with water and the resulting mixture is drunk, depending on the condition of the sores of the person (Ngema 2007, pers. comm.).

Chemical content

Little is known of the chemistry of this species. The occurrence of a bitter principle named celastrin has been reported (Benson & Margulis, 2002).

3.2.11 Scientific name : *Hypericum aethiopicum* Thunb.
Zulu name : *uNsukumbili*
Common name : St John's Wort

Figure 3.14 A flowering *Hypericum aethiopicum.*
(www.africanbulbs.com/Hypericum%20aethiopicum26-11-08.jpg)

Botanical description

Hypericum aethiopicum is a perennial shrublet of up to one meter in height, with creeping rhizhomes and erect, flowering branches. The opposite leaves are without hairs but have small transluscent oil glands. The characteristic yellow flowers occur in groups on the branch tips, followed by small dry capsules of about 10 mm long and filled with numerous dark brown shiny seeds (Van Wyk *et al.*, 1997).

Distribution

It grows in Angola (Huila), Lesotho, KwaZulu-Natal, Western Cape and has become a trouble some weed (Bromilow, 2001).

Conservation status

No information on its conservation status was found.

Medicinal uses

Its roots are used in enemas administered for backache or for pains due to kidney or abdominal complaints; while its leaves are cooked and strained, and the resulting liquid is taken to heal sores. The decoction is also used to treat venereal diseases (Hutchings *et al.*, 1996).

Data from ethnobotanical survey

The leaves are heated, ground and mixed with Vaseline. A dose of the mixture is then applied to a wound, depending on that wound (Dindi 2007, pers. comm.).

Chemical content

Although no information is available on the chemistry of *Hypericum aethiopicum* but certain chemicals have been found in *Hypericum perforatum* and other closely related species. The naphthodianthrone hypericin and hypericin like substances occur in several species of *Hypericum.* The flavonoids rutin, hyperin, isoquercetrin and biflavonoids are also present with up to 3% of a known antibacterial substance called hyperforin (Van Wyk *et al.*, 1997).

3.2.12 Scientific name : *Lippia javanica* (Burm. f.) Spreng

Zulu name : *uMsuzwane*

Common name : Fever tea

Figure 3.15 The flowering stage of *Lippia javanica*. (www.plantzafrica.com/plantklm/lippiajavan.htm).

Botanical description

This multi-stemmed, woody shrub stands erect at 1 to 2 m high. Its stems have a square appearance when looked at in cross-section. Its leaves when crushed gave off a strong lemon-like smell and are hairy with noticeable veins. This plant is also said to be one of the most aromatic of South Africa's indigenous shrubs. Small cream flowers can be found on the shrub from summer to autumn in some areas, and all year in other areas. These flowers are arranged in dense, rounded flower heads. The fruits are rather inconspicuous, small and dry (Van Wyk *et al.,* 1997).

Distribution

The plant *Lippia javanica* is widespread throughout large parts of South Africa, with the exception of the Western Cape. It grows from the Eastern Cape northwards, extending into tropical Africa, including Botswana, Swaziland, Mozambique, Malawi, Tanzania, Zambia, Tanzania, and Kenya (Pooley, 1998).

Conservation status

This plant is usually very hardy and can grow under difficult circumstances, therefore, requiring little maintenance (Van Wyk *et al.,* 1997).

Medicinal uses

This plant is well known medicinally to many African tribes, avid herbalists and herb gardeners. The leaves, twigs and occasionally the roots of the plant are used for different reasons. In general, the Xhosa people are known to drink its weaker infusion as a tea substitute and its stronger infusion for the treatment of coughs, colds and bronchial problems. They drink the leaves and stem with milk or water. In addition, the Xhosa people also use *Lippia javanica* to disinfect meat which has been infected with anthrax (Van Wyk & Gericke, 2000; Van Wyk *et al*., 1997; Hutchings *et al*., 1996).

This herb is also said to be effective against fever, especially in cases of malaria, influenza, measles and as a prophylactic against lung infections. In these cases, *Lippia javanica* is often mixed with another herb, *Artemisia afra* (Roberts, 1990). If inhaled, the smoke from *Lippia javanica* has proven to be effective against asthma, chronic coughs and pleurisy. Its leaves and stems can be burned to treat skin disorders such as heat rash, scratches, stings and bites. Here the tea is usually cooled and then applied like a lotion. Even lice and scabies can be treated with it in this manner (Van Wyk *et al.,* 1997).

Data from ethnobotanical survey

The leaves are ground and mixed with cold water. One cup of the mixture is then used as an enema once a day for sores (Mhlongo 2007, pers. comm.).

Chemical content

The icterogenic principles, pentacyclic triterpenoids, also known as lantadene A and lantadene B, have been isolated. Stearic, palmitic, myristic, oleic, arachidic, behenic and lignoceric acids, and tiacontane alkanes are yielded by the plant's leaves. The main amino acid components are alanine, asparagine, arginine and proline; while the main essential oil components are caryophyllene, linalool and p-cymene. Glucose is the only sugar component. Choline is also present (Hutchings *et al*., 1996).

3.2.13 Scientific name : *Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp

Zulu name : *Icishamlilo*

Common name : Wild verbena

Figure 3.16 Flowering stages of *Pentanisia prunelloides*. (www.plantzafrica.com/plantnop/pentanprunel.htm).

Botanical description

This plant is an erect, perennial herb of about 600 mm, with stout, hairy stems sprouting from a woody rootstock. The leaves, which have no petioles (leaf stalks), are very variable, but are usually ovate with wavy margins. The tubular blue or lilac flowers are in dense heads at the end of the stems. Flowering occurs in early summer, from August to January. The plants are long-lived and are dormant in the winter months (Johnson, 2004).

Distribution

It is widespread in grassland throughout southern Africa, from Eastern Cape to Tanzania, at altitudes from sea level up to 1980 m. It grows in well-drained soils, in full sunlight, and tolerates frost (Pooley, 1998).

Conservation status

No information on its conservation status.

Medicinal uses

Its tuberous roots and leaves are used extensively in traditional medicine to treat a wide range of ailments. Root decoctions are taken orally or as enemas, and are also applied externally for burns, swellings, rheumatism, heartburn, vomiting, fever, toothache, tuberculosis, snakebite and haemorrhoids. It is taken by pregnant women

to ensure an easy childbirth and its leaf poultices are applied for a retained placenta. Its Zulu name means: “that which puts out the fire” (Johnson, 2004).

Data from ethnobotanical survey

The plant has to be ground and mixed with cold water, and the person has to bath in the mixture. The plant is mixed with other unknown plant parts and water then administered orally or as an enema for sores. The dose was not specified (Dindi 2007, pers. comm).

Chemical content

Palmitic acid has been detected in this plant (Yff *et al.,* 2002).

3.2.14 Scientific name : *Sclerocarya birrea* (A. Rich.) Hochst

Zulu name : *uMganu*

Common name : Marula tree

Figure 3.17 *Sclerocarya birrea* with its fruits and the mature tree. (www.plantzafrica.com/plantqrs/sclerobirr.htm).

Figure 3.18 The leaves and bark of *Sclerocarya birrea.* (www.plantzafrica.com/plantqrs/sclerobirr.htm).

Botanical description

The marula is a medium-sized to large deciduous tree with an erect trunk and rounded crown. The edible fruits and the multiple uses associated with almost all parts of the marula make it one of southern Africa's most valued trees. It is one of the plants that played a role in feeding people in ancient times. Male and female flowers are borne on separate trees. Flowers are green on the tree but turn yellow after falling (February-June). The compound leaves tend to be mostly crowded at the end of the branches (Mutshiyalo & Tshisevhe, 2005).

Distribution

The marula is widespread in Africa, from Ethiopia in the north to KwaZulu-Natal in the south. In South Africa, it is more dominant in the Baphalaborwa area in Limpopo. It occurs naturally in various types of woodland, on sandy soil or occasionally on sandy loam (Mutshiyalo & Tshisevhe, 2005).

Conservation status

No information on its conservation status was found.

Medicinal uses

The powdered bark is used to treat pregnant women 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 use the male plant. Traditional healers use the hard nut in their divining dice. A decoction of the bark treats dysentery, diarrhoea and rheumatism and has a prophylactic effect against malaria. The bark is an excellent remedy for haemorrhoids. Its roots and bark are also used as laxatives. A drink made from marula leaves is used for the treatment of gonorrhea (Mutshiyalo & Tshisevhe, 2005).

Data from ethnobotanical survey

The bark is used as an enema once a day for sores (Mhlongo 2007, pers. comm.).

Chemical content

The bark of the marula tree yields tannins, tanning matter and traces of alkaloids. The fruit is rich in ascorbic acid, while juice extracts yield sesquiterpene

hydrocarbons. Kernels yield a non-drying oil and contain protein and some iodine. The oil-rich seeds contain oileic acid, myristic, stearic and amino acids with a predominace of glutamic acid and arginine (Mutshiyalo & Tshisevhe 2005).

3.2.15 Scientific name : *Solanum aculeastrum* Dun
Zulu name : *iNtuma*
Common name : Apple of sodom

Figure 3.19 Fruit of *Solanum aculeastrum.* (www.plantzafrica.com/plantqrs/solanacul.htm).

Botanical description

This branched shrub, or small tree can grow up to 1-5 m high. It is usually heavily armed with large, sharp, brown and straight to recurved, broad-based and laterally compressed prickles which are up to 15 mm long. Its young branches are covered with grey or pale-brown hairs, becoming glabrous and dark-brown with age. Its leaves are shortly petiolate, ovate, up to 150 by 130 mm, usually deeply 5-7-lobed, with their upper surface green and glabrescent. The lower surface of the leaves is thickly, whitish, tomentose, and more or less prickly on the midrib on both surfaces. The inflorescence is a few to 10-flowered racemose cyme with only the proximal flowers female-fertile. The corolla is white to pale-violet, rotate-stellate, and about 20 mm in diameter. The fruit is a smooth, globose berry, often with a warty surface, up to 60 mm in diameter, and green but ripening to yellow. Its seeds are 4 by 3 mm in size. Its chromosome number is 2n = 24 (Welman, 2004).

The lobed discolorous leaves, the mostly recurved prickles, and the large yellow fruit make this plant unique among the African species of *Solanum*. It could perhaps be confused with *S. lichtensteini*, but the latter small shrub is unarmed, or has small prickles. It starts flowering from September to July, peaking in November and March, and starts bearing fruit from April to January, peaking in June and November (Welman, 2004).

Distribution

Solanum aculeastrum subsp. *aculeastrum* occurs from tropical Africa down to South Africa. In southern Africa, this taxon is found in Limpopo, Mpumalanga, KwaZulu-Natal, Western and Eastern Cape and also in Swaziland (Welman, 2004).

Conservation status

Acocks (1988) regards this species as an undesirable plant which should be reduced in number by appropriate veld management.

Medicinal uses

The fresh fruit is boiled to treat a wide variety of afflictions including cancer, toothaches and ringworm (Wanyonyi *et al*., 2003).

Data from ethnobotanical survey

The roots of the plant are ground and mixed with other unknown plants and used as an enema once a day for sores (Mathenjwa 2007, pers. comm.).

3.2.16 Scientific name : *Trichilia dregeana* Sond.

Zulu name : *uMkhuhlu*

Common name : Natal mahogany

Figure 3.20 The mature tree of *Trichilia dregeana* and its bark. (www.plantzafrica.com/planttuv/trichildreg.htm).

Botanical description

Trichilia dregeana has beautiful dark foliage and a large, rounded crown. Impressive heights of up to 35 m have been recorded with the tall, main stem assuming a relatively straight and sometimes buttressed habit of up to 1.8 m in diameter. The grey bark is smooth in texture, but often rough and segmented around the base of the main stem on older specimens (Palgrave *et al.*, 2002).

The compound leaves can reach lengths of 70 cm and are imparipinnated with 3-5 pairs of leaflets and a terminal one. The petiole is 8-10 cm in length. The leaflets are entire, opposite to alternate, glossy and dark-green in colour, and can attain a size of 21 cm in length and 8.5 cm in width. They exhibit 8-12 pairs of side veins, petioles of up to 1 cm in length, and an undersurface that is hairless to slightly hairy, and notably paler than the upper surface (Palgrave *et al.,*2002).

Figure 3.21 The leaves of *Trichilia dregeana*.
(www.plantzafrica.com/planttuv/trichildreg.htm).

Its creamy-white flowers with petals that are velvety on both surfaces and 1. 4 – 2.4 cm in length are produced from October to December and borne in dense, branched, axillary inflorescences.

Figure 3.22 Flowering stage of *Trichilia dregeana*.
(www.plantzafrica.com/planttuv/trichildreg.htm).

Its fruits are round, velvety capsules, which are 3 cm in diameter and usually split into 3 valves. On splitting, the capsules reveal 6 very attractive seeds. These seeds, which are black in colour, are covered largely in a bright red to scarlet aril, regarded as a striking and distinctive feature of the tree. Fruiting occurs mainly between January and May (Palgrave *et al.* 2002).

Distribution

The forest mahogany is a fairly widespread species, stretching from Pondoland in the south up through Swaziland, Mpumalanga and Limpopo Province, into

Zimbabwe and northwards into tropical Africa. It is found in areas of high rainfall, for example, in coastal and evergreen montane forests. It has also been found in altitudes ranging from 15 m to 1220 m above sea level (Germishuizen & Meyer 2003). Due to its ornamental attributes, it is widely cultivated, as a result of which it is often encountered outside of its natural habitat.

Conservation status

No information on its conservation status was found.

Medicinal uses

The species is also an important medicinal plant, with the seed, oil, leaves, roots and bark being used for such purposes. Unspecified plant parts are used by the Zulu people to treat stomach complaints and backache. On the other hand, the Xhosa people are known to administer bark decoctions as enemas for kidney-related backache. The Vhavenda people also administer enemas made with the bark for kidney problems, and as stomach and blood cleansers. In Nigeria, the leaves are used in the treatment of syphilis, while in Zimbabwe; the bark is used as a purgative and a fish poison (Burring, 2004). Palmer and Pitman (1972) stated that the tree is used throughout Africa to treat a range of ailments including leprosy and sleeplessness.

Data from ethnobotanical survey

The leaves and bark is mixed with cold water and the person has to bath in the mixture (Mhlongo 2007, pers. comm.).

Chemical contents

Five limonoids have been isolated from the seeds. Trichillin A, an insect anti-feedant, was also isolated from this plant (Palgrave *et al.* 2002). Trichilea dregeanin was also found to be present in this plant (Van Wyk *et al.,* 1997).

3.2.17 Scientific name : *Warburgia salutaris* (Bertol. f.) Chiov.

Zulu name : *Isibhaha*

Common name : Pepper-bark tree

Figure 3.23 The leaves of *Warbugia salutaris*. (www.plantzafrica.com/plantwxyz/warbug.htm).

Botanical description

Warbugia salutaris is an evergreen, slender tree that grows from 5 to 10 m tall. Its dark-green, glossy leaves, with entire margins, are a paler green on the undersurface. They are simple, alternately arranged, and elliptic to lanceolate. The midrib is slightly off-centre with a tapering apex and base. The leaves have a bitter, burning, aromatic taste. This plant has white to greenish flowers of up to 7 mm in diameter. The flowers are solitary, axillary, or in tight, few-flowered heads, borne on short, robust stalks in the axils of the leaves. These flowers develop into round, oval berries which are, narrowed towards the base; they are dark green but turn purple when ripe; and they are covered with glands and are leathery in texture. The stem is covered by a rich brown bark that is also bitter and peppery (Van Wyk *et al.,* 1997).

Distribution

Warbugia salutaris is a tropical forest tree which extends southwards as far as KwaZulu-Natal, eastern and northern Gauteng and across Swaziland. It also occurs in Malawi. Its growth habitat is forests and valleys (Mbambezeli, 2004).

Conservation Status

The tree has been heavily exploited for its bark. Thus it has been propagated from cuttings in an attempt to reduce pressure on the natural populations (Van Wyk *et al.,* 1997).

Medicinal uses

Medicinally, the pepper-like, bitter stems and the root bark are used to cure many ailments. As an expectorant or when smoked, they are widely used remedies for common colds. When dried and ground, they make a snuff used to clear human nasal sinuses. When taken orally, they are believed to cure spots in the lungs. Both the stems and the root bark are remedies for malaria. When powdered and mixed with water, they are also believed to cure sores in the mouth. The wood is not well known for timber in South Africa probably because of its rarity. According to Palmer & Pitman (1973), in Kenya, the leaves of the pepper-bark are sometimes added to curries; and the wood used in building.

Data from ethnobotanical survey

The leaves are ground and mixed with other ingredients. A dose of the mixture determined on the basis of the wound or sore, is then applied onto it (Lithuli 2007, pers. comm.).

Chemical content

The bark of *Warbugia salutaris* is reported to contain tannin with 3% of mannitol. Sequiterpenoid dialdehydes isolated from the bark include warburganal, muzugadial, polygodial, mukaadial and ugandensidial (Mbambezeli, 2004).

3.2.18 Scientific name : *Ziziphus mucronata*

Zulu name : Umlahlankosi

Common name : Buffalo thorn tree

Figure 3.24 The flowers, leaves and mature tree of *Ziziphus mucronata*. (www.plantzafrica.com/plantwxyz/zizimucro.htm).

Botanical description

Ziziphus mucronata is a small to medium-sized tree, 3–10(–20) m high; with a spreading canopy. The main stem is green and hairy when young; year old branches often zigzag; the bark is reddish brown or roughly mottled grey, cracked into small rectangular blocks, revealing a red and stringy under-surface. Young stems are reddish brown Leaves are simple, alternate; ovate or broadly ovate; vary enormously in size from tree to tree, 30–90 x 20–50 mm, tapering or often mucronate apex, base strongly asymmetrical, cordate to rounded on one side; margin finely serrated, often badly eaten by insects, glossy green above, slightly hairy and paler below; 3- to 5-veined from the base; veins covered with fine hairs when young; petiole up to 20 mm long; stipules, when present, take the form of small thorns at the nodes, one straight and one hooked. Leaves turn golden yellow in autumn. Flowers are borne in dense clusters in leaf axils; green to yellow; ± 4 mm in diameter; inconspicuous (October–February). The fruit is a smooth, shiny, leathery, spherical drupe, 12–20 mm in diameter, reddish-brown or deep red when ripe, slightly sweet, the pulp is dry. The fruit sometimes stays on the plant long after the leaves have fallen (March–August). The seeds are usually solitary, elliptic and compressed (Mazibuko, 2007).

Distribution

The buffalo thorn is distributed throughout the summer rainfall areas of sub-Saharan

Africa, extending from South Africa northwards to Ethiopia and Arabia (Mazibuko, 2007).

Conservation status

No information was found concerning its conservation status.

Medicinal uses

Its bark and roots are used medicinally for the treatment of various ailments, including rheumatism, gastrointestinal complaints and snake bites (Tas *et al.,* 1991). Warm bark infusions are used as expectorants in cough and chest problems while root infusions are popular as a remedy for diarrhoea and dysentery. Decoctions of roots and leaves are applied externally to boils, sores and glandular swellings not to promote healing but for pain relief (Van Wyk *et al.*, 1997).

Data from ethnobotanical survey

The leaves and stem of the plant are crushed and the juice is used to treat ear sores (Gcabashe 2007, pers. comm).

Chemical content

Several alkaloids commonly referred to as peptide alkaloids are known from *Ziziphus* species. About 12 structurally related alkaloids from this class have been isolated from roots, stem bark and leaves of *Ziziphus mucronata*. Mucronine D is a typical example (Van Wyk *et al.,* 1997).

3.3. DISCUSSION

The sustainable management of traditional medicinal plant resources is very important not only because of their value as a potential source of new drugs but also due to society's reliance on traditional medicinal plants for health. The majority of people in the Ongoye area consult traditional healers for health care. With a few exceptions, traditional medicinal plants are gathered from the wild. Certain plant types which are the sources of supply of traditional medicines have drastically declined due to forest clearance for agriculture, afforestation of montane grasslands, uncontrolled burning of vegetation, and livestock grazing e.g. *Warbugia salutaris*.

According to the interviews that were conducted 34 plants were found to be useful in treating sores and wounds (Table 2.1 and 2.2). Eighteen were available in the wild and at the homesteads. Three of the eighteen plants were obtained from the muthi market. The people selling the plants obtain them from the wild. The information of the plants from the interviews pointed that the plants are mostly obtained from the wild. The big trees were the ones found in their homes probably because they provide them with shade e.g. *Trichilia dregeana, Albizia adianthifolia* and *Sclerocarya birrea*. Men and women participated in the survey. The results from the survey showed that women had more knowledge than men. In all the age ranges that were provided in the questionnaires the knowledge was obtained from both men and women (see Table 3.1).

Table 3.1 Age range of the participants.

Age-range (yrs)	No of people	Women	Men
15-24	7	4	3
25-34	9	6	3
35-44	19	14	5
45-54	20	11	9
55-64	21	13	8
Over 65	4	2	2
Total	80	50	30

Table 3.1 shows that the knowledge was carefully passed on verbally from generation to generation. This information was passed on as the person grew up and this is supported by the number of people in each age range. For an example at the age-range of 15-24 yrs very few people had the knowledge but as the age increases, the number of people also increases. In the age-range of 65 yrs and above the number decreases because most of the homes did not have old people and the homes that had old people, their memory was not as good. The survey also showed that young men tend to have less interest on medicinal plants as it is believed to be a thing for women because they are the caretakers in their families. As men grow up some of them develop the interest and acquire knowledge on medicinal plants. This is supported by the slowly growing numbers of men participants in the survey (Table

3.1). *Hypericum aethiopicum* (unsukumbili) was the most used plant for treating sores and wounds. Of the 80 homesteads visited, 57 survey participants were using *Hypericum aethiopicum* at the time of the survey because it works the best for them.

CHAPTER 4

ANTIMICROBIAL ACTIVITY

4.1 WOUNDS AND OTHER SKIN INFECTIONS

Infections associated with traumatic injuries such as animal or human bites, burns, cuts or the penetration of a foreign object must be carefully sampled in order to recover the relevant pathogen and results must be interpreted carefully. This is because wound infections are constantly with normal flora. A variety of pathogens can be associated with wound infections and because some of these are anaerobes, samples must be transported from the collection site under anaerobic conditions. A common pathogen associated with purulent discharge is *Staphylococcus aureus* (Madigan *et al*., 2003).

4.2 ANTIBIOTICS

An antibiotic is a toxic compound naturally produced by moulds and bacteria to target and destroy competing microorganisms. Antibiotic activity was first discovered in the 19th century when the French chemist Louis Pasteur noticed that certain bacteria have the ability to kill other microorganisms, which became known as the case of anthrax (Johnson, 2001). Antibiotics are distinguished from growth factor analogs because they are natural products rather than synthetic chemicals. One of the most important groups of antibiotics both historically and medically is the β-lactam group. The β-lactam antibiotics include the penicillins, cephalosporins and cephamycins. These antibiotics all share the presence of a characteristic structural component, the β-lactam ring. The first β-lactam antibiotic discovered is the penicillin G which is active primarily against 'Gram positive' bacteria. Its action is restricted to 'Gram positive' bacteria because the 'Gram negative' bacteria are impermeable to the antibiotic (Madigan *et al.,* 2003). Antibiotics selectively attack bacteria without harming the cells belonging to the host organism. Any given antibiotic does this in one of two general ways:

- Firstly, bactericidal antibiotics (e.g. Penicillin and Cephalosporin) kill bacteria by inhibiting cell wall synthesis and allowing cell contents to leak out. Even though human and animal cells do not have cell walls, these antibiotics do not damage cells.
- Secondly, bacteriostatic antibiotics (including tetracycline, erythromycin) stop bacteria from reproducing by inhibiting nucleic acid formation (DNA and RNA) or by inhibiting protein synthesis by cell structures called ribosomes. Antibiotics that inhibit DNA and RNA affect bacterial cells more than human or animal cells. Antibiotics that inhibit protein synthesis can cause side effects because some human and other animal ribosomes are similar to those in bacteria (Johnson, 2001).

Antibiotics can also be categorized by the type of bacteria they affect. The cell wall of some species of bacterium is made of a thick layer of peptidoglycan. Gram-negative bacteria have a thin layer of peptidoglycan combined with both an outer and inner membrane. These differences in structure can be seen when bacteria are Gram-stained. Bacteria with thick peptidoglycan can appear purple when stained and are referred to as Gram-positive. Bacteria with thin peptidoglycan appear colourless or red and are referred to as Gram-negative (Johnson, 2001).

As from 1999 the antibiotic manufacturing has increased 25 times from 907 198 164 kgs to over 22 679 954 096 kgs each year (Johnson, 2001). While these drugs are still the best defense against bacterial infections, more and more of these compounds are also being used against illnesses that cannot be cured by the other current medications. When antibiotics first appeared about 50 years ago, they were rightly hailed as a modern medical miracle. Until that time, bacterium-related infections, such as meningitis and typhoid fever, often led to death. Antibiotics have saved millions of lives and have had relatively had few side effects. Presently, the term, antibiotic, is more commonly used to refer to synthetic or partly synthetic compounds used medically against bacteria that cause illness in humans, animals and plants. Penicillin and erythromycin are two of the most widely used antibiotic drugs. The microorganisms have an amazing adaptability for mutation to adjust to the environment. The misuse of antibiotics in our medical practice is certainly a

factor in selecting resistant microorganisms. The microorganisms are responding to the normal pressures but these pressures have increased with the misuse of antibiotics (Johnson, 2001).

4.3 DESCRIPTION OF MICROORGANISMS

4.3.1 *Bacillus subtilis*

Bacillus subtilis, which is one of the food-poisoning bacteria, is a Gram-positive, facultative, aerobic, sporulating bacillus normally found in soil. One of the earliest bacteria to be described was *Vibrio subtilis*. In 1872 the organism was renamed *Bacillus subtilis* by Gordon. This organism is a charter member of a large and diverse genus which is part of the family *Bacillaceae*. This family's distinguishing feature is the production of endospores, which are round, oval or cylindrical, highly refractile structures formed within bacterial cells. The spores were first described by Cohn in *Bacillus subtilis* and later by Koch in the pathogens. Bacteriophages that infect bacillus are common in soil. The most extensively studied Bacillus phages are those associated with *Bacillus subtilis* and can grow in minimal-salt-bacterial media with glucose as a carbon source (Aymerich *et al*., 1986). *Bacillus subtilis* is normally considered as being non-pathogenic; but it has been linked to food-borne illnesses, causing diarrhoea, nausea, vomiting, and associated with rice dishes served in oriental restaurants, infection is self-limiting (Willey *et al*. 2008). *Bacillus subtilis* produces proteolitic enzyme, *subtilism*, which is an extracellularenzyme that catalyzes the breakdown of proteins into polypeptides, resembles trypsin in its action, and has been shown to be a potent occupational allergen (Willey *et al., 2008)*.

4.3.2 *Escherichia coli*

Escherichia coli are Gram-negative, non-sporulating, rodlike, facultative, aerobes which are usually found in the gastro-intestinal tracts of warm blooded organisms. *Escherichia coli* contain different types of pilus and are found in virtually every genus of Gram-negative bacteria. No matter where pili are encountered, they are usually assumed to confer some form of attachment capability upon the bacteria possessing

them. It should be noted, however, that while this assumption is useful in certain circumstances, it is not strictly valid. Many bacteria which possess medical importance have been examined for the presence of pili; but only in relatively few cases has the importance of these structures in pathogenesis been rigorously established. Pili associated with *Escherichia coli* are classified by their morphology under electron microscopic examination and by their binding properties, e.g., type 1 pili of *Escherichia coli* are rigid-appearing organelles which are seven nanometers wide and one to two micrometers long.

Certain types of pili have long been associated with the utero-toxigenic strain of *Escherichia coli* which causes diarrhoea in humans. The most common cause of urinary tract infection in humans is *Escherichia coli*. The isolates are nearly always type-1, piliated and most often, pap-piliated. *Escherichia coli* causes at least five types of gastro-intestinal diseases in humans. Pathogenicity is generally due to the presence of one or more virulence factors, including invasiveness factors, heat-lable and heat-stable enterotoxins, verotoxins and colonization factors or adhesins. Pathogenic strains are usually identified by detection of specific virulence factors or of a serotype associated with a virulence factor (Willey *et al*. 2008).

The most recently identified *Escherichia coli* disease is hemorrhagic colitis, caused by strains of serotype 0157:H7 and a few other serotypes. This disease, characterized by painful abdominal cramping and bloody diarrhoea, is caused by strains that produce verotoxin and some strains associated with haemolytic uremic syndrome. *Escherichia coli* is an emerging cause of a food-borne infection which leads to bloody diarrhoea and occasionally to kidney failure. Most cases of the illness have been associated with eating under-cooked, contaminated, ground beef. Person-to-person contact in families and childcare centers is also an important mode of transmission if hygiene is inadequate.

Escherichia coli infection can also occur after drinking raw milk and after swimming in or drinking contaminated water. The organisms live in the intestines of healthy cattle, so preventive measures on cattle farms and during meat processing are necessary. Meat can be contaminated during slaughter; but the microorganism can also be thoroughly mixed into beef when it is being ground, while the bacteria

present in a cow's udder or equipment may get into raw milk. Although the number of microorganisms required to cause disease is not known, it is suspected to be very small. Colonies of the bacteria are flat, grayish and with irregular edges, and tend with time to spread on the surface of the agar. Its cultures have a characteristic odor and a metallic sheen, which are both useful characters for identification. Variants may not have these characters; but they may appear upon sub-cultivation (Willey *et al*. 2008).

4.3.3 *Klebsiella pneumoniae*.

Klebsiella pneumonia is a Gram-negative, non-sporulating, facultative, aerobic rod that is normally found in the human gastro-intestinal tract. It is nitrogen-fixing bacteria. An adhesion to a mucosal surface is often the first step in the development of an infection. Adhesins are often also hemmaglutinins and may or may not be located on fimbriae which protrude from the surface of the bacterial cells. Strains of *Klebsiella oxytoca* may produce thick channeled fimbriae associated with mannose-sensitive hemaglutination. These strains may also produce thin non-channelled (type 3) fimbriae associated with the mannose-resistant hemaglutinins. These type-3 fimbriae can be coated with type-3 antiserum against *Klebsiella oxytoca (*K70/1) and are antigenically related to those of *Enterobacter gergoviae.* Strains of *Klebsiella oxytoca* that have not acquired any resistant determinants are naturally resistant to aminopenicillin (ampicillin) and carboxypenicillin (carbenicillin) and susceptible to other beta-lactam antibiotics. This is due to the production of a chromosomal penicillinase which is inhibited by clavulanic acid. A small zone of inhibition around 100 μg carbenicillin discs is typical of this phenotype. Acquired resistance often arises from the production of a plasmid-determined penicillinase. The strains show a higher resistance to carbenicillin and resistance to ureidopenicillins, cefalothin, cefamandole, and cefuroxime (Willey *et al*. 2008).

Klebsiella species may be found in human feces. A survey of the presence of *Klebsiella* in urban residents, hospital personnel, and newly admitted patients showed that 30-37% of individuals carried *Klebsiella*, including a 29-35% fecal carriage and a three-to-four-percent throat carriage. There was a slight increase in prevalence among long-term patients. *Klebsiella* species can cause human diseases

ranging from asymptomatic colonization of the intestinal, urinary or respiratory tract to fatal septicemia and are widely recognized as important opportunistic pathogens in humans, especially hospital patients. Infections with *Klebsiella* are caused mainly by *Klebsiella pneumoniae* and *Klebsiella oxytoca* in a proportion of about two to one. Capsular types found to be endemic in hospitals are diverse and include K2 and other K-antigen types above K-6. Features predisposing to nosocomial infection by *Klebsiella pneumoniae* or *Klebsiella oxytoca* are the same as those which dispose the *Enterobacter* infections. These include extremes of age, chronic alcoholism, diabetes mellitus or chronic cardiac, renal, pulmonary or neoplastic disease (Willey *et al*. 2008).

Strains of *Klebsiella pneumonia* and *Klebsiella oxytoca* which have not acquired any resistance are determined as naturally resistant to ampicillin and carboxypenicillin but susceptible to other beta-lactam antibiotics. This is due to the production of a chromosomal penicillinase which is inhibited by clavulanic acid (Cohen and Powderly, 2004).

4.3.4 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, non-sporulating, non-motile, usually non-capsulate, facultative, aerobic cocci. Staphylococci are widespread in nature although they are found more consistently in denser populations on the skin, skin glands and mucous membranes of mammals and birds. These microorganisms are sometimes found in the pharynx, mouth, blood, mammary glands, intestines, and upper respiratory tracts of these hosts. The largest populations are found in the region of the skin supplied with large numbers of sweat glands, and on the skin and mucous membranes surrounding openings on the body surface.

Staphylococcus is coagulase-positive and is regarded as a potentially serious pathogen. It is a major cause of mortality and is also responsible for a variety of infections. The mechanism of staphylococcal pathogenicity is extensively in the species *Staphylococcus aureus*. This aggressive and invasive species has at it disposal a variety of extra-cellular enzymes, toxins and antiphagocytic components of the cell wall to use against the host. Some principal toxins include the alpha

haemolysins, leukocidin, and perhaps of less significance, beta and gamma hemolysins. Certain strains of *Staphylococcus aureus* are capable of producing other damaging toxins such as enterotoxins and exfoliatin, and the toxin responsible for the toxic shock syndrome. The enzyme coagulase is regarded as a virulence factor in that it may inhibit the bactericidal activity of normal serum and phagocytosis. *Staphylococcus aureus* lipase may influence the non-specific, primary defense system by increasing the chemotactic response of polymorph nuclear leukocytes. The ability of *Staphylococcus aureus* to bind fibronectin and collagen exposed in open wounds in microtrauma allow this organism to readily establish itself in traumatized tissues.

Binding to laminin allows this species to localize in basal membranes in various body sites. Staphylococcal pathogenicity also depends upon the effectiveness of the host defense system, which include natural barriers of the skin and mucous membranes, and humoral and phagocytic immune responses. During the past decade, it has become increasingly apparent that patients with deficient numbers of phagocytic cells, or disorders in their function, have increased susceptibility to staphylococcal infections. Members of the genus *Staphylococcus* are Gram-positive cocci that occur singly, in pairs, tetrads, short chains and irregular grapelike clusters. These members are non-motile, non-spore-forming, and usually unencapsulated, or have limited capsular formation. *Staphylococcus aureus* grows rapidly and abundantly under aerobic conditions. Staphylococcal cell walls contain teichoic acid and peptidoglycan. They can be grouped according to their natural or genomic relationships as dertemined by DNA-DNA hybridization and the thermal stability of DNA heteroduplexes (Willey *et al*. 2008).

Table 4.1 Physical description of the bacterial strains used.

BACTERIA	STRAIN NUMBER	GRAM-POSITIVE/ GRAM-NEGATIVE	GROWTH DESCRIPTION
Bacillus subtilis	ATCC6051	Gram-positive bacilli, spore- forming and aerobic bacterium.	Colonial variation is observed. One type of colony is flat, irregular-shaped and whitish in color. The other is smaller, more circular, flat/rough and translucent.
Escherichia coli	ATCC11775, u1505s, u16406, u16403.	Gram-negative bacilli, non-spore- forming.	Colonies are glistening, smooth, translucent and 1, 5-3 mm in diameter.
Klebsiella pneumonia	ATCC13883	Gram-negative bacilli, non-spore- forming.	Two colony types can be seen. The predominant type is glistening, smooth, opaque and 2 mm in diameter. The second type is light, smaller and more translucent. Colonies vary in size.
Staphylococcus aureus	ATCC12600, P5020, 4790, T1266.	Gram-positive, spherical cell clusters, non- spore-forming.	Colonies are glistening, circular and smooth.

4.4 AGAR WELL AND DISC DIFFUSION

4.4.1 Agar well diffusion results

The results obtained from the agar well and the disc diffusion methods show high level of compatibility as seen in Tables 4.14 to 4.17.The agar well diffusion results for the plant species tested against the different microorganisms are presented in Tables 4.2 - 4.5 (appendix II).

4.4.2 Disc diffusion results

The disc diffusion results for the plant species tested against the different microorganisms are presented in Tables 4.6 – 4.9 (appendix II).

4.4.3 Discussion

Three methods were carried out for antimicrobial activity; this was to learn and compare the efficacy of each method. The tables show the inhibition results of the plant extracts from 10 mm and above. The few variations in results between the disc diffusion and agar well diffusion results can possible be explain by the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of plant extract diffusion. The plants which were bought at the Muthi Market (Empangeni) for treating sores and wounds are widely used medicinally in rural areas. Some people from the community mentioned that they buy the muthi from the market because it's not available in their area (Madonsela 2007, pers. comm). These plants did have some inhibiting effect on some of the bacteria which were tested.

Table 4.14 Comparison of results obtained for antibacterial testing against *Bacillus subtilis*.

PLANT NAMES	Agar well results				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	+	-	-	+	+	-	-	-	+	-	-
Acanthospermum austral (leaves)	+	+	+	-	+	+	+	+	-	+	-	-
Acorus calamus	-	+	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	+	-	-	-	-
Clerodendium hirsutum	-	+	-	-	-	+	+	-	-	-	-	-
Combretum erythrophyllum	+	+	+	-	+	+	-	-	+	+	-	-
Faurea saligna	-	+	-	-	-	+	-	-	-	-	-	-

PLANT NAMES	Agar well results				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Gerbera ambigua	-	+	+	-	-	+	+	-	-	+	-	-
Gunnera perpensa	+	+	+	-	+	+	-	-	+	+	-	-
Hypericum aethiopicum (leaves)	+	+	-	-	+	+	+	-	-	+	-	-
Hypericum aethiopicum (stem)	+	+	-	+	+	+	-	-	-	+	-	-
Hypoxis hemerocallidea	-	+	+	-	-	+	+	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	+	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea	+	+	-	-	+	+	-	-	-	-	-	-
Sclerocarya birrea (bark)	+	+	-	-	+	+	-	-	-	-	-	-
Solanum uculeastrum (stem)	+	+	-	-	-	+	-	-	-	-	-	-
Solanum aculeastrum (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	+	-	-
Trichilia dregeana (stem)	+	+	-	-	+	+	-	-	-	-	-	-
Trichilia dregeana (leaves)	+	+	-	-	+	+	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	+	-	-	-	+	-	-	-	-	-	-
Warburgia salutaris (stem)	-	+	-	-	-	+	-	-	-	+	-	-
Ziziphus mucronata (stem)	+	+	-	-	+	+	-	-	-	+	-	-
Ziziphus mucronata (leaves)	+	+	-	-	+	+	-	-	-	+	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.15 Comparison of results obtained for antibacterial testing against *Klebsiella pneumoniae.*

PLANT NAMES	Agar well result KP				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	-	-	-	+	-	-	-	-	+	-	-
Acanthospermum australe (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	+	-	-	-	+	-	-	-	+	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	+	-	-	-
Baccharoides adoensis (stem)	-	+	-	-	-	+	-	-	-	+	-	-
Baccharoides adoensis (leaves)	-	+	-	-	-	+	-	-	-	-	-	-
Clerodendium hirsutum	-	-	-	+	-	-	-	-	-	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	-	-	+	+	-	+
Faurea saligna Harv.	-	-	-	-	-	-	-	-	-	-	-	-
Gerbera ambigua	+	-	-	-	+	-	-	-	+	-	-	-
Gunnera perpensa	-	+	+	-	-	+	+	-	+	-	-	-
Hypericum aethiopicum (leaves)	+	-	+	-	+	-	+	-	+	+	-	-
Hypericum aethiopicum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	+	-	-	-	+	-	-	-	+	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	+	-	-	-	+	-	-	-	-	-	-
Sclerocarya birrea	+	-	-	+	-	+	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	+	-	-	+	+	-	-	-	+	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	+	-	-	-	+	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	+	-	-	-	+	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	-	-	-	-	-	-	-	+	-	-

PLANT NAMES	Agar well result KP				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Warburgia salutaris (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Ziziphus mucronata (stem)	+	-	-	-	+	-	-	-	+	-	-	-
Ziziphus mucronata (leaves)	-	-	-	-	-	-	-	-	-	-		-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.16 Comparison of results obtained for antibacterial testing against *Staphylococcus aureus*.

PLANT NAMES	Agar well result SA				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	+	-	-	+	+	-	+	+	+	-	-
Acanthospermum australe (leaves)	+	+	-	-	+	+	-	-	-	-	-	-
Acorus calamus	+	+	-	+	+	+	-	-	-	-	-	-
Albizia adianthifolia (stem)	+	+	-	-	+	+	-	-	-	-	-	-
Albizia adianthifolia (leaves)	+	+	-	-	+	+	-	-	-	+	-	-
Baccharoides adoensis	+	+	-	-	+	+	-	-	-	-	-	-
Baccharoides adoensis (leaves)	+	+	-	-	+	+	-	-	-	-	-	-
Clerodendium hirsutum	+	+	-	-	+	+	-	-	-	-	-	-
Combretum erythrophyllum	+	+	-	-	+	+	-	-	-	+	-	-
Faurea saligna	+	+	-	+	+	+	-	-	-	-	-	-
Gerbera ambigua	+	+	-	-	+	+	-	-	-	+	-	-
Gunnera perpensa	+	+	-	-	+	+	-	-	-	+	-	-
Hypericum aethiopicum (leaves)	+	+	-	-	+	+	-	-	+	+	-	-
Hypericum aethiopicum (stem)	+	+	-	-	-	+	-	-	+	-	-	-
Hypoxis hemerocallidea	+	+	-	-	+	+	-	-	-	-	-	-
Lippia javanica (leaves)	+	+	-	-	-	+	-	-	-	-	-	-
Lippia javanica (stem)	+	+	-	-	+	+	-	-	-	-	-	-
Pentanisia prunelloides	+	+	-	+	+	+	-	-	+	+	-	-
Sclerocarya birrea	-	-	-	-	+	+	-	-	-	+	-	-

PLANT NAMES	Agar well result SA				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Sclerocarya birrea (bark)	-	-	-	-	+	+	-	-	-	+	-	-
Solanum uculeastrum (stem)	+	-	-	-	+	-	-	-	-	+	-	-
Solanum uculeastrum (leaves)	+	+	-	-	-	-	-	-	-	+	-	-
Solanum uculeastrum (root)	+	+	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	+	+	-	-	+	+	-	-	-	-	-	-
Trichilia dregeana (leaves)	+	+	-	+	+	+	-	-	+	+	-	-
Warburgia salutaris (leaves)	+	+	+	-	-	+	-	-	-	-	-	-
Warburgia salutaris (stem)	+	+	+	-	-	+	+	-	-	+	-	-
Ziziphus mucronata (stem)	+	+	-	-	+	+	-	-	-	-	-	-
Ziziphus mucronata (leaves)	+	+	-	-	+	+	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.17 Comparison of the results obtained for antibacterial activity against *Escherichia coli.*

PLANT NAMES	Agar well result EC				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Acorus calamus L.	-	+	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	-	-	-	-	-	+	-	-	-	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	-	-	-	-	-	-
Faurea saligna	-	-	-	-	-	-	-	-	-	-	-	-
Gerbera ambigua	-	-	-	-	-	-	-	-	-	-	-	-
Gunnera perpensa	-	+	-	-	-	+	-	-	-	-	--	-

PLANT NAMES	Agar well result EC				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Hypericum aethiopicum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	-	-	-	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	+	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea	-	+	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	+	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (roots)	-	-	-	-	-	-	-	-	--	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (stem)	-	+	-	-	-	-	-	-	-	-	-	-
Ziziphus mucronata (stem)	-	_	-	-	-	-	-	-	-	-	-	-
Ziziphus mucronata (leaves)	-	_	-	-	-	-	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.18 Comparison of results obtained for antibacterial testing against *Staphylococcus aureus P5020.*

PLANT NAMES	Agar well result SAP5020				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	-	-	-	+	-	-	+	+	-	-	-
Acanthospermum australe (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	+	-	-	+	+	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	+	-	-	-	+	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Clerodendium hirsutum	+	+	-	-	+	+	-	-	-	+	-	-
Combretum erythrophyllum	+	+	-	-	+	+	+	-	-	+	-	-
Faurea saligna	+	+	-	-	+	+	-	-	-	-	-	-
Gerbera ambigua	+	-	-	+	+	-	-	-	-	-	-	-
Gunnera perpensa	+	+	+	+	+	+	-	+	+	+	+	-
Hypericum aethiopicum (leaves)	+	+	-	+	+	+	-	-	+	+	+	-
Hypericum aethiopicum (stem)	+	-	-	+	+	-	-	-	-	-	-	-
Hypoxis hemerocallidea	+	+	-	-	+	+	+	-	-	+	-	-
Lippia javanica (leaves)	+	+	-	-	+	+	-	-	-	+	-	-
Lippia javanica (stem)	+	-	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	+	+	-	+	+	+	-	+	-	-	-	-
Sclerocarya birrea	+	+	-	-	+	+	-	-	-	+	-	-
Sclerocarya birrea (bark)	+	+	-	+	+	+	-	-	-	+	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	+	-	-	-	+	-	-
Solanum uculeastrum (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	+	-	-	-	+	-	-
Trichilia dregeana (stem)	-	+	-	-	-	-	-	-	-	+	-	-
Trichilia dregeana (leaves)	+	-	-	+	+	+	-	-	-	-	-	-
Warburgia salutaris (leaves)	+	+	+	+	+	-	+	-	+	-	-	-
Warburgia salutaris (stem)	+	+	-	+	+	+	-	-	-	-	+	-

PLANT NAMES	Agar well result SAP5020				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Ziziphus mucronata (stem)	+	-	-	-	+	+	+	-	-	-	-	-
Ziziphus mucronata (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.19 Comparison of results obtained for antibacterial testing against *Staphylococcus aureus 4790.*

PLANT NAMES	Agar well result SA4790				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	+	-	-	-	+	-	-	-	+	-	-
Acanthospermum australe (leaves)	+	+	-	+	+	+	-	+	+	+	-	-
Acorus calamus	+	-	-	-	+	-	-	-	+	-	-	-
Albizia adianthifolia (stem)	+	-	-	-	+	-	-	-	+	-	-	-
Albizia adianthifolia (leaves)	+	+	-	+	+	+	-	+	+	+	-	-
Baccharoides adoensis (stem)	-	+	-	-	+	+	-	-	+	+	-	-
Baccharoides adoensis (leaves)	-	-	-	-	+	-	-	-	+	-	-	-
Clerodendium hirsutum	+	-	-	-	+	-	-	-	+	-	-	-
Combretum erythrophyllum	+	+	-	-	+	+	-	-	+	+	-	-
Faurea saligna	-	+	-	-	+	+	-	-	-	+	-	-
Gerbera ambigua	+	-	-	-	+	-	-	-	+	-	-	-
Gunnera perpensa	+	+	-	-	+	+	-	-	+	+	-	-
Hypericum aethiopicum (leaves)	+	-	-	+	+	-	+	-	+	-	-	-
Hypericum aethiopicum (stem)	+	-	+	-	+	-	+	+	+	-	-	-
Hypoxis hemerocallidea	+	-	-	-	-	-	-	-	+	-	-	-
Lippia javanica (leaves)	+	-	+	-	+	-	-	-	+	-	-	-
Lippia javanica (stem)	+	-	-	-	+	-	-	-	+	-	-	-
Pentanisia prunelloides	+	+	-	-	+	+	-	-	+	+	-	-
Sclerocarya birrea	+	-	-	-	+	-	-	-	+	-	-	-

PLANT NAMES	Agar well result SA4790				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Sclerocarya birrea (bark)	+	+	-	+	+	+	-	+	+	+	-	-
Solanum uculeastrum (stem)	-	+	-	-	-	+	-	-	+	+	-	-
Solanum uculeastrum (leaves)	-	-	-	-	+	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	+	-	-	-
Trichilia dregeana (stem)	+	+	-	-	+	+	-	-	-	+	-	-
Trichilia dregeana (leaves)	+	+	-	+	+	+	-	+	+	+	-	-
Warburgia salutaris (leaves)	+	-	-	+	+	-	-	+	+	-	-	-
Warburgia salutaris (stem)	+	-	-	-	+	-	-	-	+	-	-	-
Ziziphus mucronata (stem)	+	+	-	-	+	+	-	-	+	+	-	-
Ziziphus mucronata (leaves)	+	+	-	-	-	+	-	-	-	+	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.20 Comparison of results obtained for antibacterial testing against *Staphylococcus aureus T1266.*

PLANT NAMES	Agar well result SA T1266				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Acorus calamus	+	+	-	+	+	+	-	-	+	-	-	-
Albizia adianthifolia (stem)	-	+	-	-	-	+	-	-	-	+	-	-
Albizia adianthifolia (leaves)	-	-	-	-	+	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	+	+	-	-	+	+	-	-	-	+	-	-
Baccharoides adoensis (leaves)	+	+	-	-	+	+	-	-	-	+	-	-
Clerodendium hirsutum	+	-	-	-	+	-	-	-	-	-	-	-
Combretum erythrophyllum	-	+	-	-	-	+	-	-	-	-	-	-
Faurea saligna	+	+	-	+	-	-	-	-	-	-	-	-
Gerbera ambigua	-	-	+	-	+	+	-	-	-	+	-	-

PLANT NAMES	Agar well result SA T1266				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Gunnera perpensa	+	+	+	-	-	+	-	-	-	-	-	-
Hypericum aethiopicum (leaves)	-	+	-	-	+	+	-	-	-	+	-	-
Hypericum aethiopicum (stem)	+	+	-	-	+	-	-	+	-	-	-	-
Hypoxis hemerocallidea	+	-	-	-	+	+	-	+	-	+	-	-
Lippia javanica (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Lippia javanica (stem)	+	-	-	-	+	-	-	-	-	-	-	-
Pentanisia prunelloides	+	-	-	-	+	-	-	-	-	-	-	-
Sclerocarya birrea	+	-	-	+	+	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	+	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	+	-	-	-	+	-	+	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	+	-	+	-	+	-	-	-	+	+	-	-
Warburgia salutaris (stem)	+	-	+	-	+	-	-	-	+	+	-	-
Ziziphus mucronata (stem)	-	+	-	+	-	+	-	-	–	-	-	-
Ziziphus mucronata (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.21 Comparison of results obtained for antibacterial testing against *Escherichia coli U1505 s.*

PLANT NAMES	Agar well result EC U1505s				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	-	+	-	-	-	+	-	+	-	-	-	-
Acanthospermum australe (leaves)	-	+	-	-	+	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	+	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	+	-	-	-	+	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	+	-	-	-	+	-	-	-	-
Baccharoides adoensis (leaves)	-	+	-	-	+	-	+	-	-	-	-	-
Clerodendium hirsutum	-	-	+	-	-	+	-	+	-	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	-	-	--	-	-	-
Faurea saligna	-	-	-	-	-	-	-	-	-	-	-	-
Gerbera ambigua	-	-	-	-	+	-	-	-	-	-	-	-
Gunnera perpensa	-	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (stem)	+	-	-	-	-	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	+	+	-	+	-	-	-	-	-	-	-
Lippia javanica (Burm. f.) Spreng (leaves)	-	-	-	-	+	-	-	-	-	-	-	-
Lippia javanica (stem)	-	+	-	-	-	+	-	-	-	-	-	-
Pentanisia prunelloides	-	-	-	+	+	+	-	-	-	-	-	-
Sclerocarya birrea	+	+	+	+	+	+	-	+	-	-	-	-
Sclerocarya birrea (bark)	-	+	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	+	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	+	-	+	-	-	-	-	-	-	-

PLANT NAMES	Agar well result EC U1505s				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Warburgia salutaris (stem)	-	-	-	+	+	-	+	-	-	-	-	-
Ziziphus mucronata (stem)	-	-	-	-	-	+	-	-	-	-	-	-
Ziziphus mucronata (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.22 Comparison of results obtained for antibacterial testing against *Escherichia coli U16406.*

PLANT NAMES	Agar well result ECU16406				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	-	+	-	-	-	-	-	-	-	-	-	-
Acorus calamus	-	-	-	+	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	+	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	+	-	-	+	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	+	+	-	-	+	-	-	-	+	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	-	-	+	-	-	-
Faurea saligna	-	-	-	-	+	-	-	-	+	-	-	-
Gerbera ambigua	+	-	-	-	+	-	-	-	+	-	-	-
Gunnera perpensa	+	-	-	-	+	-	-	-	+	-	-	-
Hypericum aethiopicum (leaves)	+	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	-	+	-	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	+	-	-	-	-	-	-
Lippia javanica (stem)	-	+	-	-	-	-	-	-	-	-	-	-

PLANT NAMES	Agar well result ECU16406				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Pentanisia prunelloides	+	-	-	+	+	-	-	-	+	-	-	-
Sclerocarya birrea	-	+	-	-	+	+	-	-	-	+	-	-
Sclerocarya birrea (bark)	+	-	-	-	+	-	-	-	+	-	-	-
Solanum uculeastrum (stem)	+	-	-	-	+	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	+	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	+	-	-	-
Trichilia dregeana (stem)	-	+	-	+	+	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	+	-	+	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	-	+	-	+	-	-	-	-	-	-
Warburgia salutaris (stem)	+	+	-	-	+	-	-	-	-	-	-	-
Ziziphus mucronata (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Ziziphus mucronata (leaves)	+	+	-		+	-	-	-	+	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

Table 4.23 Comparison of results obtained for antibacterial testing against *Escherichia coli 16403.*

PLANT NAMES	Agar well result ECU16403				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Acanthospermum australe (stem)	+	-	-	-	+	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	+	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	+	-	-	-	+	-	-	-	-	-	-	-
Clerodendium hirsutum	-	-	-	-	-	-	-	-	-	-	-	-

PLANT NAMES	Agar well result ECU16403				Disc diffusion results				MIC			
	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot	Acet	Met	Cold	Hot
Combretum erythrophyllum	-	-	-	-	-	-	-	-	-	-	-	-
Faurea saligna	-	-	-	-	-	-	-	-	-	-	-	-
Gerbera ambigua	-	-	-	-	-	-	-	-	-	-	-	-
Gunnera perpensa	-	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (stem)	-	-	-	-	+	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	+	-	-	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	+	-	-	-	+	-	-	-	-	-	-	-
Sclerocarya birrea	+	-	+	+	+	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	-	-	-	+	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	+	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	+	-	-	+	+	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	+	-	-	-	-	-	-	-	-	-
Warburgia salutaris (stem)	-	-	-	+	+	-	-	-	-	-	-	-
Ziziphus mucronata (stem)	-	-	-	-	-	-	-	-	-	-	-	-
Ziziphus mucronata (leaves)	-	+	+	-	+	-	-	-	-	-	-	-
Neomycin	+	+	+	+	+	+	+	+	+	+	+	+

Acet = Acetone; Met = Methanol; Cold = Cold water; Hot = Hot water
+ = ≥ 10mm; - = ≤10mm; (MIC) + = ≤ 6.25; - = ≥ 6.25

On the disc diffusion and agar well methods, the methanol and acetone results were more positive than water extracts. This may have been due to the capability of methanol and acetone to dissolve both polar and non-polar compounds. The water extracts also showed some activity, although it was very low. Water can only dissolve polar compounds, which may be the cause of lower activity.

The use of American Type Culture Collection (ATCC) bacterial strains is due to the fact that they are standard cultures; and, as such, it is a source of over 60 000 authenticated, viable cultures; and the world's premier biological culture respository. This fact assures medical and biological scientists of the reliability of their culture.

Neomycin was used as a positive control because it inhibits the growth of both Gram-positive and Gram-negative bacteria (Marshal & Williams, 2003). Its selection was because of its high percentage of peptidoglycan compared to gentamycin, tobramycin, amikacin, streptomycin and kanamycin. It was used in the treatment of topical infections (Dollery, 1991).

The negative control, dimethylsulphoxide (DMSO), is a colourless liquid and an important polar solvent which dissolves both polar and non-polar compounds from a plant. It is miscible in a wide range of organic solvents including water. It has the distinctive property of penetrating the human skin very readily.

Amongst the test microorganisms used, the *Staphylococcus aureus* (ATCC 6051) was found to be the most sensitive to the different plant extracts, followed by the *Bacillus subtilis*. *Escherichia coli* were the least sensitive bacteria as it causes diarrhoea and the plants collected where specifically for treating wounds.

4.5 MINIMUM INHIBITION CONCENTRATION (MIC)

4.5.1 MIC results

The MIC results for the plant species tested against the different microorganisms are presented in Tables 4.10 – 4.13 (appendix II).

4.5.2 Discussion

The acetone extracts of *Acanthospermum australe* (leaves), *Baccharoides adoensis* (leaves) and the methanol extracts of *Acanthospermum australe* (leaves and stem) and *Solanum aculeustrum* (root) have MIC values of ≤ 6.25 mg/ml against *Bacillus*

subtilis (Table 4.10). The acetone extracts of *Albizia adianthifolia* (leaves and stem), *Combretum erythrophyllum, Gerbera ambigua, Gunnera perpensa, Hypericum aethiopicum* (leaves), *Ziziphus mucronata* (stem) and methanol extracts of *Acanthospermum australe* (stem), *Baccharoides adoensis* (stem), *Combretum erythrophyllum, Hypoxis hemerocallidea, Hypericum aethiopicum* (leaves), *Sclerocarya birrea* (bark) and *Warbugia salutaris* (leaves) have MIC values of ≤ 6.25 mg/ml against *Klebsiella pneumoniae* (Table 4.15). In the case of hot water extract of *Combretum erythrophyllum* the MIC value was ≤ 6.25 against *Klebsiella pneumoniae* (Table 4.15).

Staphylococcus aureus (ATCC 6051) was inhibited by acetone extracts of *Acanthospermum australe* (stem), *Hypericum aethiopicum* (leaves and stem), *Pentanisia prunelloides, Trichilia dregeana* (leaves) and methanol extracts of *Acanthospermum australe* (stem), *Albizia adianthifolia* (leaves), *Combretum erythrophyllum, Gerbera ambigua, Gunnera perpensa, Hypericum aethiopicum* (leaves), *Pentanisia prunelloides, Sclerocarya birrea* (bark), *Solanum aculeastrum* (stem and leaves), *Trichilia dregeana* (leaves) and *Warbugia salutaris* (stem) (Table 4.16). *Staphylococcus aureus P5020* was inhibited by the acetone extract of *Acanthospermum australe* (stem), *Gunnera perpensa, Hypericum aethiopicum* (leaves), *Warbugia salutaris* (leaves) and methanol plant extracts of *Clerodendium hirsutum, Combretum erythrophyllum, Hypoxis hemerocallidea, Gunnera perpensa, Hypericum aethiopicum* (leaves), *Lippia javanica* (leaves), *Sclerocarya birrea* (bark), *Solanum aculeastrume* (stem and root) and *Trichilia dregeana* (stem). Only one hot water extract inhibited the above mentioned strain and that is *Warbugia salutaris* (stem) (Table 4.18).

Staphylococcus aureus 4790 was inhibited by 23 acetone extracts which were *Acanthospermum australe* (leaves), *Acorus calamus, Albizia adianthifolia* (stem and leaves), *Baccharoides adoensis* (stem and leaves), *Combretum erythrophyllum, Clerodendium hirsutum, Hypoxis hemerocallidea, Gerbera ambigua, Gunnera perpensa, Hypericum aethiopicum* (leaves and stem), *Lippia javanica* (leaves and stem), *Pentanisia prunelloides, Sclerocarya birrea* (bark), *Solanum aculeastrum* (stem and root),*Trichilia dregeana* (leaves), *Warbugia salutaris* (leaves and stem), *Ziziphus mucronata* (stem) and 14 methanol plant extracts inhibited the above

mentioned strain which were *Acanthospermum australe* (stem and leaves), *Albizia adianthifolia* (leaves), *Baccharoides adoensis* (stem), *Combretum erythrophyllum, Faurea saligna, Gunnera perpensa, Pentanisia prunelloides, Sclerocarya birrea* (bark), *Solanum aculeastrum* (stem), *Trichilia dregeana* (stem and leaves), *Ziziphus mucronata* (stem and leaves) (Table 4.19). *Staphylococcus aureus T1266* was inhibited by three acetone plants extracts which are *Acorus calamus, Warbugia salutaris* (leaves and stem) and eight methanol plants extracts inhibited this strain which were *Albizia adianthifolia* (stem), *Baccharoides adoensis* (leaves and stem), *Hypoxis hemerocallidea, Gerbera ambigua, Hypericum aethiopicum* (leaves), *Warbugia salutaris* (leaves and stem) (Table 4.20). *Escherichia coli U 16406* was inhibited by nine acetone plant extracts which were *Clerodendium hirsutum, Combretum erythrophyllum, Faurea saligna, Gerbera ambigua, Gunnera perpensa, Pentanisia prunelloides, Sclerocarya birrea* (bark), *Solanum aculeastrum* (root), *Ziziphus mucronata* (leaves) and *Sclerocarya birrea* was the only methanol extract which inhibited this strain (Table 4.22).

The following three plant species have not previously been tested for their antibacterial activity: *Clerodendrum hirsutum, Faurea saligna, Gerbera ambigua. Acanthospermum austral* and *Solanum aculeastrum* were previously only tested against *Candica albicans*. Table 4.24 show some antibacterial results obtain from the literature for the same plant species used in this study, but were extracted with different solvents and tested against different micro-organisms.

Table 4.24 Known antibacterial activities of the plant species tested in this study.

Plant	***Microorganism***	***Solvent***	***Activity***	***Reference***
Acanthospermum australe	*Candida albicans*	Dichloromethane	+	Portillo *et al.* 2001
Acorus calamus	*Staphylococcus aureus*	Methanol	-	Phongpaichit *et al.,* 2005
	Escherichia coli	Methanol	+	Phongpaichit *et al.,* 2005
Albizia adianthifolia	*Staphylococcus aureus*	Methanol	-	McGaw *et al.* 2000
	Klebsiella pneumoniae	Methanol	-	McGaw *et al.* 2000
	Bacillus subtilis	Methanol	-	McGaw *et al.* 2000

Plant	*Microorganism*	Solvent	Activity	Reference
Baccharoides adoensis	*Staphylococcus aureus*	Methanol	+	Elkatib *et al.* 2006
	Escherichia coli	Methanol	+	Elkatib *et al.* 2006
Combretum erythrophyllum	*Staphylococcus aureus*	Acetone	+	Martini, 2004
	Escherichia coli	Acetone	+	Martini, 2004
Gunnera perpensa	*Staphylococcus aureus*	Acetone	+	McGaw *et al.*, 2005
Hypericum aethiopicum	*Staphylococcus aureus*	Methanol	+	Schempp *et al.* 2003
Hypoxis hemerocallidea	*Staphylococcus aureus*	Methanol	+	Muwanga, 2006
Lippia javanica	*Staphylococcus aureus*	Acetone	+	Minenzeh, 2004
	Escherichia coli	Acetone	-	Minenzeh, 2004
Pentanisia prunelloides	*Staphylococcus aureus*	Ethanol	+	Yff *et al. 2002*
	Escherichia coli	Ethanol	+	Yff *et al. 2002*
	Klebsiella pneumoniae	Ethanol	+	Yff *et al. 2002*
	Bacillus subtilis	Ethanol	+	Yff *et al. 2002*
Sclerocarya birrea	*Staphylococcus aureus*	Acetone	+	Eloff, 2001
	Escherichia coli	Acetone	+	Eloff, 2001
Solanum aculeastrum	*Candida albicans*	Methanol	+	Steenkamp *et al.* 2006
Trichilia dregeana	*Bacillus subtilis*	Ethyl acetate	+	Eldeen *et al. 2005*
	Staphylococcus aureus	Ethyl acetate	+	Eldeen *et al. 2005*
	Escherichia coli	Ethyl acetate	+	Eldeen *et al. 2005*
	Klebsiella pneumoniae	Ethyl acetate	+	Eldeen *et al. 2005*
Warbugia salutaris	*Escherichia coli*	Methanol	+	Rabe *et al. 1997*
	Bacillus subtilis	Methanol	-	Rabe *et al. 1997*
	Klebsiella pneumoniae	Methanol	-	Rabe *et al. 1997*
Ziziphus mucronata	*Staphylococcus aureus*	Ethanol	+	Adamu *et al.* 2004

In this study it was found that *Acorus calamus* possesses antimicrobial activity though a lack of antibacterial activity was previously reported (Phongpaichit *et al.,* 2005). Methanol extracts of *Acorus calamus* were tested against *Staphylococcus aureus* and *Escherichia coli* but the *Acorus calamus* was unable to inhibit *Staphylococcus aureus* (Phongpaichit *et al.,* 2005). In this research *Acorus calamus* shows activity against some *Staphylococcus aureus* strains and *Escherichia coli* strains (Table 4.10 - 4.19).

Albizia adianthifolia had no activity against *Staphylococcus aureus, Bacillus subtilis,* and *Klebsiella pneumoniae* according to McGaw *et al.* (2000), but in this study it was found to be active active against *Klebsiella pneumoniae, Staphylococcus aureus* and less effective against *Escherichia coli* (Table 4.10 - 4.19). Elkatib *et al*. (2006) reports that methanol extracts of *Baccharoides adoensis* leaves, stem and fruits were able to inhibit *Escherichia coli and Staphylococcus aureus.* As far as other test microorganisms are concerned it seems as if nothing has been done. The findings of Elkatib *et al.* (2006) correspond to the findings of this research as *Staphylococcus aureus* and *Escherichia coli* were inhibited by *Baccharoides adoensis* extracts (Table 4.10 - 4.19*).*

According to Martini (2004) *Combretum erythrophyllum* has shown antimicrobial activity against Gram positive and negative bacteria (*Staphyllococcus aureus* and *Escherichia coli*). *Combretum erythrophyllum* has shown antibacterial activity agianst *Staphylococcus aureus* and *Escherichia coli* as shown in (Table 4.10 - 4.19). *Gunnera perpensa* has been tested against *Staphylococcus aureus* using acetone extract (McGaw *et al.*, 2005). The results were positive which corresponds to the findings of this research (Table 4.10 - 4.19). *Lippia javanica* has previously been tested against *Staphylococcus aureus* and *Escherichia coli* (Table 4.24) (Minenzeh, 2004) which correspond to the findings of this research (Table 4.14 - 4.23). *Pentanisia prunelloides* was extracted with ethanol which resulted in the inhibition of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* (Yff *et al*., 2002). *Pentanisia prunelloides* was not effective against *Bacillus subtilis* (Table 4.10) but it was able to inhibit *Staphylococcus aureus, Klebsiella pneumoniae* and *E. coli* (Table 4.10 - 4.19).

The antibacterial activity of *Sclerocarya birrea* corresponds well with Eloff's (2001) results agains *Staphylococcus aureus* and *Escherichia coli* (Table 4.24). *Solanum aculeastrum* has been tested but not for the microorganisms used in this research (Table 4.24). Ethyl acetate extract of *Trichilia dregeana* was tested positive against *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli* (Eldeen *et al.* 2005) (Table 4.24). According to Rabe (1997) methanol extract of *Warbugia salutaris* was effective against *Escherichia coli* and ineffective against *Bacillus subtilis and Klebsiella pneumoniae* (Table 4.24) but in this research *Warbugia salutaris* was able to inhibit *Escherichia coli, Bacillus subtilis* and was unable to inhibit *Klebsiella pneumoniae* (Table 4.10 - 4.19). Ethanol extract of *Ziziphus mucronata* was found in the literature to be able to inhibit *Staphylococcus aureus and Escherichia coli* (Adamu *et al.,* 2004).

From the MIC results obtained in the present study, at least 10 plant species (Tables 4.20 - 4.21) with a MIC value of ≤ 3.13 mg/ml could be a good source of bioactive components with antimicrobial potency. These plants are: *Acanthospermum australe*, *Baccharoides adoensis*, *Clerodendium hirsutum*, *Gerbera ambigua*, *Gunnera perpensa*, *Hypericum aethiopicum*, *Hypoxis hemerocallidea*, *Sclerocarya birrea*, *Solanum uculeastrum* and *Warburgia salutaris*.

CHAPTER 5

PHYTOCHEMICAL SCREENING

5.1 INTRODUCTION

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. Unlike the absence of primary metabolites, the absence of secondary metabolities results not in immediate death, but in long-term impairment of the organism's survivability, or perhaps not in any significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group.

The function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases; they are also used for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc). Since these compounds are usually restricted to a much more limited group of organisms, they have long been of prime importance in taxonomic research (Mojab *et al*., 2003).

Phytochemical techniques are developing fast and there are now tools available which allow for the analysis of complex mixtures in novel ways. Traditional medicines offer a rich and largely unexplored source of therapeutic leads for the pharmaceutical industry (Carson & Crews, 2007). Presently, numerous products derived from medicinal plants are used which offer many opportunities in the context of drug discovery (Cragg & Newman, 2005; Carson & Crews, 2007; Raskin *et al*., 2002). Phytochemical aspects of such drugs, by reason of being a rapidly emerging field of research, deserve special attention. For example, plant extracts containing a variety of bioactive compounds may provide important combination therapies that simultaneously affect multiple pharmacological targets and provide clinical efficacy beyond the reach of single compound-based drugs (Schmidt *et al*., 2007; Williamson, 2001). However, this clearly requires a clear and well-defined strategy to characterize such extracts and the plants they are derived from phytochemically.

5.2 SECONDARY METABOLITES

5.2.1 Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Their name was derived from the word *alkaline* and was used to describe any nitrogen-containing base. Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste (Filippos *et al*., 2007).

5.2.2 Flavonoids

Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone). They are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfil many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Filippos *et al.,* 2007).

5.2.3 Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts: leaves, stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. Saponins dissolve in water to form a stable soapy froth; this is thought to be due to their amphiphilic nature. They are highly toxic to cold-blooded animals, due to their ability to lower surface tension. Some saponins are poisonous. They can cause skin rash in many people if swallowed. They are also used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are popular ingredients in shampoos and

cleansers. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently. They also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plant immune system acting as an antibiotic to protect the plant against microbes and fungus (Chatterrjee & Chakravorty, 1993).

5.2.4 Anthraquinones

An anthraquinone is an aromatic organic compound. It is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is semi-soluble in water but dissolves in alcohol, nitrobenzene and aniline. It is chemically fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes. They are also used as a laxative (Chatterrjee & Chakravorty, 1993)

5.2.5 Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos *et al*., 2007).

5.3 RESULTS

The results for the phytochemical screening of plants used in this study are summarized in Table 5.1. This table also indicates the presence of alkaloids, flavonoids, saponins, anthraquinones, cardiac glycosides or tannins in some species as described previously in other studies. Most of the plants surveyed produce different secondary metabolites, which were evidenced by the differences in their phytochemical results (Table 5.1).

Table 5.1 Phytochemical screening results. Results from the literature are indicated in blue.

Plant species	Alkaloids	Flavonoids	Saponins	Anthra-quinones	Cardiac glycosides	Tanins
Acanthospermum australe	+ + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- NPR	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)
Acorus calamus	+ + (Gilani *et al.*, 2006)	+ + (Gilani *et al.*, 2006)	- + (Gilani *et al.*, 2006)	- NPR	- NPR	- + (Gilani *et al.*, 2006)
Albizia adianthifolia	+ + (Burkill, 1985)	- NPR	- + (Burkill, 1985)	- NPR	- + (Burkill, 1985)	- + (Burkill, 1985)
Baccharoides adoensis	+ - (Oluwole *et al.*, 2007)	+ - (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- - (Oluwole *et al.*,2007)	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)
Clerodendium hirsutum	- NPR	+ NPR	- NPR	- NPR	- NPR	+ NPR
Combretum erythrophyllum	- NPR	- + (Martini *et al.*, 2004)	- NPR	+ NPR	+ NPR	+ NPR
Faurea saligna	- + (Martini *et al.*, 2004)	- NPR	- NPR	- NPR	- NPR	+ NPR
Gerbera ambigua	- NPR	- NPR	- NPR	- NPR	- NPR	+ NPR
Gunnera perpensa	+ + (McGaw *et al.*, 2008)	- NPR	- NPR	- NPR	+ NPR	+ NPR
Hypericum aethiopicum	- NPR	- NPR	- NPR	- NPR	- NPR	+ NPR

Plant species	Alkaloids	Flavonoids	Saponins	Anthra-quinones	Cardiac glycosides	Tanins
Hypoxis hemerocallidea	+ + (Muwanga, 2006)	NPR	NPR	NPR	+ + (Muwanga, 2006)	+ + (Muwanga, 2006)
Lippia javanica	+ NPR	- NPR	- NPR	- NPR	+ NPR	- NPR
Pentanisia prunelloides	- NPR	- NPR	- NPR	- NPR	+ NPR	+ NPR
Sclerocarya birrea	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- NPR	- NPR	+ + (Oluwole *et al.*, 2007)	+ + (Oluwole *et al.*, 2007)
Solanum uculeastrum	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- NPR	- NPR	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)
Trichilia dregeana	- + (Oluwole *et al.*, 2007)	- + (Oluwole *et al.*, 2007)	- NPR	- NPR	- + (Oluwole *et al.*, 2007)	+ + (Oluwole *et al.*, 2007)
Warbugia salutaris	- NPR	- NPR	- NPR	- NPR	- NPR	- NPR

+ = positive; - = negative, NPR = No previous report or information available in the literature.

5.4. DISCUSSION

Six of the plants were found to have alkaloids; three had flavonoids; seven had cardiac glycosides; ten had tannins; none had saponins and one had anthraquinones (Table 5.1). In this study very basic phytochemical methods were used for testing the plant extracts. The variation in the results between this study and those found in the literature confirms it. It is thus recommended that these plant extracts should be retested with either HPLC (High performance liquid chromatography) or GC (Gas Chromatography) methods to be more accurate.

Some of the plants investigated in this study have previously been tested for the presence of phytochemicals. According to Oluwole *et al.,* (2007) *Acanthospermum australe* tested positive for alkaloids, flavonoids, glycosides and tanins and negative for saponins. It was not tested for anthraquinones. In this study *A. australe* tested only positive for alkaloids (Table 5.1). *Acorus calamus* was previously tested positive for alkaloids and flavonoids and corresponds to the results of this research. Literature reports that it contains saponins and tanins but in this research it tested negative for it. Anthraquinones and glycosides have not been tested before (Gilani, *et al.,* 2006). *Albizia adianthifolia* was tested for alkaloids, saponins, glycosides, and tanins and were all found to be present (Burkill, 1985). These findings contradict with the ones in this research. Flavonoids and anthraquinones have never been tested before. Oluwole *et al*., (2007) found that *Baccharroides adoensis* had saponins, glycosides and tanins but did not have alkaloids, flavonoids and anthranoids. The results for anthraquinones and tanins correspond to the ones in this research (Table 5.1). For *Clerodendrum hirsutum* no information concerning the phytochemicals tested was found. Flavonoids were the only phytochemical to be tested in *Combretum erythrophyllum* and tested positive for it (Martini *et al.,* 2004). Alkaloids were the only phytochemical found to be tested previously in *Faurea saligna* (Table 5.1).

No results could be found in the literature for phytochemicals in the following plants *Gerbera ambigua* and *Hypericum aethiopicum*. Alkaloids are present in *Gunnera perpensa* (McGaw *et al.,* 2008). *Hypoxis hemerocallidea* was found to be having alkaloids, cardiac glycosides and tanins, the other phytochemicals were not tested (Muwanga, 2006). No information could be found concerning the testing of these phytochemicals for *Lippia javanica, Pentanisia prunelloids* and *Warbugia salutaris.* Literature revealed that *Sclerocarya birrea* tested positive for alkaloids, flavonoids, cardiac glycosides and tannins (Oluwole *et al.,* 2007). The results for the alkaloids and flavonoids contradict the ones in this research. Oluwole *et al.,* (2007) report that saponins and anthraquinones were absent in *Sclerocarya birrea* which corresponds to results in this research. *Solanum uculeastrum* was tested negative for all six phytochemicals but Oluwole *et al.* (2007) found alkaloids, flavonoids, glycosides and tanins to be present. Tanins were the only phytochemicals found to be present in

Trichilia dregeana in this study which contradicted with Oluwole *et al.* (2007) findings of the presence of alkaloids, flavonoids, and glycosides.

CHAPTER 6

BIO-AUTOGRAPHIC ASSAY

6.1 INTRODUCTION

The detection and identification of specific molecules may often be accomplished most conveniently by autographic methods. These procedures, in which nutritionally deficient bacterial cells suspended in solid minimal media show zones of growth in the presence of required nutrients, has several advantages over liquid assay methods. These include simplicity of technique and the reduction of interference from inhibitory material which may be present in crude samples as natural constituents (Lockhart, 1954).

The number of antibiotic-resistant pathogens has increased drastically in recent years. Consequently, there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy. These types of compounds have potential in decreasing the effective dose of antimicrobial drugs for therapy. *In vitro* experiments have shown that natural products and some of their components decrease the minimum inhibitory concentration (MIC) of antibiotics for different microorganisms. Bio-autographic methods combine chromatographic separation and *in situ* activity determination, facilitating the localization and target-directed isolation of active constituents in a mixture. Traditionally, the bio-autographic technique has used the growth inhibition of microorganisms to detect the anti-microbial components of extracts chromatographed on thin-layer chromatography (TLC). This methodology has been considered as the most effective assay for the detection of anti-microbial compounds (Shahverdi *et al*. 2006).

6.2 RESULTS

The plant extracts that showed the highest activity against all microorganisms tested were selected for this experiment. The TLC plates and corresponding plates from the bio-autographic assay were photographed and the R_f values were calculated using the following formula:

R_f =Distance moved by the compound/Distance moved by solvent system.

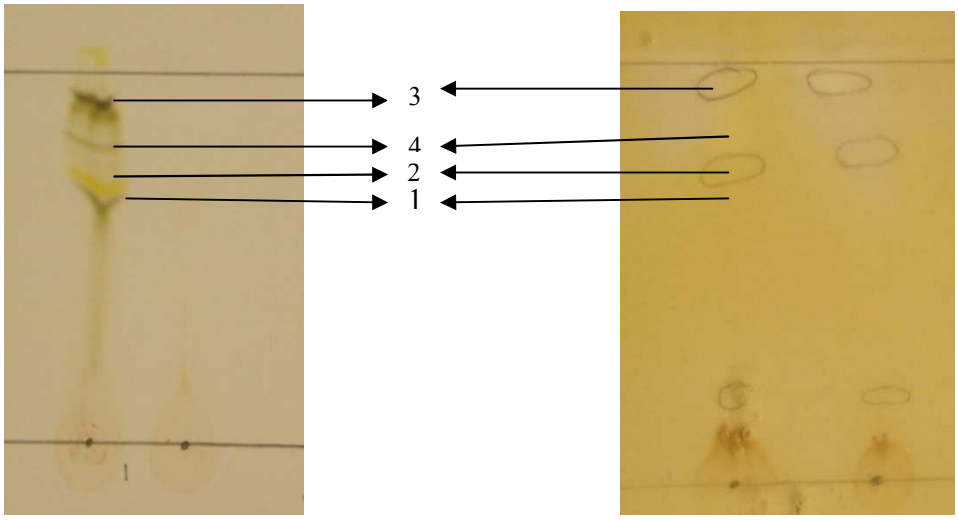

Figure 6.1 The TLC plate (left) and the bio-autographic assay results plate (right) of *Trichilia dregeana.*

1. R_f = 0.6 2. R_f = 0.8 3. R_f = 0.9 4. R_f = 0.7

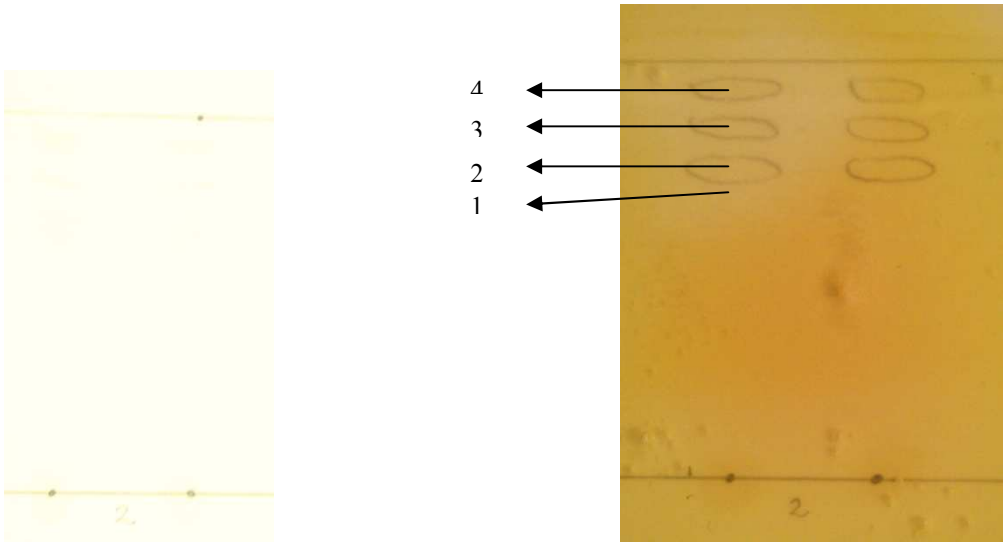

Figure 6.2 The TLC plate (left) and the bio-autographic assay results plate (right) for *Pentanisia prunnelloides.*

1. R_f =0.10 2. R_f = 0.8 3. R_f = 0.8 4. R_f = 0.9

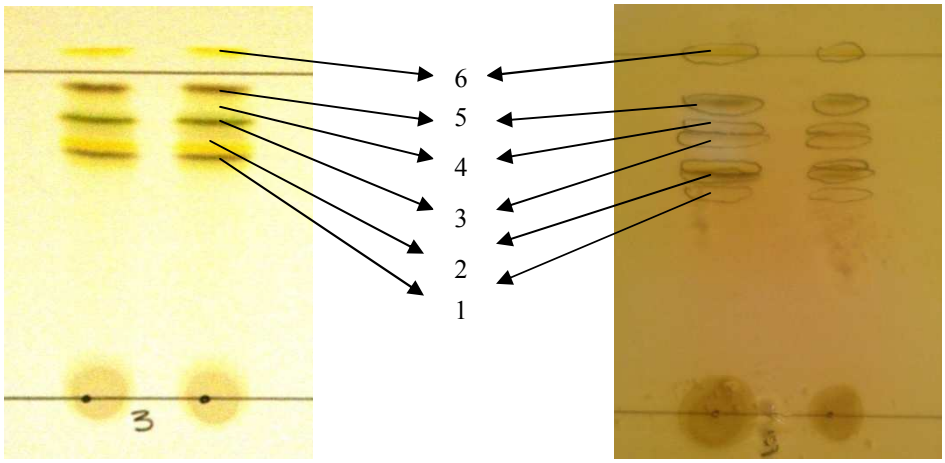

Figure 6.3 The TLC plate (left) and the bio-autographic assay results plate (right) for *Lippia javanica*.

1. R_f = 0.7 2. R_f = 0.7 3. R_f = 0.7 4. R_f = 0.5

5. R_f = 0.5 6. R_f = 0.9

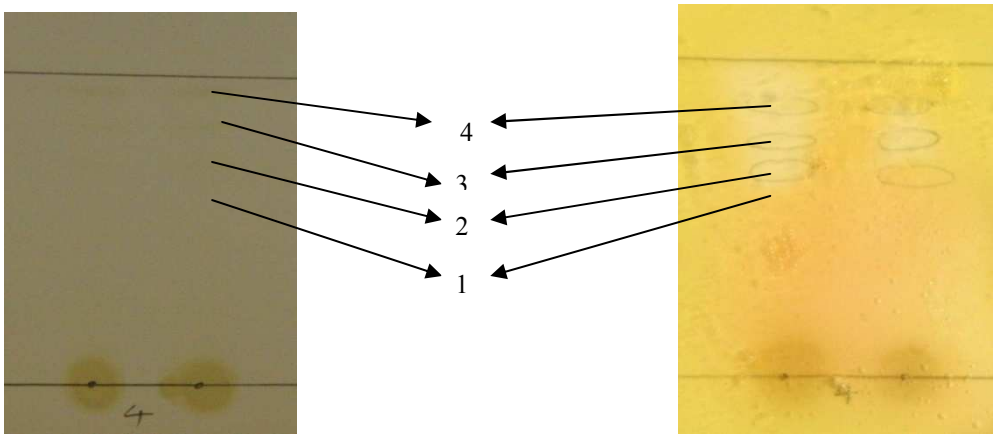

Figure 6.4 The TLC plate (left) and the bio-autographic assay results plate (right) for *Hypoxis hemerocallidea.*

1. R_f = 0.6 2. R_f = 0.7 3. R_f =0.8 4. R_f = 0.9

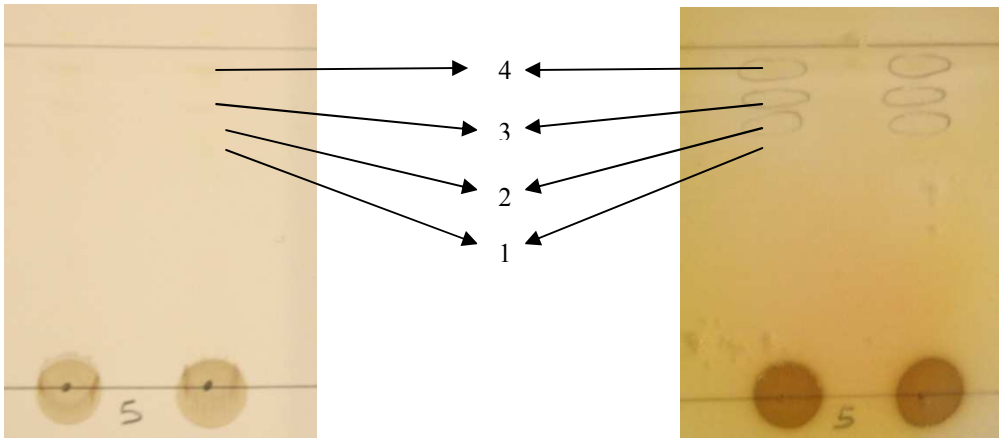

Figure 6.5 The TLC plate (left) and the bio-autographic assay results plate (right) for *Gunnera perpensa.*

1. R_f = 0.6 2. R_f =0.7 3. R_f = 0.8 4. R_f = 0.9

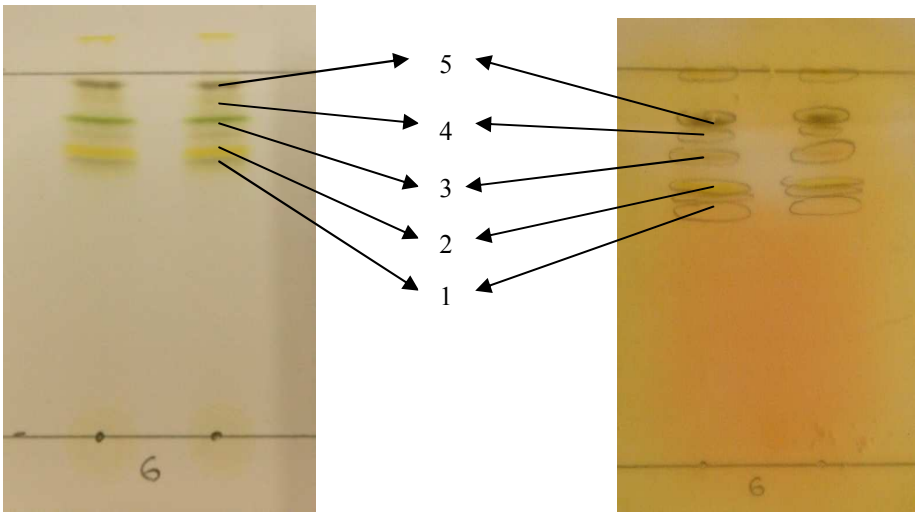

Figure 6.6 The TLC plate (left) and the bio-autographic assay results plate (right) of *Warbugia salutaris.*

R_f = 0.7 2. R_f = 0.8 3. R_f = 0.8 4. R_f = 0.9

5. R_f =1

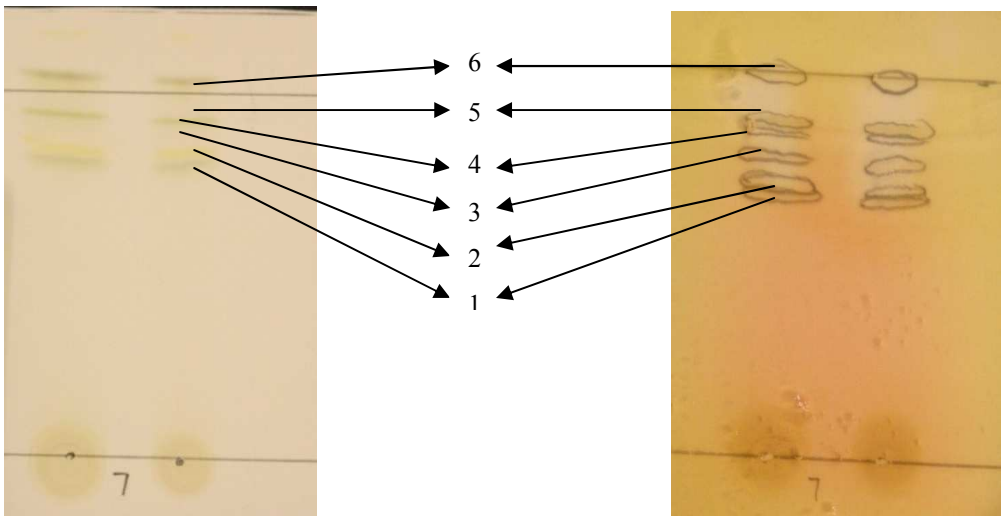

Figure 6.7 The TLC plate (left) and the bio-autographic assay results plate (right) of *Hypericum aethiopicum*.

1. R_f = 0.6 2. R_f = 0.6 3. R_f = 0.7 4. R_f = 0.8

5. R_f =0.8 6. R_f = 0.9

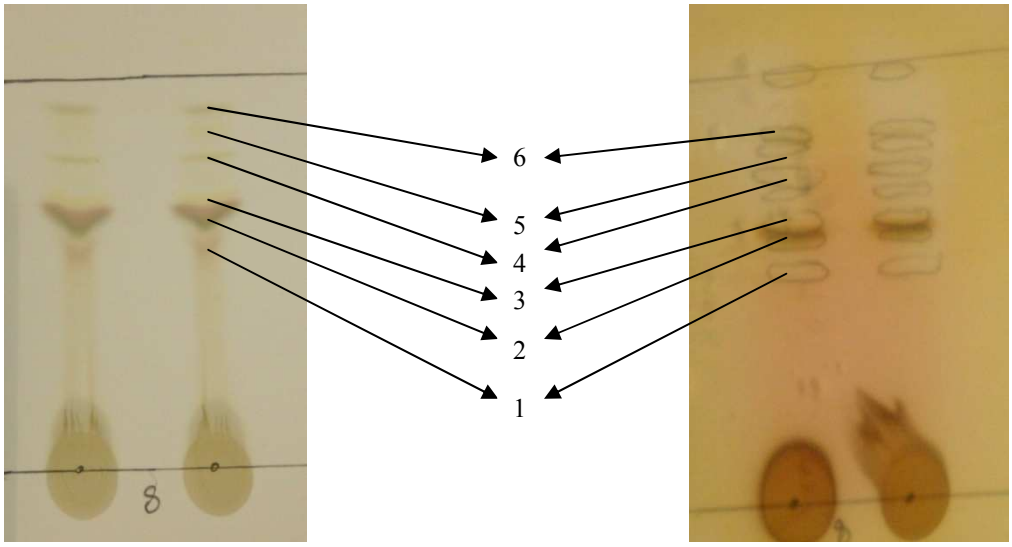

Figure 6.8 The TLC plate (left) and the bio-autographic assay results plate (right) for *Combretum erythrophyllum.*

1. R_f = 0.5 2. R_f =0.6 3. R_f = 0.7 4. R_f = 0.8

5. R_f = 0.8 6. R_f = 0.9

6.3 DISCUSSION

The bio-autoautographic assay was carried out to dertemine the presence of individual compounds with antibacterial activity, which would allow for further isolation of the active compounds. The spots that are marked with the arrows are the compounds that each plant secreats that were separated by the TLC method and the R_f values were calculated. The results obtained showed that the compounds responsible for the inhibition of microorganisms are more than one for each plant which is supported by the zones of inhibition on the plates. The results showed that the larger the variety of compounds in an extract the better the chances of inhibition, as visible in *Combretum erythrophyllum* extract (Figure 6.8). The results were interpreted as follows: the clear zones were the zones of inhibition by the plant compound and the pink color was the INT that was sprayed on the plate to allow us to see the growth of microorganisms that remained pink. It is recommended to isolate these active compounds and identify them with NMR (Nuclear magnetic resonance spectroscopy) and MS (Mass spectrometry).

CHAPTER 7

SUMMARY AND CONCLUSION

7.1 SUMMARY

Plant specimens and ethnobotanical information were collected in different areas of the uThungulu District Municipality, in the Ongoye region of KwaZulu-Natal Province, in South Africa. These areas include Makholokholo, Mhlaleni, Ntshidi, Ekuphumuleni, Macekane, Obisana, Gugushe, Ophongola, and Sinjingwane. From these areas information of 33 plant species were found; 18 plant species were collected for experimental purposes, identified, prepared for herbarium purposes, and tested for antimicrobial activity. The questionnaire was used as the main information collection tool.

The collected plants which were tested were selected on the basis of availability. Each plant was separated into different parts, namely, the leaves, stem, barks and roots. But mostly, the focus was on the parts that were mentioned by the people during the survey. The interviews revealed that *Hypericum aethiopicum* (unsukumbili) is the plant that is most trusted by the people of the Ongoye area to heal sores and wounds as it was mentioned more repeatedly during the survey than any of the other plants.

Antibacterial activity testing was carried out with ten microorganisms, four of which were ATCC strains; and six were multi-drug-resistant strains from Lancet Laboratories, Durban. Agar well diffusion and disc diffusion antibacterial assays were carried out with a view to finding out how effective the plants were against the bacteria which infect sores and wounds. The controls used were the antibiotic, neomycin (positive) and the negative control DMSO. The clear zones obtained from these methods were measured and compared to the controls in millimeters.

The serial dilution assay was conducted to determine the MIC which is described in Chapter 2. Positive and negative controls were also used for comparison purposes. At the end, the INT had to be added in order to get the pink/purple color. Comparing

the results obtained from the agar well diffusion, the disc diffusion and the MIC revealed the following seven plants, which were the most effective against the micro-organisms tested: *Hypericum aethiopicum, Gunnera perpensa, Warbugia salutaris, Hypoxis hemerocallidea, Lippia javanica, Pentanisia prunelloides* and *Trichilia dregeana.*

Preliminary TLC and the biographic assay tests were carried out on above mentioned plants to find out how many compounds were responsible for the inhibition of the bacteria tested. The results showed that the antibacterial assay can be attributed to the compounds observed at the various R_f values on the TLC separation. Also sometimes the activity of compounds is not easily observed in this assay, if the compound does not diffuse through the agar, then the activity could be masked (= false-negative).

7.2 CONCLUSION

Sustainable management of traditional medicinal plant resources is very important, not only because of their value as a potential source of new drugs but also due to community reliance on traditional medicinal plants for health. Certain vegetation types that are sources of supply of traditional medicines have drastically declined due to forest clearance for agriculture, afforestation of montane grasslands, uncontrolled burning and livestock grazing. Exclusion from core conservation areas adversely affects the traditional healers who previously gathered the medicinal plants from these sites. The supply of herbal medicines to traditional healers is affected by competing resource uses such as for building materials and fuels, timber logging, commercial harvesting for exports, and the extraction of pharmaceuticals. This creates a growing demand for fewer resources, in some cases resulting in the local disappearance of favoured and effective sources of traditional medicine and reduced species diversity. The most vulnerable species are popular, slow-growing or slow to reproduce; or species with a specific habitat requirement and a limited distribution. Although in theory, the sustainable use of bark, roots or whole plants used as herbal medicines is possible, the high levels of money and manpower required for the intensive management of slow-growing species in multiple-species systems are unlikely to be found. The cultivation of alternative sources of supply of popular, high-

conservation, priority species outside of core conservation areas is therefore essential. However cultivation of such species is not a simple solution and at present is unlikely to be profitable due to slow growth rates for most tree species and the low prices paid for traditional medicines.

Commercial gatherers of medicinal plant material for trade are poor women whose aim is not resource management but earning money. Cultivation as an alternative to the over-exploitation of scarce traditional medicinal plants was suggested over 65 years ago in South Africa for scarce and effective species such as *Warbugia salutaris.* Until 1991, no large scale cultivation had taken place. There are two main reasons for this are: (1) A lack of institutional support for the production and dissemination of key species for cultivation. (2) The low prices paid for traditional medicinal plants by herbal medicine traders and urban herbalist. If cultivation is to be successful as an alternative source of supply to improve the self-sufficiency of traditional healers and to take harvesting pressure off wild stocks, then plants have to be produced cheaply and in large quantities. Any cultivation for urban demand will be competing with material harvested from the wild that is supplied into the market by commercial gatherers who have no input costs for cultivation. Prices will therefore increase with scarcity due to transport costs, search time and long-distance trade. The prices paid to gatherers are very low, taking no account of annual sustainable off-take. In many cases, medicinal plants are also an open-access, rather than a limited-access or private resource. To make a living, commercial medicinal plant gatherers, therefore, mine rather than manage these resources.

Traditional healers are very aware of the conservation status of local traditional plant resources and can be influential in changing local opinion so as to limit over-exploitation. It is recommended that support be given to the formation of rural traditional healers associations and to the self-sufficiency of traditional healers. This might be through local health services, with the support of the World Health Organization Traditional Medicine programme. Information should be disseminated to rural communities on appropriate cultivation methods. Very little goes unnoticed in communally owned areas, so if problems arise regarding the depletion of valued local resources, traditional healers' associations or community leaders are likely to

be at least effective as forest guards and could draw on conservation or forest guard support where necessary.

It is recommended that the preparation of the extracts resemble the methods of preparation which are used by the people. In that way more positive results can be found. It is also recommended that further work be done on the isolation and the identification of the most effective antimicrobial compounds responsible for the inhibition of the micro-organisms tested. This study lends thus some support to traditional knowledge and may serve as a basis for selecting the most active medicinal plants to use in traditional medicine practices in the future.

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APPENDIX I: Research Questionnaire

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)?

Age Group:

Infants	
Children	
Adults	

APPENDIX II: Antimicrobial activity results tables

Agar-well diffusion

Table 4.2 Results of sensitivity of different bacteria against **acetone** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against acetone plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	18	13	17	-	16	10	-	6	-	10
Acanthospermum australe (leaves)	17	-	32	-	12	13	16	-	-	11
Acorus calamus	-	-	12	-	-	11	16	6	-	-
Albizia adianthifolia (stem)	-	14	10	-	10	10	-	-	-	6
Albizia adianthifolia (leaves)	-	-	13	-	10	13	-	-	-	-
Baccharoides adoensis (stem)	-	-	12	-	10	9	11	5	11	-
Baccharoides adoensis (leaves)	-	-	11	-	10	9	14	-	-	10
Clerodendium hirsutum	-	-	15	-	16	17	11	7	14	-
Combretum erythrophyllum	31	-	21	-	22	20	-	-	-	-
Dioscorea dregeana	-	-	11	-	13	17	10	-	-	-
Faurea saligna	-	-	20	-	10	-	10	-	-	-
Gerbera ambigua	-	10	18	-	14	10	-	-	13	-
Gunnera perpensa	22	-	13	-	20	27	10	9	11	-
Hypericum aethiopicum (leaves)	13	14	13	-	15	30	-	-	10	-

Sensitivity of different bacteria against acetone plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Hypericum aethiopicum (stem)	10	-	15	-	13	14	15	11	-	9
Lippia javanica (leaves)	-	-	17	-	14	12	11	-	-	-
Lippia javanica (stem)	-	-	20	-	-	15	10	-	-	-
Pentanisia prunelloides	-	-	18	-	15	23	11	9	11	-
Sclerocarya birrea	21	11	12	-	21	15	18	19	-	15
Sclerocarya birrea (bark)	20	9	21	-	16	20	-	-	10	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	11	-
Solanum uculeastrum (leaves)	17	-	-	-	-	-	-	-	-	7
Solanum uculeastrum (root)	-	-	17	-	-	8	-	-	-	-
Trichilia dregeana (stem)	21	14	13	-	-	12	-	-	-	10
Trichilia dregeana (leaves)	17	-	20	-	16	14	-	-	-	-
Warburgia salutaris (leaves)	-	-	15	-	20	15	15	-	-	-
Ziziphus mucronata (stem)	19	15	30	-	15	9	-	9	-	-
Ziziphus mucronata (leaves)	18	-	21	-	14	10	10	-	11	8
Neomycin	14	15	30	31	16	18	21	24	19	20

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Table 4.3 Results of sensitivity of different bacteria against **methanol** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

	Sensitivity of different bacteria against methanol plant extracts (mm)									
	ATTC CULTURES				**SA**	**SA**	**SA**	**EC**	**EC**	**EC**
PLANT NAMES	**BS**	**KP**	**SA**	**EC**	**P5020**	**P4790**	**T1266**	**U1505S**	**U16406**	**U16403**
Acanthospermum australe (stem)	13	_	20	_	_	19	_	10	-	-
Acanthospermum australe (leaves)	22	_	24	10	_	17	_	11	12	-
Acorus calamus	10	_	11	11	_	_	13	-	-	-
Albizia adianthifolia (stem)	_	_	14	_	_	_	15	14	-	-
Albizia adianthifolia (leaves)	_	_	18	_	_	10	_	12	-	-
Baccharoides adoensis (stem)	_	15	11	_	_	23	19	-	-	-
Baccharoides adoensis (leaves)	_	18	13	_	_	_	17	11	-	-
Clerodendium hirsutum	12	_	11	9	10	_	_	-	14	-
Combretum erythrophyllum	20	_	22	_	13	25	20	-	-	-
Dioscorea dregeana	18	10	10	8	11	_	_	14	-	-
Faurea saligna	13	_	19	_	17	17	15	-	-	-

Sensitivity of different bacteria against methanol plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Gerbera ambigua	16	_	21	_	_	_	_	-	-	-
Gunnera perpensa	22	18	16	16	13	27	23	-	-	-
Hypericum aethiopicum (leaves)	13	_	16	_	15	_	13	-	-	-
Hypericum aethiopicum (stem)	17	_	18	_	_	_	15	-	-	-
Lippia javanica (leaves)	_	_	11	-	19	_	_	-	-	-
Lippia javanica (stem)	_	_	21	-	_	_	_	21	11	-
Pentanisia prunelloides	_	16	21	13	20	17	_	-	-	-
Sclerocarya birrea	9	17	13	17	24	_	_	18	27	-
Sclerocarya birrea (bark)	10	17	18	10	20	23	_	-	-	-
Solanum uculeastrum (stem)	10	_	_	_	_	15	_	11	-	-
Solanum uculeastrum (leaves)	_	_	_	_	19	_	_	-	-	-
Solanum uculeastrum (root)	_	_	_	_	_	_	_	-	-	-
Trichilia dregeana (stem)	22	_	19	_	23	12	19	-	16	-
Trichilia dregeana (leaves)	15	_	16	_	_	15	_	-	-	-

Sensitivity of different bacteria against methanol plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Warburgia salutaris (leaves)	15	_	20	16	15	_	_	-	-	-
Ziziphus mucronata (stem)	15	_	26	_	_	12	18	-	-	-
Ziziphus mucronata (leaves)	20	_	23	_	_	13	_	16	14	11
Neomycin	17	15	30	21	18	20	18	19	13	16

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results:

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Table 4.4 Results of sensitivity of different bacteria against **cold water** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

	Sensitivity of different bacteria against cold water plant extracts (mm)									
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	9	-	7	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	15	-	5	-	-	-	-	-	-	-
Acorus calamu	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	9	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-

Sensitivity of different bacteria against cold water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	9	-	-	-	-	-	-	11	-	-
Combretum erythrophyllum	10	-	-	-	-	-	-	-	-	-
Faurea saligna	-	-	7	-	-	-	-	-	-	-
Gerbera ambigua	12	-	-	-	-	-	13	-	-	-
Gunnera perpensa	10	10	7	-	15	-	11	-	-	-
Hypericum aethiopicum (leaves)	9	14	-	-	11	10	-	-	-	-
Hypericum aethiopicum (stem)	8	-	-	-	-	13	-	-	-	-
Hypoxis hemerocallidea	11	-	9	-	-	-	-	14	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	4	-	-	-	-	-	-	-
Sclerocarya birrea	8	-	-	-	-	-	-	17	-	10
Sclerocarya birrea (bark)	6	-	-	-	-	-	-	-	-	-

Sensitivity of different bacteria against cold water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	13	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	12	-	17	6	10	17	-	11
Strychnos deccusata (stem)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	10
Neomycin	38	33	13	30	21	16	15	19	31	28

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction

Table 4.5 Results of sensitivity of different bacteria against **hot water** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against hot distilled water plant extracts (mm)										
	ATTC CULTURES				SA	SA	SA	EC	EC	EC
PLANT NAMES	BS	KP	SA	EC	P5020	P4790	T1266	U1505S	U16406	U16403
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	9	-	-	-	-	10	-	-	-	-
Acorus calamus	-	-	10	-	-	-	15	-	15	-
Albizia adianthifolia (stem)	-	-	-	-	17	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	13	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	10	14	11
Baccharoides adoensis (leaves)	6	-	-	9	-	-	-	-	-	-
Clerodendium hirsutum	-	21	-	-	-	-	-	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	9	-	7	-
Faurea saligna	-	-	17	-	-	9	-	-	-	-
Gerbera ambigua	-	-	-	-	13	-	10	-	-	-
Gunnera perpensa	-	-	-	-	12	-	-	-	-	-
Hypericum aethiopicum (leaves)	-	-	-	-	16	-	-	-	-	-
Hypericum aethiopicum (stem)	11	-	-	-	13	17	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	-	-	-	-	-	-

Sensitivity of different bacteria against hot water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	13	-	11	-	-	10	-	-
Sclerocarya birrea	-	19	-	-	-	-	18	15	11	17
Sclerocarya birrea (bark)	-	-	-	-	14	19	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	8	-	-	-
Solanum uculeastrum (leaves)	-	-	-	-	-	-	-	-	-	10
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	11	-
Strychnos deccusata (stem)	-	-	-	-	-	-	21	-	-	-
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	10
Trichilia dregeana (leaves)	-	-	15	-	11	17	-	-	19	-
Warburgia salutaris (leaves)	-	-	-	-	17	17	-	-	13	-
Warburgia salutaris (stem)	-	-	-	-	23	-	-	11	10	17
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	20	18	16	20	25	22	20	21	20	18

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Disc-diffusion assay

Table 4.6 Results of sensitivity of different bacteria against **acetone** plant extracts. DMSO and neomycin(0.1mg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against acetone plant extracts (mm)										
	ATTC CULTURES				SA	SA	SA	EC	EC	EC
PLANT NAMES	BS	KP	SA	EC	P5020	P4790	T1266	U1505S	U16406	U16403
Acanthospermum australe (stem)	20	10	19	-	21	9	-	-	-	15
Acanthospermum australe (leaves)	20	-	23	-	17	16	22	10	-	12
Acorus calamus	-	-	10	-	-	15	18	11	-	-
Albizia adianthifolia (stem)	-	10	13	-	12	12	-	-	-	9
Albizia adianthifolia (leaves)	-	-	17	-	17	15	15	-	-	-
Baccharoides adoensis (stem)	-	-	10	-	13	11	12	-	-	-
Baccharoides adoensis (leaves)	-	-	12	-	13	11	11	14	-	10
Clerodendium hirsutum	-	-	10	-	22	19	19	-	14	-
Combretum erythrophyllum	27	-	19	-	10	22	-	-	-	-
Faurea saligna	-	-	20	-	15	-	12	10	-	-
Gerbera ambigua	-	13	20	-	15	12	-	-	15	-

Sensitivity of different bacteria against acetone plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Gunnera perpensa	20	-	15	-	16	30	15	12	12	-
Hypericum aethiopicum (leaves)	10	11	15	-	15	24	-	-	12	-
Hypericum aethiopicum (stem)	10	-	17	-	16	15	16	9	-	12
Hypoxis hemerocallidea	-	-	9	-	10	19	16	9	-	11
Lippia javanica (leaves)	-	-	-	-	15	19	10	11	-	-
Lippia javanica (stem)	-	-	10	-	-	20	15	-	-	-
Pentanisia prunelloides	-	-	20	-	13	20	17	12	11	10
Sclerocarya birrea	17	9	12	-	17	19	15	12	17	18
Sclerocarya birrea (bark)	22	13	17	-	19	15	-	-	18	-
Solanum uculeastrum (stem)	-	-	18	-	-	-	-	-	17	-
Solanum uculeastrum (leaves)	19	-	-	-	-	10	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (stem)	17	17	25	-	23	11	-	-	-	-
Strychnos deccusata (leaves)	19	-	22	-	14	8	16	-	11	-

Sensitivity of different bacteria against acetone plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Trichilia dregeana (stem)	17	10	18	-	-	15	-	-	10	13
Trichilia dregeana (leaves)	19	-	15	-	19	17	-	-	-	-
Warburgia salutaris (leaves)	-	-	-	-	16	17	14	10	-	-
Warburgia salutaris (stem)	-	-	-	-	20	22	17	19	10	16
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	15	15	30	30	20	20	25	24	20	23

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Table 4.7 Results of sensitivity of different bacteria against **methanol** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against methanol plant extracts (mm)										
	ATTC CULTURES				SA	SA	SA	EC	EC	EC
PLANT NAMES	BS	KP	SA	EC	P5020	P4790	T1266	U1505S	U16406	U16403
Acanthospermum australe (stem)	13	-	18	-	-	17	-	13	-	-
Acanthospermum australe (leaves)	24	-	33	8	-	19	-	-	-	-
Acorus calamus	9	-	13	9	-	-	17	-	-	-
Albizia adianthifolia (stem)	-	-	11	-	-	-	15	12	11	-
Albizia adianthifolia (leaves)	-	-	14	-	-	13	-	-	-	-
Baccharoides adoensis (stem)	-	17	13	-	-	20	23	-	-	-
Baccharoides adoensis (leaves)	-	21	12	-	-	-	19	-	-	-
Clerodendium hirsutum	16	-	16	10	14	-	-	17	-	-
Combretum erythrophyllum	22	-	21	-	15	27	22	-	-	-
Faurea saligna	15	-	21	-	13	17	12	-	11	-
Gerbera ambigua	12	-	19	-	-	-	-	-	-	-
Gunnera perpensa	24	22	14	19	19	29	24	-	-	-
Hypericum aethiopicum (leaves)	10	-	14	-	16	-	13	-	-	-
Hypericum aethiopicum (stem)	19	-	20	-	-	-	17	-	-	-

Sensitivity of different bacteria against methanol plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Hypoxis hemerocallidea	18	13	12	8	15	-	-	-	-	-
Lippia javanica (leaves)	-	-	18	-	17	-	-	-	17	-
Lippia javanica (stem)	-	-	21	-	-	-	-	11	-	-
Pentanisia prunelloides	-	12	19	11	20	19	-	10	-	-
Sclerocarya birrea Hochst	10	15	13	21	27	-	-	10	17	-
Sclerocarya birrea (bark)	12	19	22	13	23	19	-	-	-	-
Solanum ciculeastrum (stem)	12	-	-	-	-	18	-	19	-	-
Solanum cicuculeastrum (leaves)	-	-	-	-	13	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (stem)	17	-	31	-	-	14	18	-	-	-
Strychnos deccusata (leaves)	21	-	22	-	-	10	-	-	-	-
Trichilia dregeana (stem)	20	-	14	-	21	15	22	-	-	-
Trichilia dregeana (leaves)	17	-	21	-	-	13	-	-	14	-
Warburgia salutaris (leaves)	13	-	21	15	10	-	-	-	11	-
Warburgia salutaris (stem)	15	-	19	22	16	-	-	-	-	-

Sensitivity of different bacteria against methanol plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	27	13	30	33	26	25	27	24	22	24

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Table 4.8 Results of sensitivity of different bacteria against **cold water** plant extracts. DMSO and neomycin (30µg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against cold water plant extracts (mm)										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	9	-	5	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	17	-	7	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	13	-	-

Sensitivity of different bacteria against cold water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Clerodendium hirsutum	12	-	-	-	-	-	-	-	-	-
Combretum erythrophyllum	9	-	-	-	-	-	-	-	-	-
Faurea saligna	-	-	4	-	-	-	-	-	-	-
Gerbera ambigua	15	-	-	-	-	-	-	-	-	-
Gunnera perpensa	7	13	9	-	15	-	-	-	-	-
Hypericum aethiopicum (leaves)	12	19	-	-	11	10	-	-	-	-
Hypericum aethiopicum (stem)	9	-	-	-	-	13	-	-	-	-
Hypoxis hemerocallidea	10	-	9	-	-	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	7	-	-	-	-	-	-	-
Sclerocarya birrea	9	-	-	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	7	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	15	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-

Sensitivity of different bacteria against cold water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Strychnos deccusata (stem)	-	-	-	-	10	-	-	-	-	-
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris	-	-	11	-	20	9	-	-	-	-
Warburgia salutaris (stem)	-	-	13	-	-	-	-	19	-	-
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	38	33	13	30	21	16	15	-	31	28

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Table 4.9 Results of sensitivity of different bacteria against **hot water** plant extracts. DMSO and neomycin (0.1mg/ml) as negative and positive controls respectively.

Sensitivity of different bacteria against hot distilled water plant extracts (mm)										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	-	-	-	-	-		-	11	-	-
Acanthospermum australe (leaves)	12	-	-	-	10	-	-	-	-	-
Acorus calamus	-	-	-	-	-	6	-	-	-	-
Albizia adianthifolia (stem)	-	6	-	-	12	6	-	-	-	-
Albizia adianthifolia (leaves)	-	6	-	-	12	6	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	14	-	-
Baccharoides adoensis (leaves)	10	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	-	-	25	-	25	6	12	-	6	-
Combretum erythrophyllum	3	3	12	-	12	25	-	-	3	-
Faurea saligna Harv.	-	-	50	-	25	-	12	-	-	-
Gerbera ambigua	-	6	25	-	25	12	-	-	6	-
Gunnera perpensa	3	3	25	-	3	-	12	-	3	-
Hypericum aethiopicum (leaves)	12	3	6	-	6	12	-	-	3	-
Hypericum aethiopicum (stem)	-	-	6	-	12	50	50	12	-	-
Hypoxis hemerocallidea	-	-	-	-	-	-	15	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	9	-	-	-	-

Sensitivity of different bacteria against hot water plant extracts (mm)										
PLANT NAMES	ATCC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	-	-	11	-	-	-	-	-
Sclerocarya birrea	-	16	-	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	-	-	-	-	-	-	-	-	-
Solanumuiculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (stem)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	14	-	-	-
Trichilia dregeana (leaves)	-	-	18	-	-	-	-	-	-	-
Warburgia salutaris Isibhaha (leaves)	-	-	-	-	19	-	-	-	-	-
Warburgia salutaris(stem)	-	-	-	-	10	-	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	22	20	21	23	25	30	27	21	20	21

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Average Calculation of results: $\frac{a+b}{2}$

a – Measurement of zone of inhibition in vertical direction, b – measurement of zone of inhibition in horizontal direction.

Minimum inhibitory concentrations (MIC)

Table 4.10 Minimum inhibitory concentrations results for the **acetone** plant extracts.

MIC results in mg/ml										
	ATTC CULTURES				SA	SA	SA	EC	EC	EC
PLANT NAMES	BS	KP	SA	EC	P5020	P4790	T1266	U1505S	U16406	U16403
Acanthospermum australe (stem)	-	-	3.125	-	6.25	-	50	-	-	6.25
Acanthospermum australe (leaves)	-	50	25	-	12.5	3.125	-	-	-	-
Acorus calamus	-	-	-	-	-	12.5	6.25	-	-	-
Albizia adianthifolia Usolo (stem)	-	6.25	-	-	12.5	6.25	-	-	-	-
Albizia adianthifolia (leaves)	-	6.25	-	-	12.5	6.25	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	25	-	12.5	-
Baccharoides adoensis (leaves)	-	-	-	-	-	6.25	-	-	-	-
Clerodendium hirsutum	-	-	25	-	25	6.25	12.5	-	6.25	-
Combretum erythrophyllum	3.125	3.125	12.5	-	12.5	25	-	-	3.125	-
Faurea saligna	-	-	50	-	25	-	12.5	-	-	-
Gerbera ambigua	-	6.25	25	-	25	12.5	-	-	6.25	-
Gunnera perpensa	3.125	3.125	25	-	3.125	-	12.5	-	3.125	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Hypericum aethiopicum (leaves)	12.5	3.125	6.25	-	6.25	12.5	-	-	3.125	-
Hypericum aethiopicum (stem)	-	-	6.25	-	12.5	50	50	12.5	-	-
Hypoxis hemerocallidea	-	50	-	-	-	12.5	25	-	-	-
Lippia javanica (leaves)	-	-	50	-	-	3.125	12.5	-	-	25
Lippia javanica (stem)	-	-	50	-	-	-	12.5	-	-	-
Pentanisia prunelloides	-	-	6.25	-	25	-	-	-	6.25	-
Sclerocarya birrea	25	-	-	-	12.5	6.25	25	6.25	-	25
Sclerocarya birrea (bark)	25	-	-	-	12.5	12.5	-	-	6.25	50
Solanum uculeastrum Intuma (stem)	-	-	-	-	-	6.25	-	-	12.5	-
Solanum uculeastrum Intuma (leaves)	-	-	-	-	-	6.25	-	-	-	-
Solanum uculeastrum (root)	-	12.5	-	-	25	6.25	-	-	6.25	
Strychnos deccusata (stem)	12.5	6.25	12.5	-	-	12.5	-	-	-	25
Strychnos deccusata (leaves)	25	-	12.5	-	25	6.25	50	-	6.25	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Trichilia dregeana (stem)	12.5	50	12.5	-	-	6.25	-	-	-	12.5
Trichilia dregeana (leaves)	12.5	-	6.25	-	50	12.5	-	-	-	-
Warburgia salutaris (leaves)	-	-	-	-	50	-	6.25	-	-	-
Warburgia salutaris(stem)	-	-	-	-	6.25	6.25	6.25	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	3.13	3.13	0.78	1.56	6.25	3.13	6.25	6.25	6.25	3.13

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Table 4.11 Minimum inhibitory concentrations results for the methanol plant extracts.

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	3.13	3.13	3.13	-	12.5	3.13	-	50	25	-
Acanthospermum australe (leaves)	6.25	-	-	-	-	-	-	-	-	-
Acorus calamus	25	25	25	-	-	-	25	-	-	-
Albizia adianthifolia (stem)	-	50	12.5	-	-	-	3.13	25	-	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Albizia adianthifolia Usolo (leaves)	12.5	-	6.25	-	-	12.5	-	-	-	-
Baccharoides adoensis (stem)	-	1.56	12.5	-	-	1.56	3.13	-	-	-
Baccharoides adoensis (leaves)	-	25	12.5	-	-	25	3.13	25	-	-
Clerodendium hirsutum	12.5	50	25	-	3.13	-	-	-	12.5	-
Combretum erythrophyllum	6.25	6.25	3.13	-	6.25	6.25	12.5	-	-	-
Faurea saligna	-	-	12.5	-	-	12.5	3.13	-	-	-
Gerbera ambigua	3.13	25	3.13	-	-	-	-	-	-	-
Gunnera perpensa	3.13	50	3.13	-	6.25	12.5	6.25	-	-	-
Hypericum aethiopicum (leaves)	6.25	0.785	0.78	-	6.25	-	12.5	-	-	-
Hypericum aethiopicum (stem)	6.25	-	-	-	-	-	6.25	-	-	-
Hypoxis hemerocallidea	12.5	6.25	25	-	3.13	-	-	50	-	-
Lippia javanica (leaves)	-	-	25	-	6.25	-	-	-	-	-
Lippia javanica (stem)	-	-	12.5	-	25	-	-	12.5	-	-
Pentanisia prunelloides	-	25	6.25	-	6.25	25	-	-	-	-
Sclerocarya birrea	-	12.5	6.25	-	3.13	12.5	-	25	6.25	-

MIC results in mg/ml										
PLANT NAMES	**ATTC CULTURES**				**SA P5020**	**SA P4790**	**SA T1266**	**EC U1505S**	**EC U16406**	**EC U16403**
	BS	**KP**	**SA**	**EC**						
Sclerocarya birrea (bark)	-	6.25	6.25	-	3.13	6.25	-	-	-	-
Solanum uculeastrum Intuma (stem)	-	-	12.5	-	-	-	-	50	-	-
Solanum uculeastrum Dun (leaves)	12.5	-	3.13	-	6.25	-	12.5	-	-	-
Solanum uculeastrum (root)	6.25	12.5	3.13	-	6.25	12.5	12.5	-	-	-
Strychnos deccusata (stem)	6.25	-	-	-	25	-	12.5	-	-	-
Strychnos deccusata (leaves)	3.13	-	-	-	-	-	-	25	12.5	25
Trichilia dregeana (stem)	25	50	12.5	-	6.25	50	12.5	-	25	-
Trichilia dregeana (leaves)	25	12.5	6.25	-	-	12.5	-	-	-	-
Warburgia salutaris (leaves)	-	3.13	-	-	3.13	3.13	3.13	-	-	-
Warburgia salutaris (stem)	3.13	50	3.13	-	3.13	50	6.25	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	3.13	3.13	1.56	0.78	3.13	6.25	0.78	1.56	3.13	1.56

Table 4.12 Minimum inhibitory concentrations results for the methanol plant extracts.

MIC results in mg/ml										
	ATTC CULTURES				SA	SA	SA	EC	EC	EC
PLANT NAMES	BS	KP	SA	EC	P5020	P4790	T1266	U1505S	U16406	U16403
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	12.5	-	-	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	-	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	-	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	-	-	-	-	-	-	-	-	-	-
Combretum erythrophyllum	-	-	-	-	-	-	-	-	-	-
Faurea saligna	-	-	-	-	-	-	-	-	-	-
Gerbera ambigua	50	-	-	-	-	-	-	-	-	-
Gunnera perpensa	-	-	-	-	6.25	12.5	-	-	-	-
Hypericum aethiopicum (leaves)	-	25	-	-	6.25	50	-	-	-	-
Hypericum aethiopicum Unsukumbili (stem)	-	-	-	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	-	-	-	-	-	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Lippia javanica (leaves)	25	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea (bark)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum Intuma (stem)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (leaves)	-	12.5	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (stem)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	-	-	-	-	-	-	-	-
Warburgia salutaris (leaves)	-	-	12.5	-	6. 25	-	-	-	-	-
Warburgia salutaris (stem)	12.5	-	25	-	-	-	-	-	-	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	3.13	1.56	0.78	0.78	3.13	1.56	6.25	6.25	3,13	1.56

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.

Table 4.13 Minimum inhibitory concentrations results for the hot water plant extracts.

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Acanthospermum australe (stem)	-	-	-	-	-	-	-	-	-	-
Acanthospermum australe (leaves)	-	-	-	-	-	-	-	-	-	-
Acorus calamus	-	-	-	-	-	-	50	-	-	-
Albizia adianthifolia (stem)	-	-	-	-	50	-	-	-	-	-
Albizia adianthifolia (leaves)	-	-	-	-	-	50	-	-	-	-
Baccharoides adoensis (stem)	-	-	-	-	-	-	-	-	50	-
Baccharoides adoensis (leaves)	-	-	-	-	-	-	-	-	-	-
Clerodendium hirsutum	-	-	-	-	-	-	-	-	-	-
Combretum erythrophyllum	-	6.25	-	-	-	-	-	-	25	-
Faurea saligna	-	-	50	-	-	-	-	-	-	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Gerbera ambigua	-	-	-	-	25	-	-	-	-	-
Gunnera perpensa	-	-	-	-	-	-	-	-	-	-
Hypericum aethiopicum (leaves)	-	-	-	-	12.5	-	-	-	-	-
Hypericum aethiopicum (stem)	50	-	-	-	25	25	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	-	-	-	-	-	-
Lippia javanica (leaves)	-	-	-	-	-	-	-	-	-	-
Lippia javanica (stem)	-	-	-	-	-	-	-	-	-	-
Pentanisia prunelloides	-	-	-	-	-	-	-	-	-	-
Sclerocarya birrea	-	12.5	-	-	-	-	25	-	-	25
Sclerocarya birrea (bark)	-	-	-	-	25	25	-	-	-	-
Solanum uculeastrum (stem)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum ma (leaves)	-	-	-	-	-	-	-	-	-	-
Solanum uculeastrum (root)	-	-	-	-	-	-	-	-	-	-
Strychnos deccusata (stem)	-	-	-	-	-	-	12.5	-	-	-

MIC results in mg/ml										
PLANT NAMES	ATTC CULTURES				SA P5020	SA P4790	SA T1266	EC U1505S	EC U16406	EC U16403
	BS	KP	SA	EC						
Strychnos deccusata (leaves)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (stem)	-	-	-	-	-	-	-	-	-	-
Trichilia dregeana (leaves)	-	-	50	-	-	25	-	-	12.5	-
Warburgia salutaris Isibhaha (leaves)	-	-	-	-	50	50	-	-	-	-
Warburgia salutaris (stem)	-	-	-	-	50	-	-	-	-	25
DMSO	-	-	-	-	-	-	-	-	-	-
Neomycin	313	0.78	313	0.78	1.56	3.13	3.13	1.56	3.13	1.56

BS – *Bacillus subtilis*, **KP** – *Klebsiella pneumonia*, **SA** – *Staphylococcus aureus*, **EC** – *Escherichia coli*.