The screening of medicinal plants traditionally used to treat diarrhoea, in Ongoye area, KwaZulu Natal.

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Abstract

This study focused on the investigation of plants used for the treatment of diarrhoea around Ongoye forest, KwaZulu-Natal, South Africa. The study revealed that 35 plant species in this area are used to treat diarrhoea. Acetone, methanol, cold and hot distilled water extracts from the different plant parts (bark, leaves, stems and the whole plant) were done. These plants are: *Acacia karoo, Acacia robusta, Acanthospermum australe, Aloe arborescens, Baccharoides adoensis, Callilepis laureola, Catharanthus roseus, Chenopodium ambrosioids, Chromolaena odarata, Dichrostachys cinerca, Faurea macnaughton, Hewittia malambaricaa, Hypoxis hemerocallidea, Ihlaza, Lippia javanica, Maytenus heterophylla, Melia azedarach, Psidium guajava, Schotia brachypetala, Sclerocarya birrea, Syzygium cordatum, Tetradenia riparia, Trichilia dregeana, Ungazini, Vernonia oligocephala* and *Vernonia tigna.* Above mentioned plants were screened for antibacterial activity against the following ATCC bacteria strains: *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus. Escherichia coli, 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 screened for the following phytochemicals: alkaloids, flavonoids, soponins, anthraquinones, cardiac glycosides and tannins. Most of the plant extracts showed high antibacterial activity against most of the tested micro-organisms with the diameter of inhibition zones ranging between 10 and 30 mm. Of the plants studied, the most active extracts were those obtained from the following plants: *Acacia robusta, Aloe arborescens, Baccharoides adoensis, Chromolaena odarata, Ihlaza, Lippia javanica, Psidium guajava, Syzygium cordatum, Schotia brachypetala, Tetradenia riparia,* and *Vernonia tigna. Staphylococcus aureus* was the bacterium that was mostly inhibited by almost all the plant extracts, followed by *Klebsiella pneumoniae, Escherichia coli* and *Bacillus subtilis.* the least inhibited bacteria strains were *Shigella sonnei, Shigella flexneri* and *Salmonella typhii.* The MIC values for active extracts ranged between 1 mg/ml and 0.4 mg/ml. The results obtained appeared to confirm the antibacterial potential of the plants investigated, and their potential in the treatment of diarrhoea in the Ongoye area.

Dedication

To my family, especially my mom (Sabina Mlambo) and my late older brother (Zakhele Mlambo).

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Contents

1. Chapter One: Introduction.....1

1.1 Overview of the use of plants to treat microbial infection.....1

1.2 *Objectives*.....5

2. Chapter Two: Ethnobotany.....7

2.1 Introduction.....7

2.2 *Ethnobotanical survey*.....9

2.3 Collected plants 15

2.4 Plants reported to be used to treat diarrhoea.....17

2.5 *Conservation status*.....73

2.6 Discussion.....74

3. Chapter Three: Antimicrobial efficacy.....77

3.1 *Introduction*.....77

3.2 Bacteria description.....80

3.2.1 Description of ATCC micro-organisms.....80

3.2.2 *Escherichia coli*.....80

3.2.3 *Staphylococcus aureus*.....82

3.2.4 *Bacillus subtilis*.....84

3.2.5 *Klebsiella pneumoniae*.....85

3.3 Description of tant micro-organisms collected at Lancet Laboratory.....86

3.4 *Pathogens used*.....87

3.5 Standard experimental procedures.....90

3.5.1 Plant extraction.....90

3.6 *Antibacterial assays*.....93

3.6.1 Culture maintenance.....93

3.6.2 Disk-diffusion technique.....94

3.6.3 *Agar-well diffusion technique*.....95

3.6.4 Minimum inhibition concentration (MIC).....95

3.7 Results.....96

3.8 Discussion.....132

4. Chapter Four: Phytochemical screening.....137
4.1 Introduction.....137
4.2 Phytochemical screening assays.....138
4.2.1 Alkaloids.....138
4.2.2 Flavonoids.....138
4.2.3 Saponins.....138
4.2.4 Anthraquinones.....138
4.2.5 Cardiac Glycosides.....139
4.2.6 Tannins.....139
4.3 Results.....140
4.4 Discussion.....141
5. Chapter Five: Bio-autographic assay.....145
5.1 Introduction.....145
5.2 Bio-autographic technique.....146
5.3 Results.....147
5.4 Discussion.....153
6. Chapter Six: Conclusion.....154
References.....157
Appendix A.....179
Appendix B.....182
Appendix C.....192
Appendix D.....201

List of Figures

2.1 *Acacia karoo* flowers....17
2.2 *Acacia robusta* tree....19
2.3 *Acridocarpus natalius* flowers and pods....21
2.4 *Alepidea amatymbica* flowers and leaves....23
2.5 *Aloe arborescens* growing between rocks....25
2.6 *Azima tetracantha* branch....27
2.7 *Baccharoides adoensis* in flower....28
2.8 *Callilepis laureola* flowers....30
2.9 *Cantharanthus roseus* flowers....32
2.10 *Chenopodium ambrosioides* leaves....34
2.11 *Chromolaena odorata* flowers and plant....36
2.12 *Cyphostemma hypoleucum* leaves....38
2.13 *Dichrostachys cinerea* tree consisting of flowers....39
2.14 *Ficus sur* tree with fruits....41
2.15 *Helichrysum odoratissimum* plant with flowers....43
2.16 *Hypoxis hemerocallidea* plant with flowers....45
2.17 *Lippia javanica* tree with flowers....48
2.18 *Gymnosporia buxifolia* branch....50
2.19 *Melia azedarach* tree with flowers and fruits....52
2.20 *Psidium guajava* fruits....54
2.21 *Prunus persica* tree with fruit....56
2.22 *Sclerocarya birrea* tree with fruits....58
2.23 *Schotia brachypetala* tree with flowers....60
2.24 *Strychnos henningsii* seeds....62
2.25 *Syzygium cordatum* tree with fruit....64
2.26 *Tetradenia riparia* tree with flowers....66
2.27 *Trichillia dregeana* tree....68
2.28 *Vernonia oligocephala* tree with flowers....70
2.29 *Vernonia tigna* tree with flowers....71
3.1 The four way streaking method....94

3.2 Antibacterial activity of bark, tubers and plants extracted with acetone...96
3.3 Antibacterial activity of leaves extracted with acetone.....97
3.4 Antibacterial activity results of stems extracted with acetone.....98
3.5 Antibacterial activity results of bark, tubers and plants extracted with methanol.....99
3.6 Antibacterial activity results of leaves extracted with methanol.....100
3.7 Antibacterial activity results of stems extracted with methanol.....101
3.8 Antibacterial activity results of bark, tubers and plants extracted with cold distilled water.....102
3.9 Antibacterial activity results of leaves extracted with cold distilled water.....103
3.10 Antibacterial activity results of stems extracted using cold distilled water.....104
3.11 Antibacterial activity results of bark, tubers and plants extracted with hot distilled water.....105
3.12 Antibacterial activity results of leaves extracted with hot distilled water.....106
3.13 Antibacterial activity results of stems extracted with hot distilled water.....107
3.14 Antibacterial activity results of bark, tubers and plants extracted with acetone.....108
3.15 Antibacterial activity results of leaves extracted with acetone.....109
3.16 Antibacterial activity results of stems extracted with acetone.....110
3.17 Antibacterial activity results of bark, tubers and plants extracted with methanol.....111
3.18 Antibacterial activity results of leaves extracted with methanol.....112
3.19 Antibacterial activity results of stems extracted with methanol.....113
3.20 Antibacterial activity results of bark, tubers and plants extracted with cold distilled water.....114
3.21 Antibacterial activity results of leaves extracted with cold distilled water.....115

3.22 Antibacterial activity results of stems extracted with cold distilled water....116
3.23 Antibacterial activity results of bark, tubers and plants extracted with hot distilled water....117
3.24 Antibacterial activity results of leaves extracted with hot distilled water....118
3.25 Antibacterial activity results of stems extracted with hot distilled water....119
3.26 Minimum Inhibition Concentration determination for bark, tubers and plants extracted with acetone....120
3.27 Minimum Inhibition Concentration determination for leaves extracted with acetone....121
3.28 Minimum Inhibition Concentration determination for stems extracted with acetone....122
3.29 Minimum Inhibition Concentration determination for bark, tubers and plants extracted with methanol....123
3.30 Minimum Inhibition Concentration determination for leaves extracted with methanol....124
3.31 Minimum Inhibition Concentration determination for stems extracted with methanol....125
3.32 Minimum Inhibition Concentration determination for bark, tubers and plants extracted with cold distilled water....126
3.33 Minimum Inhibition Concentration determination for leaves extracted with cold distilled water....127
3.34 Minimum Inhibition Concentration determination for stems extracted with cold distilled water....128
3.35 Minimum Inhibition Concentration determination for bark, tubers and plants extracted with hot distilled water....129
3.36 Minimum Inhibition Concentration determination for leaves extracted with hot distilled water....130
3.37 Minimum Inhibition Concentration determination for stems

extracted with hot distilled water.....131
5.1 The presentation of a TLC plate for the calculation of R_f values.....147
5.2 Bio-autographic assay of the crude dichloromethane\methanol extract of *Syzygium cordatum* against *S. aureus*.....148
5.3 Bio-autographic assay of the crude dichloromethane\methanol extract of *Acacia robusta* against *S. aureus*..... 148
5.4 Bio-autographic assay of the crude dichloromethane\methanol extract of *Aloe arborescens* against *S. aureus*.....149
5.5 Bio-autographic assay of the crude dichloromethane\methanol extract of *Psidium guajava* against *S. aureus*.....149
5.6 Bio-autographic assay of the crude dichloromethane\methanol extract of *Vernonia tigna against S. aureus*.....150
5.7 Bio-autographic assay of the crude dichloromethane\methanol extract of *Tetradenia riparia against S. aureus*.....150
5.8 Bio-autographic assay of the crude dichloromethane\methanol extract of *Lippia javanica against S. aureus*.....151
5.9 Bio-autographic assay of the crude dichloromethane\methanol extract of *Schotia brachypetala against S. aureus*.....151
5.10 Bio-autographic assay of the crude dichloromethane\methanol extract of *Chromolaena odarata against S. aureus*.....152
5.11 Bio-autographic assay of the crude dichloromethane\methanol extract of *Baccharoides adoensis* against *S. aureus*.....152

List of Tables

2.1 Ethnobotanical survey....12

2.2 Localities and voucher specimen for the plants used against stomach ailments....15

2.3 Plants bought from Eshowe muthi markets....17

3.1 Sensitivity pattern against different antibiotics of *Escherichia coli*....88

3.2 Sensitivity pattern against different antibiotics of *Staphylococcus aureus*....89

3.3 Yield of the different plant extracts....91

4.1 Screening of crude plant extracts for the presence of six secondary metabolites....140

B1 Results of sensitivity of different bacteria against acetone plant extracts....182

B2 Results of sensitivity of different bacteria against methanol plant extract....185

B3 Results of sensitivity of different bacteria against cold distilled plant extracts....188

B4 Results of sensitivity of different bacteria against of hot distilled water plant extracts....190

C1 Results of sensitivity of different bacteria against of acetone plant extracts....192

C2 Results of sensitivity of different bacteria against of methanol plant extracts....194

C3 Results of sensitivity of different bacteria against of cold distilled water plant extracts....196

C4 Results of sensitivity of different bacteria against of hot distilled water plant extracts....199

D1 Minimum inhibitory concentrations of medicinal plants extracted using acetone....201

D2 Minimum inhibitory concentrations of medicinal plants

extracted using methanol.....203
D3 Minimum inhibitory concentrations of medicinal plants extracted using cold distilled water.....205
D4 Minimum inhibitory concentrations of medicinal plants extracted using hot distilled water.....207

"If we knew what it was we were doing, it would not be called research, would it?" Albert Einstein.

CHAPTER ONE

1. Introduction

1.1 Overview of the use of plants to treat microbial infection

The subject of medicinal plant drugs is a highly active field of scientific study all over the world and is an important part of human history, culture and tradition. Medicinal plants and plant-derived medicines are widely used in traditional cultures all over the world. People are relying on these medicinal plants as natural alternatives to synthetic chemicals (Van Wyk and Wink 2004). As more and more natural remedies are being commercialized, there is a need for a user friendly but scientifically accurate reference guide to the plants and their products. It is likely that many important new remedies will be discovered and commercialized in the future, by following the leads provided by traditional knowledge and experience.

Medicinal plants are often associated with witchcraft and superstition because the scientific basis of the healing action of plants is not known. One example is a concept in the Doctrine of Signature which was based on the assumptions that the appearance of the plants may give clues to their medicinal properties (Van Wyk and Wink 2004). People who use traditional remedies may not understand the scientific rationale behind these medicines, but they know from personal experience that some medicinal plants can be highly effective if used as therapeutic doses. Since there is now a better understanding of how the human body functions, there is therefore a better position to fully appreciate the healing power of plants and their potential as multi-functional chemical entities for treating complicated health conditions. Medicinal plants contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health.

Herbal medicine is part of everyday life in many countries in Europe and to this day it is popular as a method to treat ailments. It is often considered to be supportive rather than curative. The use of herbal teas and herbal mixtures is particularly popular in Germany, France, Italy, Great Britain and Switzerland.

The spread of traditional Chinese medicine to other continents has undoubtedly contributed to the current popularity of herbal medicine throughout the world. The ancient system of medicine is actually a practical and holistic set of guides to maintain balance and harmony and to ensure a long and happy life. In common with Western herbal teas and African Traditional medicine, Chinese herbs are usually given in fixed mixtures of up to 20 herbs, which are carefully prepared according to traditional recipes contained in ancient compendia (Van Wyk and Wink 2004).

Today, medicinal plants play a great role in human health services worldwide. Many people in the modern world are turning to herbal medicine. For example, in the USA about 25% of all prescriptions dispensed in public pharmacies in 1973 contained drugs extracted from higher plants and about 64% of the total global population remains dependent on traditional medicine for their healthcare needs (Wondimu *et al.*, 2007). It is shown in many literature sources that India, Korea, Japan, China and Malaysia are the leading countries in the world using traditional medicines. For example in India, approximately 7500 medicinal plant species are traditionally used. It is reported that among 2000 drugs that have been used in India and Nepal, about 1500 are of plant origin (Wondimu *et al.*, 2007).

Popular interest in the use of medicinal plants has grown considerably in the second half of the 20th century. Studies of the chemistry and pharmacology of natural products from many parts of the world has resulted in increasing concern about the potential loss of biodiversity and the necessity of guaranteeing the sustainable use of natural resources. Few studies have been designed to test hypotheses related to the knowledge and use of medicinal plants. Most studies have caused more interest in local communities to examine patterns of use and selection of folk medicines, the transmission and erosion of folk knowledge and the influence of economic and social variables on plant use (De Albuquerque *et al.*, 2007).

The World Health Organization (WHO, 2003) incorporates studies of traditional medicine practices in its diarrhoeal disease control program. It is

estimated that 80% of the population in developing countries is unable to afford pharmaceutical drugs and rely on traditional medicines, mainly plant based, to sustain their primary health care needs. Several studies have evaluated the effectiveness of some traditional medicines in treating diarrhoea on all the different continents (Lin *et al.*, 2002). The South Africa Essential Drug Programme has adopted WHO recommendations for the drugs to be used to treat and manage diarrhoea, but still people seek help from traditional healers. Assurance of the safety, quality and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. Both general consumers and health care professionals need up to date, authoritative information on the safety and efficacy of medicinal plants (WHO, 2003). The main objectives of the WHO traditional medicine activities are to facilitate integration of traditional medicine into the national health care systems by assisting with policies on traditional medicine, to promote the proper use of traditional medicine by developing and producing international standards, technical guidelines and methodologies and to act as a clearing-house to facilitate information exchange in the field of traditional medicine (WHO, 2002 -2005).

Traditional medicine is regarded as a very important part of primary health care in South Africa and it is also regarded as a holistic healing system. The indigenous people of South Africa have relied on herbal medicine for all aspects of primary health care. It is estimated that between twelve and fifteen million South Africans still use traditional remedies from as many as seven hundred indigenous plant species (Balick *et al.,* 1994., Campbell, 2007., Mander *et al.*, 2006., Mander *et al.*, 2007., Mathabe *et al.*, 2006., Meyer and Flayer, 1995 and Rabe and Van Staden, 1997). Traditional healing is widely practiced in South Africa. It is estimated that 80% of the black population is consulting with traditional healers (Jager *et al.*, 1996, Eloff, 1998., Fyhrquist *et al.*, 2002). This further raises the need for a scientific evaluation of the methods used by these healers. It is necessary to establish the efficiency and safety of traditional treatment (Jonathan *et al.*, 2000). Although many rural communities now have access to mobile clinics and hospitals, there is still, to a large extent, the belief in herbal medicine. Although free health care has

become entrenched in South Africa's constitution, many rural people still rely on the cheaper traditional healing methods rather than the expensive treatments by Western practitioners (Meyer and Folayem, 1995). But the information given is contradictory because recent research conducted by Mander (2007) showed that traditional medicine was more expensive than treatment at local government clinics, dispelling the myth that traditional medicine is a cheaper alternative (Mander *et al.*, 2007). The value of ethno-medicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine because the search for new, potential medicinal plants is more successful if the plants are chosen on an ethno medical basis. It has been estimated that 74% of the pharmacologically active, plant-derived components were discovered after the ethnomedical uses of the plants started to be investigated in the years 1997-2008 (Fyhrquist *et al.,* 2002).

There is scope for more research to be done on the usage of South African plants because there is not enough documentation that has been done on the usage of medicinal plants (Van Wyk and Wink 2004) and very little scientific information is available. It is only recently (1997-2008) that a number of findings have emerged on the chemistry and biological activity of plants used in traditional healing (Van Vuuren, 2008). The books mostly used as references on South African medicinal plants were written by the following authors: Hutchings *et al.*, 1996, Iwu, 1993, Van Wyk and Malan, 1997, Van Wyk *et al.*, 2000 and Van Wyk and Wink, 2004. Medicinal plants are an important part of the South African cultural heritage. According to Van Wyk and Wink (2004) there is still a need for detailed documentation on the use of medicinal plants in South Africa, as there are only a few plants that have been tested against diarrhoea in articles conducted by Lin *et al*, 2002., Mathabe *et al.,* 2006., Teke *et al.*, 2007 and Van Vuuren, 2008. There is thus an urgent need for more research on the uses of medicinal plants in view of the vulnerability of oral traditional knowledge. In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, documentation of African medicinal plants is an urgent matter. It is therefore believed that with research and formal documentation

the usage of Africa's indigenous systems of medicine will one day play a powerful role in the healing of the world (Mathabe *et al.*, 2006).

South Africa's eastern region has a subtropical climate with a high diversity of plants. It is the home of the native Zulu kingdom known for it's warriors as well as it's traditional medicinal plants. Traditional medicine plays an important role in health care (especially in the rural areas of KwaZulu-Natal) for the treatment of illnesses. Some of these ailments are diarrhoea, dysentery and unexpected abdominal pains. It is generally the women that have extensive knowledge of medicinal plants due to their role as care givers of children. The women's knowledge includes the nutritional value and storage of food (Zobolo and Mkabela, 2006). However not much research has been done on the plants they use to treat these ailments (Hutching *et al.*, 1996). It would be interesting to search for plants with antidiarrhoeal and antimicrobial activities that could be used against any type of diarrhoeal disease. A range of medicinal plants with antidiarrhoeal and antimicrobial properties have been widely used by traditional healers in KwaZulu-Natal. However, the therapeutic potentials of some of these medicines have not been scientifically evaluated (Farnsworth, 1994, Havagiray *et al.*, 2004).

1.2 Objectives

The main objectives of the study are:

- To document the Ethnobotanical knowledge on the usage of indigenous plants for treating diarrhoea in the Ongoye area through structured questionnaires.
- To identify the collected plants and make vouchers of the specimens.
- To test medicinal plants sold for treating diarrhoea at the muthi markets closest to Ongoye forest e.g. Eshowe and Empangeni, with the plants growing in the homesteads.
- To test plant extractions for antibacterial activities against ATCC and collected multi drug-resistant bacterial strains.

- To do Minimum Inhibition Concentration (MIC) for those plant extractions with potential antimicrobial activity.
- To do chemical analysis of different extracts using the bio-autographic assay.
- To investigate the phytochemical properties of the plants.
- To make recommendations of possible plants which can be investigated further for the development of potential drugs against microbial infections.

CHAPTER TWO

Ethnobotany

2.1 Introduction

Ethnobotany is the study of how people of a particular culture and region make use of plants. Since the beginning of civilization, people have used plants as medicine (Van Wyk and Wink, 2004). Perhaps as early as Neanderthal man, plants were believed to have healing powers. The earliest recorded uses are found in Babylon circa 1770 BC in the Code of Hammurabi and in ancient Egypt people believed medicinal plants to have utility even in the afterlife of their Pharaohs. Plants have been recovered from the Giza pyramids and can be found on display in the Access Excellence Resources Center (Van Wyk and Wink, 2004). A discussion of human life on this planet would not be complete without a look at the role of plants. A complete record of the many thousands of plant species used for human functioning would fill volumes, yet historians have often tended to dismiss plants as less than fundamental in history. In recent years, however, there has been a scientific interest in the fundamental role plants play in many cultures, including medicinal purposes (Veilleux and King, 1996).

Since the beginning of the discipline, ethnobotanists have been concerned with the threat of losing traditional culture and their knowledge of plant uses and relations. Over the last three decades of the past century, work has centered on the need to catalogue the knowledge of plants in a race with the fast disappearance of natural sources, mainly Tropical Forests (Ramirez, 2007). History is our bridge from the past to the future. The key role of women in the next millennium can be stronger if people know something about the past from where we have come. There is a full history of women in relation to science culture (Parsons, 1951). In rural regions of the tropics, divisions of labour among subsistence communities predominate. Men are often engaged in hunting, fishing, livestock herding and timber extraction; activities that would take them to relatively undisturbed habitants distant from their settlements. Women are more likely to be involved in managing local resources, such as home gardens, swiddens (area cleared for temporary

cultivation) and other disturbed habitats relatively near the home (Momsen, 2004., Voeks, 2006). Women, as primary educators in indigenous communities, have sustained their frameworks and associated knowledge systems for millennia, even while undergoing major social upheavals as a result of transformative forces beyond their control (Zobolo and Mkabela, 2006). In Northern India women and girls are exclusively responsible for collection of fodder, leaves, herbs and grasses (Singh, 1988). The Maya women of the Guatemala highlands educate their children (daughters) through the chores of the garden (Keys, 1999). The African homestead gardens (Muzis) contain a mixture of spiritual, protective and medicinal plants (Zobolo and Siebert, 2005).

Indigenous women have extensive knowledge of medicinal plants due to their role as caretakers of children (Kothari, 2003). Groups of women who collected medicinal plants from the wild expressed their desire to record the information so that their daughters could use it in the future as in the case of Zer Malik (interviewee), who noted that their daughters were not interested in traditional remedies and were turning to modern allopathic medicine (FAO, 1997). Women are also knowledgeable about nutrition and storage of foods (Kelkar, 1995). Rural women in South Africa have for decades played a central role in sustaining, managing and making use of plants. In areas rich in useful plants, women manage most of the resources that are used and even grow these plants around the house (Howard, 2003). The recognition and restoration of indigenous women's knowledge through support of their effort to pass it on to future generations would enhance sustainable use of natural resources (Mikkelsen, 2005). Nearly all adults, especially women, are competent to treat illness of the first type, drawing on an extensive knowledge of the therapeutic properties of *materia medica*, above all plants. Women generally treat the same disease in more than one way by using different plants. This depends mainly upon plant availability in their territory and the wider the choice, the better the chance of a cure. But some plants are used for almost all complaints all over the world, for example *Eremophila* spp. These plants are so valued that in the past they were dried and stored for future applications (Yaniv and Bachrach, 2005).

The World Health Organization (2003) has estimated that 80% of the population in developing countries is unable to afford pharmaceutical drugs and rely on traditional medicines, mainly plant based, to sustain their primary health care needs. The increasing global acceptance of complementary and alternative medicine has been the major reason for the steep increase in the demand for medicinal plants from countries like Nigeria, China, Mexico and India, which are rich in biological diversity. In Nigeria, medicinal plants are very important natural resources not only because their continued availability can assure health security for millions, but also because of the potential to generate economic benefits (Agunu *et al.*, 2005) as are South Africa's medicinal plants.

2.2 Ethnobotanical survey

An Ethnobotanical survey was conducted in the rural homesteads of the Uthungulu District Municipality namely : **Makholokholo** Gps no: S28 °, 45 min, E31 ° , 44 min, Alt 118 m ; **Mhlaleni** Gps no: S28 °, 47 min, E31 °, 45 min, Alt 98 m ; **Ntshidi** Gps no: S28 ° 47 min, E31 °, 43 min, Alt 106 m; **Ekuphumuleni** Gps no: S28 °, 47 min, E31 °, 45 min, Alt 98 m; **Macekane** Gps no: S28 ° 48 min, E31 °, 46 min, Alt 95 m; **Obisana** Gps no: S28 °, 49 min, E31 °, 48 min, Alt 119m; **Gugushe** Gps no: S28 °, 50 min, E31 °, 45 min, Alt 306 m; **Ophongola** Gps no: S28 °, 49 min, E31 °, 47 min, Alt 240 m and **Sinjigwane** Gps no:S28 °, 45 min, E31 °, 44 min, Alt 88 m. The selection of this area was based on a survey done in this district by Zobolo and Mkabela (2006), who found that various medicinal and food plants were grown in homestead gardens. Their survey also revealed that indigenous knowledge possessed by elderly women was not adequately transferred to children for preservation. This survey was conducted by interviewing people from approximately 80 homesteads (Muzi) on their usage of plants to treat diarrhoea. The interviews were conducted in Zulu and the questionnaires were designed to obtain the following information: Zulu names of plants used, plant parts used, collection of plant material, mode of preparation and administration (appendix A). The results of the interviews on the ethnobotanical survey are shown in Table 2.1. Voucher specimens were prepared and deposited at the University of Zululand Herbarium (ZULU).

Some of the plants which were reported to be used to treat diarrhoea were bought from Eshowe and Empangeni muthi markets. All the collected specimens from the homestead and the muthi markets were used for antibacterial activity testing.

Table 2.1

Ethnobotanical survey

Table 1. Medicinal plants used in the Ongoye area to treat diarrhoea and the people who were interviewed.

Scientific names\ Family names	Zulu names	Uses	Parts Used	Interviewees	Age group	Locality
Acacia karoo **((MORACEAE)**	Umunga	Stomach problems and flu	Bark	Mr Mhlongo	45-54	Ekuphumuleni
Acacia robusta **((MORACEAE)**	Umngamanzi	Diarrhoea	Leaves	Mr Mayeza	25-34	Macekane
Acanthospermum australe **(ASTERACEAE)**	Umgwaqeni	Diarrhoea	Plant	Nosipho Zungu	15-24	Ntshidi
Acridocarpus natalitius **(MALPIGHIACEAE)**	Umabophe	Diarrhoea	Bark	Mr Mayeza	25-34	Macekane
Alepidea amatymbica **(APIACEAE)**	Ikhathazo	Diarrhoea	Roots	Mrs Luthuli	45-54	Gugushe
Aloe arborescens **(ASPHPDELACEAE)**	Inhlaba	Diarrhoea and sores	Leaves	Mrs Sikhakhane	45-54	Makholokholo
Baccharoides adoensis **(ASTERACEAE)**	Inyathelo	Diarrhoea	Leaves	Mr Mtshali	35-44	Ntshidi
Callilepis laureola **(ASTERACEAE)**	Impila	Diarrhoea	Roots	Mrs Luthuli	55-64	Gugushe
Catharanthus roseus **(APOCYNACEAE)**	Imbali emhlophe	Diarrhoea and toothache	Leaves	Mrs Mzobe	45-54	Mhlaleni
Chenopodium ambrosioides **(CHENOPODIACEAS)**	Insukumbili	Diarrhoea and sores	Plant	Mrs Nkwanyane	35-44	Gugushe
Chromolaena odarata **(ASTERACEAE)**	Usandanezwe	Diarrhoea	Leaves	Mrs Gumede	45-54	Mhlaleni
Clerodendrum glabrum **(VERBENACEAE)**	Umqaqongo	Diarrhoea	Leaves	Mrs Gumede	45-54	Mhlaleni
Cyphostemma cirrhosum **(VITACEAE)**	Udekane	Diarrhoea	Leaves	Mrs Thungo	35-44	Ekuphumuleni
Dichrostachys cinerca **(MIMOSACEAE)**	Ugagane	Diarrhoea and steaming to get ride of acne	Bark	Mrs Khumalo	55-64	Obisana

Table 1. (Continued)

Scientific names\ Family names	Zulu names	Uses	Parts Used	Interviewees	Age group	Locality
Ficus sur (MORACEAE)	Umkhiwane	Diarrhoea	Leaves	Mr Mayeza	25-34	Macekane
Helichrysum odoratissimum (ASTERACEAE)	Imphepho	Diarrhoea	Plant	Nosipho Zungu	15-24	Ntshidi
(CONVOLVULACEAE)	Intandelo	Diarrhoea and sores	Plant	Mr Khumalo	35-44	Opongola
Hypoxis hemerocallidea (HYPOXIDACEAE)	Ilabatheka	Diarrhoea	Roots	Mrs Luthuli	55-64	Gugushe
Lippia javanica (VERBENACEAE)	Umsuzwane	Diarrhoea	Leaves	Mr Mhlongo	35-44	Gugushe
Maytenus heterophylla (CELASTRACEAE)	Isibhubu	Diarrhoea and eaten as fruit	Leaves	Mrs Mhlongo	65	Mhlaleni
Melia azedarach (MELIACEAE)	Umsilinga	Repellent of mosquitoes and stomach problems	Leaves	Mrs Ndawonde	45-54	Makholokholo
Physalis viscose (SOLANACEAE)	Uqumqumu	Diarrhoea	Leaves	Mrs Mhlongo	65	Makholokholo
Portulacaria afra (PORTULACEAE)	Umndibili	Diarrhoea and homestead fencing	Leaves	Mr Mhlongo	65	Mhlaleni
Psidium guajava (MYRTACEAE)	Umgwava	Eaten as the fruit and for diarrhoea	Leaves	Mrs Miya	35-44	Macekane
Prunus persica (ROSACEAE)	Umphentshisi	Eaten as the fruit and for stomach problems	Leaves	Mrs Ngcobo	45-54	Makholokholo
Schotia brachypetala (FABACEAE)	Umgxamu	Diarrhoea and back pain	Bark	Mrs Mhlongo	55-64	Sinjigwane
Schkuhria pinnata (ASTERACEAE)	Unsakansaka	Diarrhoea and chest problems	Leaves	Mrs Mzobe	45-54	Mhlaleni
Sclerocarya birrea (ANACARDIACEAE)	Umganu	Diarrhoea and steaming to get ride of acne	Bark	Mr Mkhize	25-34	Ntshidi
Senecio quinquelobus (ASTERACEAE)	Usinini	Diarrhoea	Leaves	Mrs Thungo	25-34	Ekuphumuleni

Table 1. (Continued)

Scientific names\ Family names	Zulu names	Uses	Parts Used	Interviewee	Age group	Locality
Strychnos henningsii **(LOGANIATIGNA)**	Umqalothi	Diarrhoea	Leaves	Mr Dindi	45-54	Mhlalenii
Syzygium cordatum **(MYRTACEAE)**	Umdoni	Diarrhoea	Leaves	Mrs Nsele	45-54	Gugushe
Tetradenia riparia ***(LAMIACEAE)***	Iboza	Stomach problems and flu	Leaves	Sfiso Buthelezi	25-34	Makholokholo
Trichilia dregeana **(MELIACEAE)**	Umkhuhlu	Diarrhoea and sores	Leaves	Mr Mayeza	25-34	Macekane
Vernonia oligocephala **(ASTERACEAE)**	Uhlambihloshana	Diarrhoea	Leaves	Mr Dindi	65	Opongola
Vernonia tigna **(ASTERACEAE)**	*Uhlunguhlungu*	*Diarrhoea*	Leaves	Mr Dindi	45-54	Ekuphumuleni
Unidentified species	Udleleni	Diarrhoea	Plant	Mr Zungu	45-54	Ntshidi

2.3 Collected plants

Plants collected from the homesteads, reported to have the potential to treat diarrhoea are shown in table 2.2.

Table 2.2 Localities and voucher specimen for the plants used against stomach ailments.

Species	Voucher Specimen	Locality
Acacia karoo Hayne **(MORACEAE)**	NP Mlambo and NS Mthethwa 19 (ZULU); [3132 DC]	Ongoye area, Ukuphumuleni, Garden of Mr Mhlongo.
Acacia robusta E Meyer **(MORACEAE)**	NP Mlambo and NS Mthethwa 11 (ZULU); [3132 DC]	Ongoye area, Macekane, Garden of Mr Mayeza.
Acanthospermum australe (Loefl.) O. Kuntze **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 2 (ZULU); [3132 DC]	Ongoye area, Ntshidi, Garden of Nosipho Zungu Family.
Acridocarpus natalitius A. Juss. Var. natalitius **(MALPIGHIACEAE)**	NP Mlambo and NS Mthethwa 29 (ZULU); [3132 DC]	Ongoye area, Macekane, Garden of Mr Mayeza.
Aloe arborescens Mill **(ASPHPDELACEAE)**	NP Mlambo and NS Mthethwa 16 (ZULU); [3132 DC]	Ongoye area, Macekane, Garden of Mrs Sikhakhane.
Baccharoides adoensis Sch. Bip.Ex Walp. **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 28 (ZULU); [3132 DC]	Ongoye area, Ntshidi, Garden of Mr Mtshali.
Catharanthus roseus (L.) G. Don. **(APOCYNACEAE)**	NP Mlambo and NS Mthethwa 27 (ZULU); [3132 DC]	Ongoye area, Mhlaleni, Garden of Garden of Mrs Mzobe.
Chenopodium ambrosioides L. **(CHENOPODIACEAS)**	NP Mlambo and NS Mthethwa 17 (ZULU); [3132 DC]	Ongoye area, Gugushe, Garden of Mrs Nkwanyane.
Chromolaena ambrosioides L. **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 4 (ZULU); [3132 DC]	Ongoye area, Gugushe, Garden of Mrs Nkwanyane.
Dichrostachys cinerca (L.) Wight & Am. **(MIMOSACEAE)**	NP Mlambo and NS Mthethwa 20 (ZULU); [3132 DC]	Ongoye area, Obisane, Garden of Mrs Khumalo.
Faurea macnaughton (E Phillips) **(PROTEACEAE)**	Not collected shortage of material	Ongoye area, Gugushe, Garden of Mrs Nkwanyane.

Table 2.2 continued

Species	Voucher Specimen	Locality
Hewittia malambarica (L.) Suiesh (HYLIDAE)	No voucher specimen	Ongoye area, Opongola, Garden of Mr Khumalo.
Hypoxis hemerocallidea L. **(HYPOXIDACEAE)**	NP Mlambo and NS Mthethwa 1 (ZULU); [3132 DC]	Ongoye area, Macekane, Garden of Mrs Masikane.
Lippia javanica (Burm.f.) Spreng **(VERBENACEAE)**	NP Mlambo and NS Mthethwa 10 (ZULU); [3132 DC]	Ongoye area, Gugushe, Garden of Mr Mhlongo.
Maytenus heterophylla (Eckl. & Zehy.) N.K.B. Robson **(CELASTRACEAE)**	NP Mlambo and NS Mthethwa 24 (ZULU); [3132 DC]	Ongoye area, Mhlaleni, Garden of Mrs Mhlongo.
Melia azedarach L. **(MELIACEAE)**	NP Mlambo and NS Mthethwa 3 (ZULU); [3132 DC]	Ongoye area, Makholokholo, Garden of Mrs Ndawonde.
Psidium guajava L. **(MYRTACEAE)**	NP Mlambo and NS Mthethwa 5 (ZULU); [3132 DC]	Ongoye area, Macekane, Along the street of Macekane.
Schotia brachypetala Sond. **(FABACEAE)**	NP Mlambo and NS Mthethwa 29 (ZULU); [3132 DC]	Ongoye area, Sinjigwane, Garden of Mrs Mhlongo.
Sclerocarya birrea (A. Rich.) Hochst. Subsp. Caffra (Sond.) Kokwaro **(ANACARDIACEAE)**	NP Mlambo and NS Mthethwa 18 (ZULU); [3132 DC]	Ongoye area, Ntshidi, Garden of Mr Mkhize.
Senecio quinquelobus DC **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 23 (ZULU); [3132 DC]	Ongoye area, Ukuphumuleni, Garden of Mrs Thungo.
Syzygium cordatum Hochst. Ex. C. Krauss. **(MYRTACEAE)**	NP Mlambo and NS Mthethwa 25 (ZULU); [3132 DC]	Ongoye area, Gugushe, Garden of Mrs Nsele.
Tetradenia riparia (Hochst.) Codd **(LAMIACEAE)**	NP Mlambo and NS Mthethwa 21 (ZULU); [3132 DC]	Ongoye area, Makholokholo. Garden of Botany, University of Zululand.
Trichilia dregeana Sond. **(MELIACEAE)**	NP Mlambo and NS Mthethwa 13 (ZULU); [3132 DC]	Ongoye area, Macekane, Garden of Mayeza.
Vernonia oligocephala (DC) Sch. Bip. ex. Walp **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 9 (ZULU); [3132 DC]	Ongoye area, Opongola, Garden of Mr Dindi.
Vernonia tigna Klatt **(ASTERACEAE)**	NP Mlambo and NS Mthethwa 6 (ZULU); [3132 DC]	Ongoye area, Ekuphumuleni, Garden of Mr Dindi.

ZULU = University of Zululand Herbarium

Table 2.3 Plants bought from Eshowe muthi markets.

Species	Locality
Ihlaza⋆	Ongoye area, Eshowe muthi market, [3132 DC]
Callilepis laureola DC.	Ongoye area, Eshowe muthi market, [3132 DC]
Ungazini⋆	Ongoye area, Eshowe muthi market, [3132 DC]

⋆**Ihlaza** and **Ungazini** plants have not been identified.

2.4 Plants reported to be used to treat diarrhoea (Table 2.1).

This section discussed the plants reported by people around Ongoye forest to be used for the treatment of diarrhoea.

Family: FABACEAE
Scientific name: *Acacia karroo*
Common name: Sweet thorn
Zulu name: *Umunga*

Figure 2.1 *Acacia karroo* flowers (www.plantafrica.com/plantab/acaciakar.htm).

Table 2.3 Plants bought from Eshowe muthi markets.

Species	Locality
Ihlaza⋆	Ongoye area, Eshowe muthi market, [3132 DC]
Callilepis laureola DC.	Ongoye area, Eshowe muthi market, [3132 DC]
Ungazini⋆	Ongoye area, Eshowe muthi market, [3132 DC]

⋆**Ihlaza** and **Ungazini** plants have not been identified.

2.4 Plants reported to be used to treat diarrhoea (Table 2.1).

This section discussed the plants reported by people around Ongoye forest to be used for the treatment of diarrhoea.

Family: FABACEAE
Scientific name: *Acacia karroo*
Common name: Sweet thorn
Zulu name: *Umunga*

Figure 2.1 *Acacia karroo* flowers (www.plantafrica.com/plantab/acaciakar.htm).

Botanical Description

Acacia karroo is a small to medium, sometimes deciduous tree growing up to 20 m in height. The dark brown to almost black bark is rough and somewhat flaky, revealing a reddish colour under the bark. When the trunk is damaged, a clear gum exudes from it. The leaves are divided into about five pairs of leaflets and each is again divided into ten or more pairs of smaller leaflets of about 5 mm long. The flowers are borne in attractive yellow, ball shaped heads. The fruit is a long narrow spirally twisted pod (Pooley, 2003).

Distribution

Acacia karroo is a common tree in South Africa (Kahiya *et al.*, 2003., Saphwan *et al.*, 2004), occurring in all provinces but absent only from the Cape Peninsula. This tree is an indicator of sweet veld or an indicator of water in arid areas (Pooley, 2003). *Acacia karroo* may be found from the Western Cape through to Zambia and Angola (Joffe, 2001).

Medicinal Uses

The bark and the leaves are reported to be used for diarrhoea and dysentery. The gum, bark and leaves are also used as an emollient and astringent for colds, conjunctivitis and haemorrhage (Pooley, 2003). The plant has been reported to reduce protein degradation in the rumen and increase protein flow to post-ruminal sites where they can improve the supply and absorption of amino acids (Kahiya *et al.*, 2003). Seeds are roasted and used as a coffee substitute (Van Wyk and Van Wyk, 1997). In tropical regions, leguminous trees such as *Acacia* species have been reported to be a valuable source of forage for herbivores where there are constraints in feed quality production and diversity. These legumes are two to three times richer in crude protein than grass and cereal grains and can meet the maintenance and production requirements of ruminants (Kahiya *et al.*, 2003).

Data from the ethnobotanical survey

The bark of *Acacia karroo* is reported to be used for treatment of both diarrhoea and flu.The bark is crushed, boiled and filtered to prepare a strong decoction, of which adults drink half a cup and children only take a spoonful of

this decoction. No side effects have been reported from the usage of the decoction (Mhlongo 2007, pers. comm).

Chemical content

The bark and the leaves are rich in tannins, but no detailed analyses are available. Two biologically interesting gallotannins, known to control leaf movements, have been identified from *A. karroo* (Van Wyk and Gericke, 2000, Saphwan *et al.*, 2004). The bark was used in the past for tanning due to its high tannin content (Pooley, 2003).

Conservation status

Acacia karroo is not endangered.

Family: FABACEAE
Scientific name: *Acacia robusta*
Common name: Splendid thorn
Zulu name: *Umngamanzi*

Figure 2.2 *Acacia robusta* tree (www.bidorbuy.co.za/item/10178584/splendid Tho...).

Botanical description

Acacia robusta is a medium to large deciduous thorn tree up to 25 m in height. It has a very robust appearance; the branches grow upright and are thick, resulting in a flat crown. Flowers are pale yellow puff balls which form

Conservation status

Acacia robusta is not endangered.

Family: MALPIGHIACEAE
Scientific name: *Acridocarpus natalius*
Common name: Rain spider
Zulu name: *Umabophe*

Figure 2.3 *Acridocarpus natalius* flowers and pods (www.plantzafrica.com/plantab/acridnatal.htm).

Botanical Description

Acridocarpus natalius is a small tree or a scrambling shrub, a twiner or a rambling shrub on other vegetation. The tree is 1 to 5 m high. The stems and leaves are sometimes covered with velvety reddish pink hairs that fall off later. The leaves are alternate, simple, shiny dark green and leathery. The bark is grey and rough. The flowers are bright yellow or golden yellow. The fruit has three but more often two large, prominently veined wings that are joined (Leistner, 2000, Ngwenya *et al.*, 2003).

Distribution

Acridocarpus natalius is a common member of coastal forest, bushveld and sand forest (Palmer and Pitman, 1972). The plant is sometimes found on rocky outcrops or open grassland, occurring in the northern Eastern Cape in the Pondoland region, along the KwaZulu-Natal eastern region to Maputaland,

Swaziland, Mpumalanga's lowveld, Limpopo and Mozambique (Leistner, 2000, Ngwenya *et al.*, 2003, Golding, 2002).

Medicinal and Spiritual Uses

Roots are mixed with other ingredients to make a mixture called *intelezi* which is used to hinder court procedure. The use of this plant results in the case being postponed indefinitely or being brought to a speedy end. The roots are also used as *intelezi* for sprinkling around the homestead during a thunderstorm and to cleanse the whole family after the lightning has struck the homestead. Also to strengthen fighting sticks before traditional competitions (Palmer and Pitman, 1972, Leistner, 2000., Ngwenya *et al.*, 2003).

Data from ethnobotanical survey

The bark of *Acridocarpus natalius* is crushed, boiled and filtered to make a decoction for treating diarrhoea. Children drink a cup and adults two cups of the decoction. No side effects have been reported from the usage of the plant (Mayeza 2007, pers. comm).

Conservation status

Acridocarpus natalius plant is threatened by habitat degradation and grazing. A taxon is critically endangered when prone to extinction in the wild in the immediate future (Golding, 2002).

Family: APIACEAE
Scientific name: *Alepidea amatymbica*
Common name: larger tinsel flower
Zulu name: *Ikhathazo*

Figure 2.4 *Alepidea amatymbica* flowers and leaves (www.plantzafrica.com/plantab/alepidamat.htm).

Botanical description

Alepidea amatymbica is a robust, erect plant up to 2 m tall which grows in grassland; the leaves form a loose rosette with the flower spike rising above the surrounding grasses. The margins of the leaves are prominently toothed, each tooth ending in a bristle. The inflorescence is widely branched, with a number of small, star-shaped, white flowers (Gelfand *et al.*, 1985, Pooley, 1998, Nonjinge and Tarr, 2003).

Distribution

The plant is common in the summer rainfall grasslands of southern Africa and extends up the east coast as far as Zimbabwe and northwards into Kenya and Ethiopia (Gelfand *et al.*, 1985, Pooley, 1998, Scott-shaw, 1999).

Medicinal and Spiritual Uses

The roots are eaten raw or cooked to treat colds, coughs and influenza (Van Wyk *et al.*, 2000). Doses are a teaspoonful for a child and a tablespoonful for an adult. Root infusions are administered to children with coughs and colds

and treatment may also be with snuff from the powdered root or by inhaling smoke from burning roots. Roots are widely used for stomach and respiratory ailments by the Sotho and Xhosa people. It is also used for rheumatism, applied to wounds as styptics and chewed for sore throats. Roots are used for diarrhoea, abdominal pain, headaches, to repel bees and as a protective charm in Zimbabwe (Gelfand *et al.*, 1985, Somova *et al.*, 2001). A small dose of powdered roots is reported to act as a tonic but large doses have a purgative effect (Hutchings *et al.*, 1996). The dry rhizome and roots are smoked, or powdered and taken as a snuff by diviners and healers to assist in divination and communication with the ancestors. Smoking the roots results in mild sedation and vivid dreams. Elderly people powder the dry rhizome and take it as a snuff, or smoke the roots for headaches. The rhizome is carried as a lucky charm (Van Wyk and Gericke, 2000).

Data from ethnobotanical survey

Alepidea amatymbica is grown in the home gardens and also sold in the muthi market. The plant grows mostly in the forest during the summer season. To prepare the medicine for diarrhoea treatment *Alepidea amatymbica* roots are crushed, boiled and then filtered. *Alepidea amatymbica* could also be mixed with other plants such as *Hypoxis hemerocallidea* and *Callilepis laureola*. The medicine prepared from this plant is administered by mouth and as enemas. The dosage when administered by mouth is half a cup for children, one cup for adults and is taken three times a day. The dosage when administered as enemas depends on the person. No side effects have been reported from the usage of the plant (Luthuli 2007, pers. comm).

Chemical content

The rhizomes and roots contain high concentrations of several diterpernoids of the kaurene. The major compounds are dehydrokaurenoic acid and kaurenoic acids, of which ent-16-kauren-19-oic acid is usually present in the highest quantity (Van Wyk and Gerickle, 2000, Somova *et al.*, 2001).

Conservation status

Alepidea amatymbica plant is at low risk or near threatened; a taxa which does not qualify for conservation status, but which is close to qualifying for vulnerability (Scott-Shaw, 1999).

Family: LILIACEAE
Scientific name: *Aloe arborescens*
Common name: Krantz aloe
Zulu name: *Inhlaba*, *Inkalane*

Figure 2.5 *Aloe arborescens* growing between rocks (van Wyk and Smith, 2005).

Botanical Description

Aloe arborescens is a branched shrub or small tree with some obliquely disposed leaf rosettes. It only occurrs in high rainfall mountain grassland and forest areas, usually in rocky places. Leaves are curved, dull-greyish or bluish green with spinelles on both surfaces. The flowers are usually in unbranched, erect spikes (Van Wyk and Van Wyk, 1997, Matsuda *et al.*, 2008).

Distribution

Aloe arborescens is distributed all over South Africa (Van Wyk and Van Wyk, 1997, Gutterman and Chauservolfson, 2000., Beppu *et al.*, 2006).

Medicinal uses

Aloe arborescens have been reported to treat gastrointestinal complaints, skin injuries and burns. Various pharmacological and therapeutic activities of *Aloe* species have been studied, and there have been many reports of anti-inflammatory effects (Matsuda *et al.*, 2008).

Data from ethnobotanical survey

The leaves of *Aloe arborescens,* which are not sold in muthi markets, are used to treat diarrhoea. The mode of preparation is crushing the leaves, adding cold water and then filtering. The doses are teaspoonful for children and half a cup for adults. No side effects have been reported from the usage of the plant (Gumede 2007, pers. comm., Khoza 2007. pers. Comm., Sikhakhane 2007. pers. Comm., Zungu 2007, pers. comm).

Chemical content

Aloe species contains aloin, aloenin as anthranoid (Matsuda *et al.*, 2008), and phenolic compounds (Mill *et al.*, 1999, Gutterman and Chauservolfson, 2000, Beppu *et al.*, 2006).

Conservation status

Aloe arborescens is exceptionally common and is not threatened (Van Wyk and Smith, 2003).

Family: SALVADORACEAE
Scientific name: *Azima tetracantha*
Common name: Needle bush
Zulu name: *Ihlazane*

Figure 2.6 *Azima tetracantha* branch (www.zimbabweflora.co.zw/speciesdata/species.php?species_id=144260).

Botanical Description

Azima tetracantha is a spiny scrambling shrub consisting of spines in whorls of 4 and 4 angled branches. The leaves are oblong, leathery, and sharp-tipped at the apex. Flowers occur in axillary catkin like inflorescences and are greenish, sometimes flushed pink; sexes are separated on different plants. The fruits are thinly fleshy sub-spherical with a pointed tip, yellowish to white when ripe (Hutchings *et al.*, 1996, Hyde and Wursten, 2007).

Distribution

The plant is distributed all over the world and is especially common in Zimbabwe (Hyde and Wursten, 2007).

Medicinal Uses

The plant is used for snake bites in East Africa and in Peru as expectorants and anticatarrhals (Bennett *et al.*, 2004). Sap of the plant is used for toothache; it is inserted into the wound after tooth removal and also as a

disinfectant (Hutchings *et al.*, 1996). The plant is also used as food and for various herbal medicines in Africa, India and Madagascar (Bennett *et al.*, 2004).

Data from ethnobotanical survey

This plant was bought at Eshowe muthi Market. The medicine is prepared by crushing the leaves, boiling and filtering. Children drink half a cup and one cup is necessary for adults. No side effects have been reported from the usage of the plant (Dlamini 2007, pers. comm).

Chemical content

Four dimeric piperidine alkaloids, azime, azcarpine and carpaine have been isolated. Extracts from the roots and leaves did not yield any alkaloids or triterpenoids compounds (Hutchings *et al.*, 1996). The plant contains a high concentration of *N*-methoxy-3-indolylmethyl-glucosinolate (Bennett *et al.*, 2004).

Conservation status

Azima tetracantha is not endangered.

Family: ASTERACEAE

Scientific name: *Baccharoides adoensis* (= *Vernonia adoensis*)

Zulu name: *Inyathelo, uhlonyane*

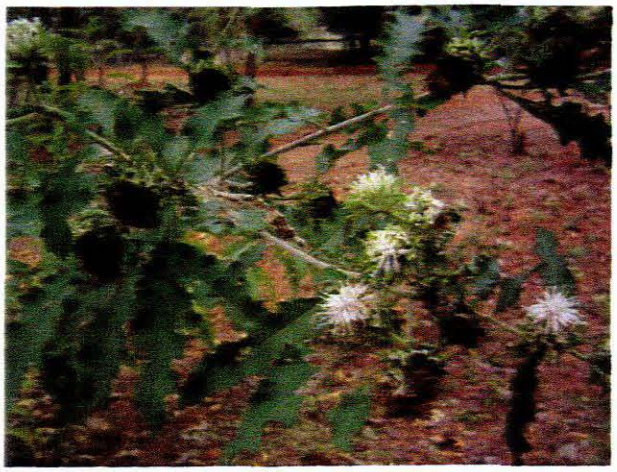

Figure 2.7 *Baccharoides adoensis* in flower (www.metafro.be/prelude/view country?cc=ZW).

Botanical Description

Baccharoides adoensis is a shrub of up to 2 m high. The roots are usually taproots, sometimes fibrous. The stem is usually erect, sometimes prostrate to ascending. The leaves are usually alternate or opposite each other, sometimes in basal rosettes. The fruits are dry with relatively thick, tough pericarps sometimes beaked and winged. The fruit consists of one seed (Nergard *et al.*, 2004).

Distribution

The plant is distributed from Senegal to Nigeria, extending across Africa to Ethiopia and is also found in the open grassland of KwaZulu-Natal (Nergard *et al.*, 2004).

Medicinal Uses

The roots are used in the Malian folk medicine for the treatment of gastritis, gastro duodenal ulcers, as an aid to ameliorate digestion and as a wound healing remedy. The plant is used for stomach pains and wound healing (Nergard *et al.*, 2004). In South Africa it is used to treat stomach, chest and skin complaints, head lice and back pain (Pooley, 1998).

Data from ethnobotanical survey

Baccharoides adoensis was collected from the surrounding area and is also cultivated in home gardens. It is sometimes planted as a fence around homesteads. This plant is not sold in the muthi market. There is no specific time for collection of the plant parts. The decoction resulting from crushed, boiled and filtered leaves is drunk by children (half a cup) and adults (one cup) to treat diarrhoea. This medicine was reported to have no side effects (Mtshali 2007, pers. comm).

Chemical content

Two polysaccharides, pectin and a pectic arabinogalactan were isolated from the dried powdered roots. Several acidic polysaccharide fractions were isolated from the roots and the glaucolides were isolated from the aerial parts (Nergard *et al.*, 2004).

Conservation status

Baccharoides adoensis is not endangered.

Family ASTERACEAE
Scientific name: *Callilepis laureola*
Common name: Ox-eye daisy
Zulu name: *Impila*

Figure 2.8 *Callilepis laureola* flowers (www.aluka.org/action/showCompilationPage?doi=10.55555).

Botanical Description

Callilepis laureola is a perennial herb and it mostly occurs in grassland. The leaves are very variable with 3 veined margins entire or toothed (Pooley, 1993). *Callilepis laureola* have solitary fowerheads and large woody tubers (Pooley, 1998).

Distribution

The plant grows mostly in grassland, from the Eastern Cape to Mozambique (Pooley, 1993).

Medicinal and Spiritual Uses

Leaves are used in traditional medicine to treat tapeworm, snakebites, whooping cough, infertility, to ensure easy childbirth, to kill maggots in cattle wounds and as a protective charm (Pooley, 1993, Van Wyk *et al.*, 2000). A

paste of the crushed roots is applied directly to open wounds, and then covered with a bandage. The tuberous root of the plant is used in traditional medicine by the Zulu and Xhosa people of South Africa (Steenkamp *et al.*, 2004). Although there are no approved medical uses of *Impila* from a health regulatory standpoint, the plant is widely used among the Zulu people and appears to serve as a multi-purpose remedy. Reports indicate that *Callilepis laureola* is used to treat stomach problems. A tonic made from the root is also taken by young girls in the early stages of menstruation. The greatest and most valued attribute of this plant, however, appears to lie in its "protective powers" in warding off "evil spirits". For example, an *Impila* decoction consumed before festivals is thought to offer protection from "those harboring evil intentions" (Popat *et al.*, 2001).

Data from ethnobotanical survey

Callilepis laureola was bought at the muthi market. The plant is available mostly during the summer season in the forest. The plant part used for the treatment of diarrhoea is the roots. The crushed, boiled and filtered roots of *Callilepis laureola* are used to treat diarrhoea. Oral administration doses are half a cup for children and a cupful for adults. The dosage depends on the person when the medicine is taken as enemas. No side effects have been reported from the usage of the plant (Luthuli 2007, pers. comm).

Chemical content

Callilepis laureola has been reported to be very toxic and has been found to cause fatal liver necrosis in humans. Generalized symptoms of intoxication include abdominal pain, vomiting, diarrhoea, a disturbed level of consciousness, convulsions, and acute liver and renal failure leading to severe hypoglycemia and metabolic acidosis (Popat *et al.*, 2001). The plant contains an acidic form of atractyloside. The glucosidic nature of the atractylosides has been confirmed and the compounds isolated include three further kaurenoid glycosides, carboxy atractloside, two 6-isovalerates, two new thymol derivatives and a new ketol (Hutchings *et al.*, 1996, Steenkamp *et al.*, 2004).

Conservation status

Callilepis laureola is not endangered.

Family: APOCYNACEAE
Scientific name: *Cantharanthus roseus*
Common name: Periwinkle
Zulu name: *Imbali emhlophe; ikhwinini*

Figure 2.9 *Cantharanthus roseus* flowers (en.wikipedia.org/wiki/catharanthus_roseus).

Botanical description

Cantharanthus roseus is an evergreen shrub or herbaceous plant growing 1 m tall. The leaves are oval to oblong, 2.5-9 cm long and 1-3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole of 1-1.8 cm long arranged in opposite pairs. The flowers are white to dark pink with a darker red centre. The fruit is a pair of follicles (Huxley, 1992).

Distribution

It originates from Madagascar, but can now be found in tropical and subtropical places all over the world. In South Africa it has become a weed, particularly in KwaZulu-Natal and Mpumalanga (Van Wyk and Van Wyk, 1997).

Medicinal Uses

Cantharanthus roseus is used as a tea substitude. Tea from the flowers is used for blood cleansing and the milk sap is used for insect bites and warts (Hutchings *et al.*, 1996). Leaves are used for menorrhagia and rheumatism in parts of southern Africa and as a galactagogue. In Madagascar leaves and roots are used as purgatives, emetics and depuratives. The plant is traditionally used to treat diabetes and rheumatism and the isolated alkaloids are used in modern cancer therapy (Van Wyk and Wink, 2004., Wu *et al.*, 2004). Leaves are also used for tooth aches and for urinary tract infections (Hutchings *et al.*, 1996, Wu *et al.*, 2004).

Data from ethnobotanical survey

The leaves of *Cantharanthus roseus*, which are not sold in the muthi market, are used to treat diarrhoea and toothache. For treating diarrhoea, cold water is added to crushed leaves and then filtered. Adults drink a cup and children only half a cup of this medicine. However, a paste prepared from adding hot water to crushed leaves is used as a remedy for toothache. No side effects have been reported from the usage of the plant (Nzobe 2007, pers. comm).

Chemical content

The plant contains active indole alkaloids including catharathine; lochnerine; vindoline; vincristine (used to treat child leukaemia and Hodgkin's disease); vinblastine; reserpine and ajmaline (Watt and Breyerbrandwijk, 1962., Wu *et al*, 2004). A calmodulin-like protein was isolated from suspension cultured cells of *Catharanthus* (Hutchings *et al.*, 1996, Laszlo and Dicosmo, 2001).

Conservation status

Cantharanthus roseus is not endangered.

Family: CHENOPODIACEAS
Scientific name: *Chenopodium ambrosioides*
Common name: Wormseed
Zulu name: *Insukumbili*

Figure 2.10 *Chenopod ambrosioides* leaves (www.uni-graz.at/~katzer/engl/Chen_amb.htm/).

Botanical description

Chenopodium ambrosioides is an herbaceous perennial with erect stems of up to a meter in height, sprouting from a woody rootstock. The leaves are about 60 mm long with characteristically serrated margins, making the plant easy to identify. Small yellow flowers are borne in sparse clusters towards the end of the branches. The leaf stalks are long, flat and the leaf margins are toothed or lobed (Hutchings *et al.*, 1996, Van Wyk *et al.*, 2000).

Distribution

The plant is distributed all over Africa (Hutchings *et al.*, 1996, Ketzis *et al.*, 2002).

Medicinal Uses

The plant has been reported to be administered as enemas for intestinal ulceration. Seeds, or sometimes the plant decoctions are used as insecticides in the Transkei. Infusions are taken for colds and stomach ache by the Sotho people and unspecified parts are used as a vermifuge. Plant decoctions are

used topically for eczema and poultices are used to remove sand worms in unspecified areas of southern Africa. In Zimbabwe leaves are used in medicines for madness, convulsions, uterine pain, chest pain and fevers in infants. *Chenopodium ambrosioides* is cultivated as a snake repellent in Zimbabwe. Leaves are used in the treatment of leprosy in Nigeria. Sap is used for nervous complaints and as a vermifuge and abortifacient in Mauritius. The plant has been reported to cause hay fever and stock poisoning in Queensland (Hutchings *et al.*, 1996). The plant is strongly aromatic with a pungent, bitter taste (Hutchings *et al.*, 1996). *Chenopodium ambrosioides* and its essential oils have a long history of use to treat intestinal parasites, particularly ascaris (maw worm) and hookworm in adults and children (Van Wyk and Wink, 2004).

Data from ethnobotanical survey

Chenopodium ambrosioides is used to treat both diarrhoea and sores. *Chenopodium ambrosioides* mostly grows in wetlands and is available in the muthi markets in its fresh form. The mixture of crushed leaves and alum, in cold water, is prepared and administered orally and as an enema. Oral administration doses are a spoonful for children and half a cup for adults, twice a day. No side effects have been reported from the usage of the plant (Nkwanyane 2007, pers. comm).

Chemical content

All parts of the plant contain spooning, especially the roots (Hutchings *et al.*, 1996). The main chemicals found in *Chenopodium ambrosioides* include alpha-pinene, aritasone, ascaridole, butyric-acid, d-camphor, essential oils, ferulic-acid, geraniol, l-pinocarvone, limonene, malic-acid, menthadiene, menthadiene hydroperoxides, methyl-salicylate, myrcene, p-cymene, p-cymol, safrole, spinasterol, tartaric-acid, terpinene, terpinyl-acetate, terpinyl-salicylate, triacontyl-alcohol, trimethylamine, urease, and vanillic-acid. The essential oil in the seed and flowering plant are highly toxic (Kliks, 1985, Ketzis *et al.*, 2002).

Conservation status

Chenopodium ambrosioides is not endangered.

Family: ASTERACEAE
Scientific name: *Chromolaena odorata*
Common name: Parafin weed
Zulu name: *Usandanezwe*

Figure 2.11 *Chromolaena odorata* flowers and plant (www.geocities.com/wessaaliens/species/chromo.htm).

Botanical Description

Chromolaena odorata has leaves that are triangular, with three conspicuous veins and smells strongly of turpentine or paraffin when crushed. The flowers are tufted mauve to whitish. *Chromolaena odorata* is a scrambler when it grows among trees (Windly and Moore, 2006).

Distribution

Chromolaena odorata was first recorded in the South Western Cape in 1858 where it did not survive. It was probably reintroduced into Natal in about 1947 in packing material contaminated with the seed that was off-loaded at Durban harbor. It soon established itself in 1962 and was reported as spreading virulently along the coastal areas. The plant is now a major perennial weed in the coastal region of KwaZulu-Natal and all eastern lowveld areas (Browmilow, 2001).

Data from ethnobotanical survey

Chromolaena odorata is mostly available in the summer season and grows wild all over disturbed areas, such as roadsides. The crushed, boiled and filtered leaf decoction is used to treat diarrhoea. Children take a spoonful and adults a cup of this decoction. No side effects have been reported from the usage of the plant (Gumede 2007, pers. Comm., Masikane 2007, pers. Comm., Ntuli 2007, pers. comm).

Chemical content

The plant is poisonous (Browmilow, 2001). *Chromolaena odorata* afforded a new flavonoid, 5,7- dihydroxy-6,4-dimethoxyflavanone (Pisutthanan et al., 2006).

Conservation status/ Control measures

The *Chromolaena odorata* is an alien invasive species. Its control is difficult and costly because it is capable of vigorous re-growth from stem coppices, root suckers and seed. Large plants must be cut down and a suitable herbicide should be applied to the stump or re-growth. Follow-up inspections and treatment are essential to ensure that all traces have been eliminated. A beetle is raising hope of biological control, but so far it has failed to establish itself where it was released in KwaZulu-Natal. The work continues with various other species of insects (Browmilow, 2001).

Family: VITACEAE
Scientific name: *Cyphostemma hypoleucum*
Common name: Double-barrel vine
Zulu name: *Udekane, Umbombo*

Figure 2.12 *Cyphostemma hypoleucum* leaves (Pooley, 2005).

Medicinal Uses

The plant decoction is administered as enemas for febrile conditions (Hutchings *et al.*, 1996). Nothing much has been reported on the plant.

Distribution

Cyphostemma hypoleucum is distributed mostly in southern Africa (Hutchings *et al.*, 1996).

Data from ethnobotanical survey

Cyphostemma hypoleucum prefers to grow mostly in forest and in dry lands. The plant is used to treat diarrhoea and flu. To treat diarrhoea, warm water or milk is added to crushed stems and leaves (shoots), filtered and administered in enema doses of half a cup for children and one cup for adults. No side effects have been reported from the usage of the plant (Ngema 2007, pers. Comm., Thungo 2007, pers. comm).

Conservation status

Cyphostemma hypoleucum plant is at lower risk (Golding, 2002).

Family: MIMOSACEAE

Scientific name: *Dichrostachys cinerea*

Common name: Sickle bush

Zulu name: *Ugagane*

Figure 2.13 *Dichrostachys cinerea* tree with flowers (www.hear.org/pier/species/dichrostachys_cinerea.htm).

Botanical description

The plant is a thorny, small, fast-growing woody bush or tree (1.5-6 m) which invades fields and wastelands (Yayneshet *et al.,* 2008). The branches bear short, thorn ended twigs. Leaves are bipinnate. The flowers are two-coloured spikes with the upper part pink and consisting of staminodes, the lower part is yellow and is made up of fertile flowers. Pods are crowded. The seeds are obovate, dark brown 4 mm long (Hutchings *et al.*, 1996, Tassin, 1999).

Distribution

Dichrostachys cinerea is common from southern and tropical Africa to India and in Australia (Hutchings *et al.*, 1996). The plant grows mostly among the common woody browse plants that naturally grow in many arid and semi-arid rangelands in Sub-Saharan Africa (Yayneshet *et al.,* 2008, Mlambo *et al.*, 2004).

Medicinal Uses

The tree is very widely used in southern and tropical Africa for a variety of ailments. These include usage of the roots for abdominal pains, diarrhoea, coughs and toothache, to stop bleeding and as a diuretic. The fruits are used to treat sores and scabies. Bark is used for all sorts of skin infections and for post-partum pain, elephantiasis and as a ritual cleanser (Eisa *et al.*, 2000). Extracts from the stem and branches have shown positive antibacterial activity against *Staphylococcus aureus* and mild antimicrobial activity against *Escherichia coli* (Eisa *et al.*, 2000). Hutchings and Van Staden (1994) have found indications of bacterial or cytotoxic activity. The bark is used to treat dysentery, tooth-aches and elephantiasis. The leaves are a laxative and are used to treat gonorrhoea and boils. It is also a remedy for removing poison from snake bites (Rulangaranga, 1989). The seeds are used for feeding goats (Mlambo *et al.*, 2004, Yayneshet *et al.*, 2008). The roots are astringent and used in treating rheumatism and renal troubles and in the treatment of wounds (Eisa *et al.*, 2000).

Data from ethnobotanical survey

The plant prefers to grow in forests and there is no specific time for the collection of the plant parts. *Dichrostachys cinerea* bark, which is also available in the muthi markets is used to treat diarrhoea and as a mosquito repellent. The medicine is prepared by crushing the bark, boiling and filtering it and is administered by mouth and as an enema. The dosage when taken by mouth is half a cup for children and a cup for adults. The dosage when taken as an enema depends on the person. No side effects have been reported for the plant (Khumalo 2007, pers. comm).

Chemical content

The presence of the alkaloids and saponins has been found in the roots and leaves (Hutchings and Van Staden, 1994). *Dichrostachys cinerea* contains a high amount of crude proteins, Na, K, Fe, and Zn concentrations (Yayneshet *et al.*, 2008). The plant has been reported to contain tannins, triterpenes and sterols (Eisa *et al.*, 2000, Smith *et al.*, 2005, Mlambo *et al.*, 2008).

Conservation status

Dichrostachys cinerea plant is at low risk (Golding, 2002).

Family:	MORACEAE
Scientific name:	*Ficus sur*
Common name:	*Cape fig*
Zulu name:	*Umkhiwane*

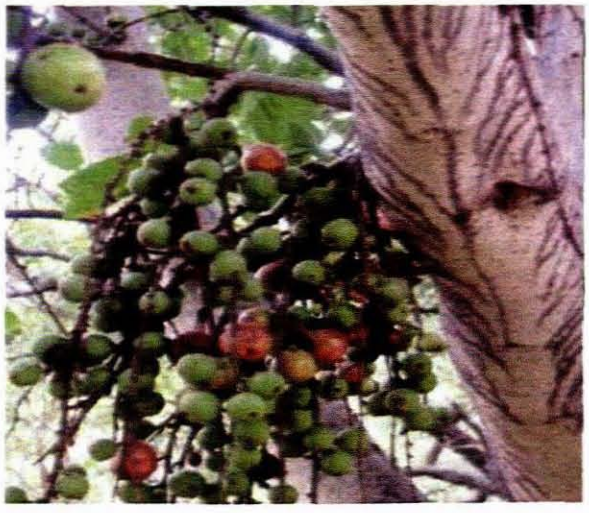

Figure 2.14 *Ficus sur* tree with fruits (Pooley, 1993).

Botanical description

Ficus sur is a large, fast-growing, evergreen tree, with large, oval, green leaves borne on a massive, spreading crown. In South Africa figs are produced from September to March and are borne in large clusters mostly low down on the trunk and can even appear at ground level arising from the roots (Pooley, 1993).

Distribution

This species is widely distributed from North Africa to the Western Cape in South Africa. *Ficus sur* is usually found on riverbanks or in riverine forests but can also be found in drier woodlands. The plant is restricted to frost-free areas with moderate rain (Kunle *et al.*, 1999, Von Breitenbach *et al.*, 2001).

Medicinal Uses

Roots and bark decoctions are administered for suspected ulceration of the lungs possibly referring to pulmonary tuberculosis. Leaf and bark infusions are used as bovine galactagogues. The roots are used for treating diarrhoea (Pooley, 1993., Kunle *et al.*, 1999). Root decoctions are also used for retaining placentas in cows (Pooley, 1993). In Zimbabwe root infusions are used for treating sterility in men, infertility in women and for uterine pain and swollen legs (Hutchings *et al.*, 1996). *Ficus sur* is also reported to be used in the treatment of malaria and fever (Muregi *et al.*, 2003, Muregi *et al.*, 2007).

Data from ethnobotanical survey

The plant has been reported to be both collected in the wild, most commonly in the forest and cultivated in homesteads. The plant is not sold in the muthi market. Flowers of *Ficus sur* are eaten as fruits, while leaves, mostly available in summer, are used to treat diarrhoea. The medicine is prepared by adding cold water to crushed leaves, filtering, and is given/administered in dosage of a cup to adults and half a cup to children. There are no side effects from drinking *Ficus sur* decoctions (Mayeza 2007, pers. comm).

Chemical content

Traces of ascorbic acid are found in the fruit, while leaves, stem and fruit give positive tests for sterols. The bark is resinous and very bitter and reported to contain tannin (Hutchings *et al.*, 1996). *Ficus sur* contains chloroquine (Muregi *et al.*, 2003, Muregi *et al.*, 2007).

Conservation status

Ficus sur is not endangered.

Family:	ASTERACEAE
Scientific name:	*Helichrysum odoratissimum*
Common name:	Everlastings
Zulu name:	*Imphepho*

Figure 2.15 *Helichrysum odoratissimum* plant with flowers (www.plantzafrica.com/planthij/helichrysodor.htm).

Botanical description

Helichrysum odoratissimum is classified as a perennial herb or shrublet (Germishuizen and Meyer, 2003). The plant grows mostly in poor stony or sandy grassland; it invades over grazed areas and road sides. *Helichrysum odoratissimum* has densely aromatic hairy or woolen leaves and persistent flower heads (Hutchings *et al.*, 1996).

Distribution

Helichrysum odoratissimum ranges from the Soutpansberg in Limpopo through the highlands of Mpumalanga and the West of Swaziland to the Midlands and Uplands of KwaZulu-Natal, Free State, Lesotho, the Cape Drakensberg mountains and coastal areas of the Eastern Cape, across the Cape fold mountains of the Cedarberg, to Giftberg in Vanrhynsdorp and as far as the Cape Peninsula. *Helichrysum odoratissimum* is also found in the mountains of Mozambique, Zimbabwe, Malawi and further north (Van Wyk and Gerickle, 2000, Joffe, 2003).

Medicinal and Spiritual Uses

The smoke of *Helichrysum odoratissimum* is inhaled as part of local Shamanic rituals or used as a ritual incense to drive away evil spirits (Hansel *et al.*, 1980, Joffe, 2003., Germishuizen and Meyer, 2003). *Imphepho* has been used to treat numerous diseases that are often ascribed to the working of these aforementioned spirits namely: coughs, colds, fever, infections and headaches (Hutchings *et al.*, 1996). Some people boil the plant and use it as a facial ointment for pimples. In Lesotho people burn this plant to fumigate rooms. It is also effective in repelling parasites and insects thus ensuring a good night's rest (Joffe, 2003).

Data from ethnobotanical survey

Helichrysum odoratissimum prefers to grow in dry lands. The plant is sold in its dry form in the muthi markets. The medicine for treating diarrhoea is prepared by cutting the plant into small pieces, adding water and filtering. Children are given half a cup and adults one cup to drink once a day, for diarrhoea. There are no side effects that have been reported for the plant (Zungu 2007, pers. comm).

Chemical content

The chemicals 3, 5-dihydroxy-6, 7, 8-trimethoxyflavone, 3-0-methylquercetin, and helichrysetin were isolated from the flowers (Prod, 1989). The following chemicals have been isolated from the plant: glucosides e.g. arbutin, sitosterol and glucoside. Monoterpenes e.g. pinene, terpineol and limonene. Sesquiterpenes e.g. viridiflorol, long chain carboxylic acid e.g. hexadecanoic acid and tetradecanoic acid (Swarts, 2006).

Conservation status

Helichrysum odoratissimum is not endangered.

Family: HYPOXIDACEAE
Scientific name: *Hypoxis hemerocallidea*
Common name: African potato
Zulu name: *Inkomfe*

Figure 2.16 *Hypoxis hemerocallidea* plant with flowers (www.plantzafrica.com/planthij/hypoxis.htm).

Botanical description

Hypoxis hemerocallidea has a corm that is hard, fleshy, mucilaginous and is white or yellow-orange within. Sliced corms, when exposed to the atmosphere, turn black with oxidation. In most species leaves are arranged one above the other in three rows that radiate outwards. Flowering stems appear within the leaves and are unbranched, with 2-12 flowers per stalk. Flowers are symmetrical (Gillmer and Symmonds, 1999, Van Wyk *et al.*, 2000). The leaves are erect, soft, sickle shaped, keeled, with prominent ribs, tapering to tips with dense white hair on the lower surface (Hutchings *et al.*, 1996).

Distribution

The genus *Hypoxis* has an estimated 90 species world-wide and is distributed on all the continents except Europe. In the Flora of southern Africa (FSA) region that includes South Africa, Namibia, Botswana, Lesotho and Swaziland, there are about 30 species, the majority confined to the eastern

parts of the subcontinent. The taxonomy of the genus has always presented a challenge due to a lack of distinct diagnostic characteristics that readily define species and infraspecific taxa (Van Wyk and Gericke, 2000, Singh, 2007).

Medicinal and Spiritual Uses

The juice from the rootstock is applied to burns. Corms are used as receptacles into which some blood from the forehead of the patient is placed and then buried. The white farmers in southern Africa use this plant to treat symptoms of benign prostate hypertrophy. The plant is administered orally or as enemas in the Transkei for patients who cannot speak, possibly as a result of shock (Hutchings *et al.*, 1996).

Leaves are used to make lasting rope, bulbs are used to blacken floors. This plant has been reportedly used to treat dizziness and mental disorders, and in western medicine, to treat cancer, inflammation and HIV (Pooley, 1993). The corm of the African potato plant (*Hypoxis hemerocallidea)* is widely used in traditional African medicine for the treatment of many general ailments, e.g., allergies, ulcers, arthritis, hypercholesterolaemia and infertility (Erlwanger and Cooper, 2008).

Hypoxis hemerocallidea seems to be an effective and non-toxic way of improving cell mediated immunity in several different situations where cell mediated immunity is compromised. It is a non-toxic and relatively cheap approach to these conditions (Nair *et al.*, 2007). African potato has been specifically promoted by the South African Minister of Health for improving cell mediated immunity (South African development Committee, 2000). Many compounds found in the African potato are known to possess antimicrobial, antioxidant and/or anti-inflammatory activity (Steenkamp *et al.*, 2006).

Data from ethnobotanical survey

Hypoxis hemerocallidea grows in forests and drylands. This plant is also sold in the muthi markets. Leaves and corms are used to treat diarrhoea and also used by people with HIV and Aids. The corm is crushed, boiled and filtered, to prepare the medicine that is administered orally and as enemas. The oral

dosage is a teaspoonful for children and one spoonful for adults. The dosage depends on the person when administered as enemas. No side effects have been reported on the plant (Luthuli 2007, pers. comm., Masikane 2007, pers. comm).

Chemical content

The major constituent of the corms of *Hypoxis* is the pentenyne glycoside hypoxoside. The corms are reported to contain hypoxosode, ß- sitisterol, sterolin and monoterpene glycosides (Hutching *et al.*, 1996, Nair *et al.*, 2007). African potato has also been reported to contain tannins (Steenkamp *et al.*, 2006).

Conservation status

Hypoxis hemerocallidea plant is data deficient. This is when there is inadequate information to make a direct or indirect assessment of its risk of extinction based on its distribution and population status. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that the future research will show that threatened classification is appropriate (Golding, 2002).

Family: VERBENACEAE

Scientific name: *Lippia javanica*

Common name: Fever tea/Lemon bush

Zulu name: *Umsuzwane*

Figure 2.17 *Lippia javanica* tree with flowers (www.plantzafrica.com/plantklw/lippiajavan.htm).

Botanical description

Lippia javanica is a multi-stemmed, erect, woody shrub, which is up to 2 m in height. The stems are square-shaped. The leaves are hairy with noticeable veins and when crushed give off a strong lemon-like smell. It is one of the most aromatic of South Africa's indigenous shrubs. The small cream flowers can be found on the shrub from summer to autumn in some areas and in others are produced throughout the year. These flowers are arranged in dense, rounded flower heads. The fruit are rather inconspicuous, small and dry (Van Wyk and Malan, 1997, Pooley, 1998, Van Wyk and Gericke, 2000).

Distribution

This plant is widespread throughout large parts of South Africa where it is used extensively in traditional herbal preparations (Viljoen *et al.*, 2005), with the exception of the Western Cape. The plant grows from the Eastern Cape northwards extending into tropical Africa including Botswana, Swaziland, Mozambique, Malawi Tanzania, Zambia, and Kenya (Van Wyk and Malan, 1997., Pooley, 1998., Van Wyk and Gerickle, 2000).

Medicinal and Spiritual Uses

Lippia javanica is well known medicinally to many African tribes and to many avid herbalists and herb gardeners (Pooley, 1998). Hot leaf infusions are widely used for coughs and colds, most frequently as inhalants but also taken orally. Leaves are also used to treat febrile rashes and are sometimes smeared on the body as a protection against dogs and crocodiles. It is also used in bathing and poultices where leaves are applied to warm the lower limbs. Cold leaf infusions are taken for a condition referred to as gangrenous rectitis. Weak leaf and stem infusions are taken for coughs, colds and bronchial ailments and with the addition of *Artemisia afra* are also used for fevers and measles by the Xhosa.

Plants are used to disinfect suspected anthrax-infested meat. Leaves are used for variety of ailments including asthma, headaches, febrile and respiratory complaints, convulsions, weak joints, cataracts and sore eyes in Zimbabwe (Pooley, 1993, Hutchings and Van Staden, 1994., Van Wyk and Gericke, 2000). The leaves are strongly aromatic and have antimicrobial properties (Manenzhe *et al.*, 2004, Viljoen *et al.*, 2005). *Lippia javanica* leaves were screened for repellent activities against *Anopheles* sp. (Manenzhe *et al.*, 2004, Omolo *et al.*, 2004).

Data from ethnobotanical survey

Lippia javanica leaf is used to treat diarrhoea and headache. The medicine is prepared by adding water to the leaves and filtering it or a person can also chew the leaves as a mode of administering it. The dosage depends on the person. No side effects have been reported from the usage of the plant (Mhlongo 2007, pers. comm).

Chemical content

Lippia javanica contains essential oil, with neral, geranial and photocitral A as the characteristic compounds. Also present are other monoterpenes as well as some sesquiterpenoids (Manenzhe *et al.*, 2004., Omolo *et al.*, 2004), flavonoids include apigenin, luteolin and 6-hydroxylated flavones, together

with their methyl ester (Van Wyk and Wink, 2004) and aromatic compounds such as myrcene, myrcenone and limonene (Viljoen *et al.*, 2005)

Conservation status

Lippia javanica is not endangered.

Family:	CELASTRACEAE
Scientific name:	*Gymnosporia buxifolia (= Maytenus heterophylla)*
Common name:	Common spike-thorn
Zulu name:	*Isibhubu*

Figure 2.18 *Gymnosporia buxifolia* branch (www.worldbotanical.com/african plants. htm).

Botanical description

Gymnosporia buxifolia has common spike thorns. It is a small, evergreen, drought resistant tree with drooping branches. The leaves are often in tufts, thinly leathery and dull green mainly on the upper half. The flowers are white in colour with many flowered axillary heads and a strongly unpleasant smell. Fruits are a globose capsule of about 5 mm in diameter, rough white with reddish brown patches (Van Wyk and Van Wyk, 1997). The large spike makes this a good perimeter plant. This plant attracts birds which eat the numerous berries it produces (Hutchings *et al.*, 1996).

Distribution

The plant is distributed all over Africa (Hutchings *et al.*, 1996, Orabi *et al.*, 2001).

Medicinal and Spiritual Uses

The bark infusions are administered as emetics and enemas. Leaf infusions are administered to stock animals for diarrhoea (Orabi *et al.*, 2001). The plant is used by Sotho people, who mix it with part of a snake, as a snakebite remedy and it is also rubbed on the body as a love charm. Roots and thorn decoctions are taken for chest colds and coughs by the Tswana, Koba and Subiya. Roots decoctions are used to treat for haemorrhoids, urine retention and venereal disease in Botswana. In various areas of East Africa, root decoctions are taken as anthelmintics and for epilepsy and used for abscesses, hernias and syphilis. In West Africa, leaves and roots are used for viral infections and as anti-inflammatories. Extract from the roots shows marked anti-inflammatory activity in rats and mice (Hutchings *et al.*, 1996). In Kenya the plant is used as mosquito repellent, and to treat fever and joint pains (Muthaura *et al.*, 2007). *Gymnosporia buxifolia* is also used as an antibacterial agent (Orabi *et al.*, 2001).

Data from ethnobotanical survey

Gymnosporia buxifolia grows mostly in dry lands and in the forest. The plant has been reported to be used for the treatment of diarrhoea and flu in both goats and people. To prepare the medicine, hot or cold water is added to crushed plant or leaves and filtered. The common spike-thorn plant is not sold in the muthi market. The medicine is administered orally, where the dosage is two spoons for children and half a cup for adults, once a day. No side effects have been reported from the usage of the plant (Dlamini 2007, pers. comm., Masikane 2007, pers. comm., Mrs Mhlongo 2007, pers. comm).

Chemical content

The plant contain the following chemicals: dihydroagarofuran alkaloid, 1β-acetoxy-9α-benzoyloxy-2β, 6α-dinicotinoyloxy-β-dihydroagarofuran, together with the known compounds β-amyrin, maytenfolic acid, 3α-hydroxy-2-

oxofriedelane-20α-carboxylic acid, lup-20(29)-ene-1β,3β-diol, -4'-methyl*epi*gallocatechin, and (−)-*epi*catechin (Orabi *et al.*, 2001).

Conservation status

Gymnosporia buxifolia is not endangered.

Family:	MELIACEAE
Scientific name:	*Melia azedarach*
Common name:	Syringe, Persian Lilac
Zulu name:	*Umsilinga*

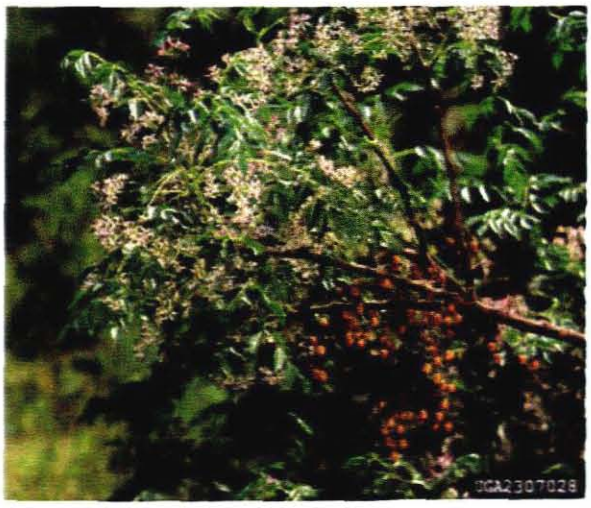

Figure 2.19 *Melia azedarach* tree with flowers and fruits (www.issg.org/database/species/ecology.asp?si=636&sts=sss).

Botanical description

Melia azedarach is described as a small to medium-sized shrub or tree. Branches are stout, with purplish bark dotted with buff-colored lenticels. Leaves are twice to three time's compound, alternate and puberulent to glabrous. Leaflets are 2-8 cm long, serrate or crenate, dark green above, often with sparse hairs along the veins and lighter and generally smooth below (Van Wyk and Van Wyk, 1997, Pooley, 2003). The fruits are poisonous (Pooley, 2003).

vanillic glucose; vanillic aldehyde; transcomanainic acid and vanillic acid (Hutchings *et al.*, 1996). Limonoids and 12-hydroxyamoorastatin compounds have been isolated from the *Melia azedarach* fruits (Coria *et al.*, 2008).

Conservation status

Melia azedarach is not endangered.

Family:	MYRTACEAE
Scientific name:	*Psidium guajava*
Common name:	Guava
Zulu name:	*Umgwava*

Figure 2.20 *Psidium guajava* fruits (www.hort.purdue.edu/newcrop/morton/guava.html).

Botanical description

Psidium species are evergreen trees with opposite leaves, fluffy white flowers and rounded to oblong edible berries (Hutching *et al.*, 1996).

Distribution

The guava has been cultivated and distributed by man, birds and animals for such a long time that its origin is uncertain, but it is believed to originate from southern Mexico (Morton, 1987). The guava has become naturalized in many parts of the world and is widely cultivated as a commercial fruit crop (Hutching *et al.*, 1996, Singh and Pal, 2008).

Medicinal Uses

Guava leaves are commonly used in South African traditional medicine, mainly to treat gastrointestinal disorders (such as diarrhoea), diabetes (Kaushik *et al.*, 2008), fever (including malaria), coughs, ulcers, boils and wounds (Hutchings *et al.*, 1996., Van Wyk and Wink, 2004., Gutieriez *et al.*, 2008., Patthamakanokporn *et al.*, 2008). The plant has also been used extensively as a hypoglycaemic agent (Patthamakanokporn *et al.*, 2008).

Data from ethnobotanical survey

The guava tree is cultivated in home gardens and can grow naturally in forests, where plant growth ranges from drylands to wetlands. Guava fruits are edible. Leaves which are mostly available in summer and can be collected anytime of the day are used to treat diarrhoea. The medicine is prepared by adding either cold or boiling water to crushed leaves and then filtering. The cold water prepared medicine is administered orally, while the one prepared by boiling water is administered as an enema. Oral dosage is not specified and enema dosage also depends on the person using it or the patient. No side effects have been reported from the usage of the plant (Dindi 2007, pers. comm., Dlamini 2007, pers. comm., Gumede 2007, pers. comm., Lindi Shobede 2007, pers. comm., Miya 2007, pers. comm., Mkhize 2007, pers. comm., Mrs Mhlongo 2007, pers. comm., Ndlovu 2007, pers. comm., Ngema 2007, pers. comm., Nguni 2007, pers.comm., Ntombifikile 2007, pers. comm., Khoza 2007, pers. comm).

Chemical content

The family Myrtaceae is known for various ethereal oils found in most or all of the unlignified shoot tissues of the shoots. These oils (sesquiterpenoids, triterpenoids etc.) are abundant, scattered, small schizogenous secretory cavities. Scattered tanniferrous cells are often present and the plants nearly always contain proanthocyanins and usually also ellagic acid and gallic acid. Guava leaf is rich in tannins and other phenolic compounds, of which amritoside is of particular interest. Another biologically interesting compound is guajaverin, a glycoside of quercetin. Tannins are known for intestinal astringents and haemostatic. The tannins presence can plausibly explain the

therapeutic value of the plant against diarrhoea and dysentery (Van Wyk and Wink, 2004, Gutieriez *et al.*, 2008). *Psidium guajava* fruits have high antioxidant activity and are totally phenolic (Salazar *et al.*, 2006, Patthamakanokporn *et al.*, 2008).

Conservation status

Psidium guajava is not endangered.

Family: ROSACEAE
Scientific name: *Prunus persica*
Common name: Peach Tree
Zulu name: *Umphentshisi*

Figure 2.21 *Prunus persica* tree with fruit (en.wikipedia.org/wiki/Peach).

Botanical Description

Prunus persica is a deciduous fruit tree. The leaves are lanceolate and the flowers are produced in early spring before the leaves; they are solitary or paired. The fruit is a drupe with a single large seed encased in hard wood with yellow or whitish flesh. The seed is red-brown, oval shaped and 1.5–2 cm long (Huxley, 1992, Szalay *et al.*, 2000., Simas *et al.*, 2008).

Distribution

This plant is distributed all over the world as it is a cultivated fruit tree (Brown, 1995, Szalay *et al.*, 2000).

Medicinal Uses

The leaves are used internally to treat gastritis, whooping cough, and cough bronchitis. Leaves also ease vomiting and morning sickness during pregnancy (Brown, 1995).

Data from ethnobotanical survey

Plant growth ranges from drylands to wetlands. The leaves, which are mostly available in summer and collected at anytime of the day, have potential to treat diarrhoea. The medicine is prepared by pouring either boiling or cold water to crushed leaves and then filtering. The medicine prepared by cold water is administered orally and the medicine prepared by boiling water is administered as enemas. Both oral and enema doses are not specific. No side effects have been reported from the usage of the plant (Bafana Mhlongo 2007, pers. comm., Gogo Mkhize 2007, pers. comm., Mrs Dlamini 2007, pers. comm., Mrs Gumede 2007, pers. comm., Mrs Ngcobo 2007, pers. comm., Mrs Ngema 2007, pers. comm., Mrs Nkwanyane 2007, pers. comm).

Chemical content

Prunus persica has been reported to contain the following: tannins, hydrogen cyanide, fatty acids (Brown, 1995), polysaccharide and uronic acids (Kardosova and Machova, 2006, Simas *et al.*, 2008). *Prunus persica* fruit has high antioxidant activity (Kardosova and Machova, 2006).

Conservation status

Prunus persica is not endangered as it is cultivated as a fruit tree.

Family: ANACARDIACEAE
Scientific name: *Sclerocarya birrea*
Common name: Marula tree
Zulu name: *Umganu*

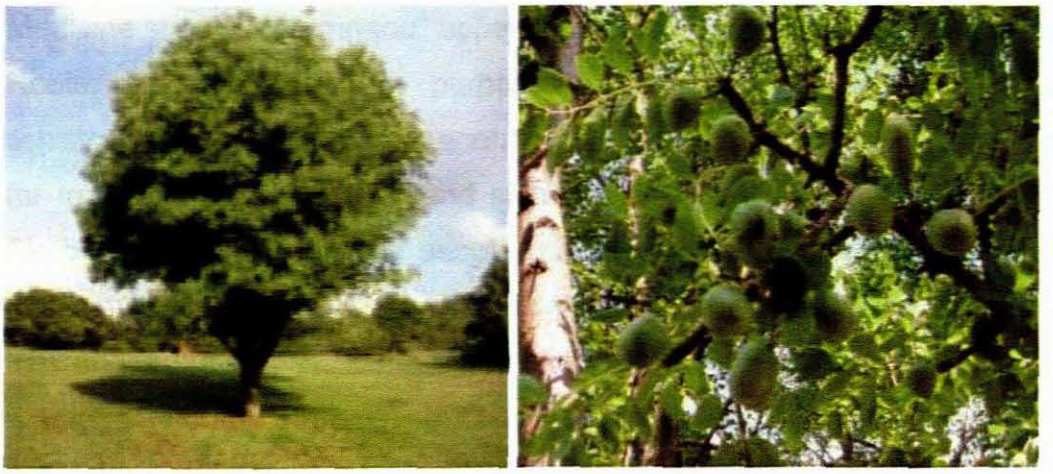

Figure 2.22 *Sclerocarya birrea* tree with fruits (www.plantzafrica.co.za/plantqrs/sclerobirr.htm).

Botanical description

The Marula is an erect tree of up to 15 meters tall, with a round crown and a rough flaky mottled bark. The leaves are divided into 10 or more pairs of sharply pointed leaflets. The compound leaves are grey-green in colour, but pale yellow prior to being shed. The flowers are borne in small oblong clusters. Male and female flowers occur separately, usually but not always on separate trees. Male flowers are more conspicuous. Female flowers are round to oval, green when young becoming butter- yellow at later stages (Roodt, 1998). The fruit is much sought after for its delicious pulp, high vitamin C content and tasty, edible nuts. The tree is best known for its golf ball sized fruit which it bears during summer (Roodt, 1998, Van Wyk *et al.*, 2000., Akinnifesi *et al.*, 2008).

Distribution

Plants in this family grow in both tropical and subtropical environments. The marula is widespread in Africa from Ethiopia in the north to KwaZulu-Natal in the south. In South Africa the plant is more dominant in the Limpopo province

(Venter and Venter, 1996, Eloff, 2001). The marula does not flourish in deep sand and therefore occurs predominantly on firm soil.

Medicinal and Spiritual Uses

Bark decoctions are administered as enemas for malaria (Roodt, 1998). Medicine known as "umganu" appears to be widely used for abdominal pains. Roots are used for many purposes in Zimbabwe including heart pain, schistosomiasis, sore eyes, menorrhaghia etc. In east Africa the bark is used for toothaches, constipation and stomach disorders (Hutchings *et al.*, 1996, Eloff, 2001). The Botswana people use an infusion of the fruit to wash tick-infested livestock. The Zulus and Tongas call the marula tree the marriage tree and a brew of the bark is administered during a cleansing ritual before marriage (Roodt, 1998). The Marula tree is used as an anti-plasmodial, antimalarial (Gathirwa *et al., 2008*) and as an antibacterial efficacy (Eloff, 2001).

Data from ethnobotanical survey

Marula grow mostly in forest and in drylands. The bark, which can be collected from the wild or bought from the muthi markets, is used to treat diarrhoea. According to informants, the crushed bark is boiled and then filtered to make the medicine that is administered with half a cup given to children and one cup to adults, as an enema. No side effects have being reported from the usage of the plant (Dlamini 2007, pers. comm., Khumalo 2007, pers. comm., Mayeza 2007, pers. comm., Mkhize 2007, pers. comm., Ndawonde 2007, pers. comm., Xulu 2007, pers. comm., Zama Mhlongo 2007, pers. comm., Zungu 2007, pers. comm).

Chemical content

This family of plants is commonly tanniferous, with tanniferous cells or elongate sacs in the parenchyma tissues usually producing procyaninns but they seldom have ellagic acid. The plant produces 5-deoxyflavonys, biflavonyls and at least sometimes, accumulates quebrachitol. The bark yields 3.5-20.5% tanning matter and traces of alkaloids. The fruit is rich in ascorbic acid, benzoic acid, cinnamic acids and the juice extracts yield 33% of

sesquiterpene hydrocarbons (Hutchings *et al.*, 1996, Roodt, 1998., Ndhlala *et al.*, 2007). The Marula tree was reported not to be toxic (Gathirwa *et al.*, 2008). The plant contains flavanoids, phenolic compounds including flavonols, catechins, proathocyanidins, anthocyanidins and isoflavonoids (Ndhlala *et al.*, 2007).

Conservation status

Sclerocarya birrea is not endangered.

Family: CAESALPINACEAE
Scientific name: *Schotia brachypetala*
Common name: Weeping-boer-bean
Zulu name: *Umgxamu*

Figure 2.23 *Schotia brachypetala* tree with flowers (gardening.mweb.co.za/0510.htm).

Botanical description

Schotia brachypetala is a medium to large tree with wide-spreading branches. The plant has a single trunk that sometimes branches low down. The tree can reach the height of 22 m. The bark is rough and brown or grey brown. The flowers are rich deep red and are produced in massive amounts. The fruit is hard, flattened woody, dark brown and contains flattened pale brown seeds (Pooley, 1993).

Distribution

Schotia brachypetala occur in warm dry areas in the bushveld, in deciduous woodlands and in shrub forests most often on the banks of rivers and streams. It is distributed from the Eastern Cape through KwaZulu-Natal, Swaziland, Mpumalanga, Northern Province and into Mozambique and Zimbabwe (Coates, 1977, McGaw *et al.*, 2002).

Medicinal and Spiritual Uses

Bark is used in red bark mixtures known as *ikhubalo* to ward off evil spirits and to cure unspecified ailments. These mixtures are often taken orally and also washed with during purification rites after the death of a relative. The mixtures are also used to strengthen the body and to steam the face. Bark infusions are taken as emetics for pimples, while decoctions are taken for heartburn and after excessive beer drinking. The roots are used for dysentery and diarrhoea treatment (Hutching *et al.*, 1996, McGaw *et al.*, 2002., Mathabe *et al.*, 2006). The bark is used to make the red dye for *sangomas*. In Zimbabwe roots are also used for ailments known as *chidyiso*. Powder from the leaves is applied to tropical ulcers and smoke from the leaves is inhaled for nosebleeds. Bark is used to wash and to reduce swelling of the body (Hutching *et al.*, 1996). *Schotia brachypetala* bark is used for treatment of mental health problems (Stafford *et al.*, 2007).

Data from ethnobotanical survey

Schotia brachypetala prefers to grow in dry lands. The mode of medicine preparation to treat diarrhoea is crushing the bark, boiling and then filtering. *Schotia brachypetala* can also be mixed with *Sclerocarya birrea* for diarrhoea treatment. The medicine prepared from this plant is only administered as enemas. The dosage depends on individuals, and must be taken only once a day. No side effects have been reported from the usage of the plant. *Schotia brachypetala* has also been reported as being used for back pains (Mfana Mhlongo 2007, pers. comm., Mr Mhlongo 2007, pers. comm., Mrs Mhlongo 2007, pers. comm).

Chemical content

Wood dust and roots are said to contain tannins (Hutchings *et al.*, 1996). The plant has been reported to contain serotonin, noradrenaline, and adrenaline compounds. *Schotia brachypetala's* medicinal value is due to the numerous secondary metabolites it contains including linolenic acid, methyl-5,11,14,17-eicosatetraenoate, furocoumarins, furoquinolines and acridone alkaloids (McGaw *et al.*, 2002., Stafford *et al.*, 2007).

Conservation status

Schotia brachypetala is not endangered.

Family: LOGANIACEAE
Scientific name: *Strychnos henningsii*
Common name: Red bitterberry, Natal Teak, Coffee-bean Strychnos
Zulu name: *umQalothi*

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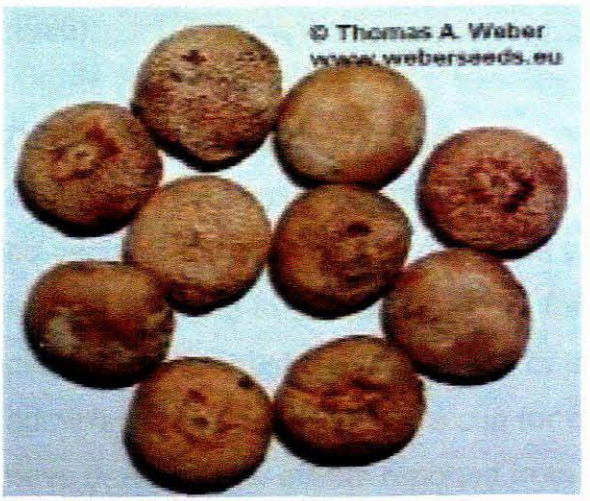


Figure 2.24 *Strychnos henningsii* seeds (www.weberseeds.eu).

Botanical description

Strychnos henningsii is a small, erect, much-branched tree, 2-12 m tall with a clean green-reddish stem. The bark is peeling, the crown compact with dark green, glossy foliage. The leaves are opposite, subsessile, ovate, 2.5-6.5 cm long and 0.8-4.5 cm wide, margins are entire, leaf tips are acuminate. The

fruits are about 1.9 cm long and 6-11 mm broad and they are red, brown or orange when ripe (Hutchings *et al.*, 1996).

Distribution

Strychnos henningsii is distributed in Angola, Kenya, Mozambique, South Africa, Swaziland, Tanzania and Uganda (Tits *et al.*, 1991., Massiote *et al.*, 1991).

Medicinal Uses

Strychnos henningsii is used in African medicines for treating various ailments including rheumatism, gastrointestinal complaints and snake bites (Tits *et al.*, 1991). Boiled roots are used for diarrhoea treatment. Pulverised bark is taken in doses of 10 g in a tablespoon of cold water for nausea. Bark is chewed for stomach complaints, unspecified parts are used for tapeworm. Bark is used as a bitter appetizer in eastern Pondoland and has also been used as a purgative and colic remedy in unspecified parts of Africa (Hutchings *et al.*, 1996).

Data from ethnobotanical survey

Strychnos henningsii prefers to grow in dry lands. The plant parts reported to be used for the treatment of diarrhoea are the leaves and the bark. This plant is also available in its fresh and dry form at the muthi market. The mode of preparation is crushing the leaves or bark, boiling and filtering it. The oral administration dosage is half a cup for children and one cup for adults. There have been no side effects reported from the plant (Mayeza 2007, pers. com).

Chemical content

Most of the 22 alkaloids isolated from the stem, bark, twigs, seeds and leaves were located in the stem bark and none of them were found in more than one part of the plant. Only a few of the stem bark alkaloids have been tested for biological activity with one having convulsive, hypotensive and cardiac depressant activity, while two others showed anticancer potential (Massiote *et al.*, 1991., Tits *et al.*, 1991., Hutchings *et al.*, 1996).

Conservation status

Strychnos henningsii is not endangered.

Family: MYRTACEAE
Scientific name: *Syzygium cordatum*
Common name: Water berry
Zulu name: *Umdoni*

Figure 2.25 *Syzygium cordatum* tree with fruit (www.plantzafrica.com/plantqrs/syzygcord.htm).

Botanical description

Syzygium cordatum is an evergreen, water-loving tree, which grows to a height of 8-15 m. This tree is often found near streams, on forest margins or in swampy spots. The leaves are elliptic to circular, bluish green on top and a paler green below. Young leaves are reddish. The flowers are white to pinkish and fragrant. The fruit are oval berries, red to dark-purple when ripe (Van Wyk and Van Wyk, 1997).

Distribution

Syzygium cordatum occurs along stream banks from KwaZulu-Natal northwards to Mozambique. The plant grows in forest margins, in bush or open grass and sometimes in high countries (Van Wyk and Van Wyk, 1997, Steenkamp *et al.*, 2007).

Medicinal Uses

The tree is known for its many uses. The fleshly fruit is slightly acidic in flavour and is eaten by people, monkeys, bush-babies and birds. The berries are sometimes used to make an alcoholic drink. The powdered bark is used as a fish poison. In central Africa the tree is known as a remedy for stomach ache and diarrhoea. It is also used to treat respiratory ailments, diabetes and tuberculosis (Van Wyk and Gericke, 2000, Musabayane *et al.*, 2005, Mathabe *et al.*, 2006). *Syzygium cordatum* is used for the treatment of infectious diseases in Venda and acts as an antifungal agent (Steenkamp *et al.*, 2007).

Data from ethnobotanical survey

Syzygium cordatum grows in drylands and forests. Fresh and dry material is also available at the muthi market. The plant parts reported to be used for the treatment of diarrhoea are the leaves and the bark. Either the bark or the leaves are crushed, boiled and filtered to prepare a medicine to cure diarrhoea. Medicine administration is either orally or as an enema. Oral doses are half a cup for children and one cup for adults. Enema dosage depends on the person. There have been no side effects reported on the plant (Nsele 2007, pers. comm., Ntombifikile 2007, pers. comm).

Chemical content

This family is known for various ethereal oils found in most or all of the unlignified tissues of the shoots. These oils (e.g. sesquiterpenoids, triterpenoids etc.) are abundant, scattered, small shizogenous secretory cavities. Scattered tanniferrous cells are often present. The plants nearly always contain proanthocyanidins and usually also ellagic acid and gallic acid. Chemicals compounds from wood and bark include friedelin; eifreidelinol, ß-sitostorol; arjunolic acid; gallic acid (hexahydroxydiphenic acid); glucose and gallic-acid-ellagic-acid complex (Candy *et al.*, 1968., Hutchings *et al.*, 1996).

Conservation status

Syzygium cordatum is not endangered.

Family: LAMIACEAE
Scientific name: *Tetradenia riparia*
Common name: Ginger bush
Zulu name: *Iboza*

Figure 2.26 *Tetradenia riparia* tree with flowers (www.mobot.org.../pibloc.asp?/oc=TEMP).

Botanical description

This handsome shrub belongs to the mint family and has a strong resinous aroma. *Tetradenia riparia* has bright green, velvety leaves, 4-6 cm long, which are slightly sticky to the touch and have neatly scalloped margins. The plant reaches about 5 meters in height and the flowers appear in the winter season. Male and female flowers are borne on separate plants, males in open panicles that from a distance form a pale lilac-pink mist. Female flowers are similar but more compact (Hutchings *et al.*, 1996).

Distribution

Tetradenia riparia is distributed in the eastern parts of South Africa and it extends into Namibia and Angola northwards to Ethiopia (Van Wyk and Van Wyk, 1997).

Medicinal Uses

Leaf infusions are reported to be effective against malaria (Hutchings *et al.*, 1996 and Omolo *et al.*, 2004). The patient can take a single dose at bed time and recover the next day. Cold water leaf decoctions, infusions and pounded leaves are taken for chronic coughs and sore throats. Warm infusions of pounded leaves are used as an emetic. Strong decoctions prepared from boiling leaves and stems should not be taken for more than four days, the dose should be limited to one spoon a day and should not be given to children. Leaves are also chewed for dengue fever. The Tswana people use leaves for fever and to calm patients and for gall sickness in cattle (Hutchings *et al.*, 1996).

Data from ethnobotanical survey

The plant has been reported to grow mostly in dry lands. *Tetradenia riparia* has been reported to treat diarrhoea, fever and flu, where leaves are used to treat diarrhoea. The mode of preparation is crushing the leaves or squeezing to obtain the juice and mixing with *Lippia javanica* leaves. The oral dosage is a teaspoonful for children, a spoonful for adults and can be taken every day. No side effects have been reported from the usage of the plant (Mkhize 2007, pers. comm., Mrs Mhlongo 2007, pers. comm., Mrs Mkhize 2007, pers. comm., Ndawonde 2007, pers. comm., Sfiso Buthelezi 2007, pers. comm).

Chemical content

Alkaloids, triterpenoid friedelin and essential oil have been isolated from *Tetradenia riparia* plant (Hutchings *et al.*, 1996, Omolo *et al.*, 2004). A new α-pyrone, tetradenolide, was isolated from the leaves (Van Puyvelde and De Kimpe, 1998).

Conservation status

Tetradenia riparia is not endangered.

Family: MELIACEAE
Scientific name: *Trichilia dregeana*
Common name: Forest natal mahogany
Zulu name: *Umkhuhlu*

Figure 3.27 *Trichilia dregeana* tree (www.plantzafrica.com/planttur/trichildreg.htm).

Botanical description

Trichilia dregeana can reach a height of about 35 m with the tall main stem assuming a relatively straight and sometimes buttressed habit, 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. The compound leaves can reach lengths of 70 cm. The leaflets are entirely opposite to alternate, glossy and dark green in colour. The creamy-white flowers are produced from October to December. The fruits are round, velvet capsules, 3 cm in diameter that split usually into three valves. On splitting the capsules reveal six very attractive seeds, these being black and covered largely by a bright red to scarlet aril, a striking and distinctive feature of the tree. Fruiting occurs between January and May (Germishuizen and Meyer, 2003, Venter and Venter, 2002, Eldeen *et al.*, 2007).

Distribution

Trichilia dregeana is a widespread species, stretching from Pondoland, KwaZulu-Natal in the south, through Swaziland, Mpumalanga and Limpopo

province into Zimbabwe and northwards into tropical Africa. The plant is found in areas of high rainfall in coastal and mountain evergreen forest (Germishuizen and Meyer, 2003, Eldeen *et al.*, 2007).

Medicinal Uses

The decoctions are made from the pieces of the bark, about the length and breadth of two fingers, pulverized and mixed into two cups of hot water and are administered as enemas for stomach disorders and intestinal complaints (Hutchings *et al.*, 1996., Pooley, 1993). The infusion of the bark or leaf is used for lumbago, rectal ulceration in children and dysentery. Oil from the seeds is rubbed into incisions made over a broken limb, followed by the application of the roasted and powdered root and the leaves are worn in burial rituals. Enemas made from bark are administered for kidney ailments, as stomach and blood cleansers and also for intestinal worms (Pooley, 1993, Eldeen *et al.*, 2005., Eldeen *et al.*, 2007). In Zambia, powdered bark is applied rectally for an ailment known as *Chiufa* disease. The roots are used for fever and as purgatives and bark for indigestion. Bark is also used for procuring abortions and as a fish poison in Zimbabwe. This plant is reported to be very poisonous. Enemas made from the plant are said to produce sweating and vomiting and have been suspected of causing death (Hutchings *et al.*, 1996).

Data from ethnobotanical survey

Trichilia dregeana has been reported to grow commonly in the forest. The bark which is used to treat diarrhoea is also available in the muthi markets. There is no specific time for the collection of the plant. The mode of preparation is crushing the bark, adding cold water and then filtering. The prepared medicine is administered orally, with doses of half a cup for children and a cupful for adults, three times a day. No side effects have been reported from the usage of the plant (Mayeza 2007, pers.comm).

Chemical content

The compounds cycloart-23-ene-3, 25-diol and limonoids have been isolated from the plant *Trichilia dregeana* (Mulholl and Taylor, 1980, Eldeen *et al.*, 2007).

Conservation status

Trichilia dregeana is not endangered.

Family: ASTERACEAE
Scientific name: *Vernonia oligocephala*
Common name: Groenamara
Zulu name: *Uhlambihloshana*

Figure 2.28 *Vernonia oligocephala* tree with flowers (www.zimbabweflora.co.zw/speciesdata/species.php?species=Vernonia%20oligocephala&sref=71867).

Botanical Description

Vernonia oligocephala is an herbaceous perennial with erect, flowering branches developing from a woody rootstock. The leaves are elliptic in shape, usually not more than twice as long as they are broad, with a sharp point and a very short stalk. The bright violet flower heads are about 10 mm in diameter and are borne in large groups towards the branch tips (Pooley, 2003).

Distribution

The plant is widespread in the glassland regions of South Africa. *Vernonia oligocephala* may be confused with *V. natalensis*, which has much the same distribution and flowers at the same time. In the latter, however, the leaves are narrower and similar on both sides (Misra and Jakupovic, 1984, Hutchings *et al.*, 1996., Pooley, 2003).

Medicinal uses

Infusions are taken as stomach bitters to treat abdominal pain and colic. Other ailments treated include rheumatism, dysentery and diabetes (Hutchings *et al.*, 1996, Pooley, 2003).

Data from ethnobotanical survey

Vernonia oligocephala has been reported to treat diarrhoea and sores. To treat diarrhoea the medicine is prepared by crushing the leaves, adding water and filtering it. It is orally administered. The dosage depends on the person. No side effect has been reported on the plant (Dindi 2007, pers. comm).

Chemical content

A larger number of different sesquiterpenoid lactones have been isolated from *Vernonia oligocephala*, including germacranolides and glaucolides (Misra and Jakupovic, 1984, Pooley, 2003).

Conservation status

Vernonia oligocephala is not endangered.

Family: ASTERACEAE
Scientific name: *Vernonia tigna*
Common name: Mountain vernonia
Zulu name: *Uhlunguhlungu*

Figure 2.29 *Vernonia tigna* with flowers (www.sntc.org.sz).

Botanical Description

Vernonia tigna is a subshrub tree of about 2 m in height with straight, erect and branched stems. The stems are downy throughout. Leaves are discolorous and narrowly winged on both surfaces (Pooley, 2003).

Distribution

Vernonia tigna grows mostly in woodlands and wooden grassland often in drainage lines. The plant is originally from Central America but is now found in many regions of southern Africa (Pooley, 2003).

Medicinal Uses

Vernonia tigna is used traditionally to treat colds, stomach ache, hysteria, epilepsy and to ensure an easy birth (Pooley, 2003). Infusions are administered as enemas for intestinal ulceration (Watt and Breyer Brandwijk, 1962). Leaves are used in medicine for madness, convulsions, uterine pain, chest pain and fevers in infants. The plants are cultivated as snake repellents in Zimbabwe (Gelfand *et al.*, 1985).

Data from ethnobotanical survey

Leaves of *Vernonia tigna* which are sold in the muthi markets in dry form are used to treat diarrhoea. The mode of preparation is crushing the leaves, adding cold water and filtering. This medicine is orally administered with doses of half a cup for children and one cup for adults, three times a day. No side effects have been reported on the plant (Dindi 2007, pers. comm., Mayeza 2007, pers. comm).

Chemical content

All parts of *Vernonia tigna* contain saponins, especially the roots. Other components isolated from the plant include quercetin, oxalic, malic, succinic acid, glucose and two triterpenoid glycoside, chenopodoside A and B (Watt and Brayer- Brandwijk, 1962., Pooley, 2003).

Conservation status

Vernonia tigna is not endangered.

2.5 Conservation status

Many medicinal plants are under threat due to debarking, over-collection and destructive harvesting practices. Several plants are highly endangered and prone to extinction. Most of the plant material used in traditional medicine is harvested in the wild by gatherers, who usually collect everything they think they can sell without regard to how the plants will survive for the future. More than half of the plant material sold as traditional medicine consists of bulbs, rhizomes or bark. When underground parts are harvested, the whole plant is removed, and too often a tree is ring-barked, resulting in the death of that tree. It has become increasingly difficult to collect enough material from the wild and several species can no longer be found because of destructive harvesting. If a plant is threatened, there is limited stock material left, or if the seeds are difficult to germinate or the species cannot be propagated via normal methods. Clonal propagation through tissue culturing might be an alternative way of increasing plant production.

The plants can be replanted in nature to restore the environment or can be used for commercial production of medicinal plants. The need for medicinal plants is not decreasing but is rather increasing. It is therefore necessary to find other ways to produce the amount of plant material that is needed. In recent years traditional healers have started to grow medicinal plants in their own gardens. These solutions can help solve the problem, but are not enough (Golding, 2002, Heywood and Iriondo, 2003). Of the plants collected, 80% are not endangered and harvesting was sustainable because most of these plants are grown in homestead gardens namely *Acacia karoo, Acacia robusta, Aloe arborescens, Azima tetracantha, Baccharoides adoensis, Callilepis laureola, Cantharanthus roseus, Chenopodium ambrosioides, Ficus sur, Helichrysum odoratissimum, Lippia javanica, Maytenus heterophylla, Melia azedarach, Psidium guajava, Prunus persica, Schotia brachypetala, Strychnos henningsii, Syzygium cordatum, Tetradenia riparia, Trichilia dregeana, Vernonia oligocephala* and *Vernonia tigna*. Mostly bark and leaves are used in the preparation of remedies. The bark was harvested at anytime of the year and also any time of the day. It has been reported that the debarking accelerates the death of many trees (Grace *et al.*, 2002). It was very difficult to obtain the

bark from other trees such as Marula (*Umganu*) because of debarking. It is thus necessary to encourage conservation among the communities and traditional healers, an idea promoted by many researchers (Kala, 2000, Shinwari and Gilani, 2003, Heywood and Iriondo, 2003). In the present study *Acridocarpus natalius, Alepidea amatymbica, Cyphostemma hypoleucum, Dichrostachys cinerea, Hypoxic hemerocallidea* and *Sclerocarya birrea* were threatened. There was limited stock material left and this needs to be conserved. If the seeds are difficult to germinate or species cannot be propagated via normal methods, then clonal propagation in tissue culture might be an alternative way of producing lots of plants.

2.6 Discussion

The aim of the ethnomedical survey and documentation was to catalogue the plants used traditionally against diarrhoea. The results of this study showed that 35 different plant species are traditionally used in treatment of diarrhoea among the community. Of the 35 plant species, 19 plant species have been previously reported in literature to have the potential to treat diarrhoea. They are: *Acacia karoo* (Pooley, 2003); *Alepidea amatymbica* (Gelfand *et al.*, 1985 and Somova *et al.*, 2001); *Aloe arborescens* (Matsuda *et al.*, 2008); *Baccharoides adoensis* (Nergard *et al.*, 2004); *Callilepis laureola* (Popat *et al.*, 2001); *Chenopodium ambrosioides* (Hutchings *et al.*, 1996); *Cyphostemma cirrhosum* (Hutchings *et al.*, 1996); *Dichrostachys cinerea* (Eisa *et al.*, 2000); *Ficus sur* (Pooley, 1993 and Kunle *et al.*, 1999); *Maytenus heterophylla* (Orabi *et al.*, 2001) ; *Melia azedarach* (Hutchings *et al.*, 1996., Madibela and Kelemogile, 2008); *Psidium guajava* (Kaushik *et al.*, 2008); *Sclerocarya birrea* (Hutchings *et al.*, 1996., Eloff, 2001); *Schotia brachypetala* (Hutchings *et al.*, 1996., McGaw *et al.*, 2002., Mathabe *et al.*, 2006); *Strychnos henningsii* (Hutchings *et al.*, 1996); *Syzygium cordatum* (Van Wyk and Gerickle, 2000., Musabayane *et al.*, 2005., Mathabe *et al.*, 2006); *Trichilia dregeana* (Pooley, 1993., Hutchings *et al.*, 1996); *Vernonia oligocephala* (Hutchings *et al.*, 1996., Pooley, 2003) *and Vernonia tigna* (Pooley, 2003). This study records for the first time 16 plants which have not been documented before in the literature as being used to treat diarrhoea, they are: *Acacia robusta, Acanthospermum australe. Acridocarpus natalius, Azima tetracantha, Cantharanthus roseus,*

Chromolaena odarata, Clerodendrum glabrum, Faurea macnaughton, Helichrysum odoratissimum, Hypoxis hemerocallidea, Physalis viscose, Portulacaria afra, Prunus persica, Schkuhria pinnata and *Senecio quinquelobus*. The total percentage of plants documented for the treatment of diarrhoea is 54% and 46% on non documented plants. Some of the plants were reported to have synergism properties e.g *Alepidea amatymbica* could be mixed with other plants such as *Hypoxis hemerocallidea* and *Callilepis laureola*. Synergism is evident where the combined action of the test substances exceeds the effects of the individual components (ESCMID, 2000).

In the present research, the main interest lies with the rich sources of medicinal plants that are found and used in the Ongoye area for the treatment of diarrheoa. This corresponds well with the knowledge that traditional medicine is an integral part of their culture and that approximately 80% of black South Africans go to traditional healers when sick. In recent years the Government has realized the need to recognize and formalize traditional healing (WHO, 2003). If traditional medicine is going to play a more formal role in primary health care it is necessary that it becomes safer and that the remedies used are optimized for efficiency.

Traditional healers and other people use whole plants or different plant parts (leaves, bark, roots and stems) for the preparation of remedies to treat diarrhoea, with leaves and bark being the most commonly used plant parts. This study revealed that women (61.4%) possessed more knowledge than men (38.6%) and that men are more familiar with the non popular plants used against the treatment of diarrhoea. Older people still rely on traditional treatment whilst the younger people regard the indigenous knowledge as primitive, outdated and are thus not interested in it (Zobolo and Mkabela, 2006). Formal medical facilities are available but most people still rely greatly on indigenous medicinal plants. However informants reported that a person is taken to the doctor after the indigenous intervention has failed. This study revealed that most women had knowledge of Zulu names of plants and their uses in traditional healthcare practice, especially in treating coughs, colds, flu,

stomach aches and diarrhoea. The indigenous knowledge possessed by women is not adequately transferred to children for preservation. More research needs to be done to find ways of preserving indigenous knowledge and to make the younger generation interested in acquiring this knowledge.

CHAPTER THREE

Antimicrobial efficacy

3.1 Introduction

World wide, infectious diseases are the number one cause of death accounting for approximately one half of all deaths in tropical countries. Infectious diseases have since 1981 become the third highest cause of death in the world with an increase of 58% (Pinner *et al.*, 1996, Agunu *et al.*, 2005).

Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing. Infectious diseases have become the leading causes of death and have increased dramatically. This fact is alarming given that it was once believed that we would eliminate infectious disease by the end of the *millennium* (WHO, 1996). The increase is attributed to the increase in diarrhoea, respiratory tract infections and HIV/Aids. Intestinal infection is the most common cause of diarrhoea worldwide and is estimated to be responsible for the death of 3-4 million individuals each year (WHO, 1996, Agunu *et al.*, 2005). Diarrhoea continues to be the leading causes of mortality and morbidity (especially in children) in developing countries including South Africa (Lin *et al., 2002*, Teke *et al., 2007*).

The emergence of multiple drug resistant strains of diarrhoeagenic pathogens has made the treatment of dysentery more difficult. In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhoea. A range of medicinal plants with anti-diarrhoeal properties have been widely used by the traditional healers of different tribes in South Africa (Lin *et al.*, 2002). The effectiveness of many of these antidiarrhoeal traditional medicines however has not been scientifically evaluated (Mathabe *et al.*, 2006). Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy (Eloff, 1998, Farnsworth, 1994).

Diarrhoea is defined as a loose, watery stool which occurs more than three times a day. Diarrhoea can cause dehydration, which means the body lacks enough fluids to function properly. Dehydration is particularly dangerous in children and the elderly and it must be treated promptly to avoid serious health problems. Diarrhoea can be caused by the following: bacterial infections, viral infections, food intolerances, parasites, reaction to certain medicine, intestinal disease and functional bowel disorders (Madigan *et al.*, 2003). Prolonged diarrhoea can be a sign of other problems. Most HIV patients are infected by bacterial and fungal infections because their immune system is unable to protect them against these infections due to malnutrition. Other contributing factors are the increase in antibiotic resistance in nosocomial and community acquired infections, for example, multi-drug resistant tuberculosis. The most dramatic increase of multi-drug resistant tuberculosis is occurring in the 25-44 year age groups. These negative health trends call for renewed interest on infectious diseases in medical and public health communities and renewed strategies on treatment and prevention. Due to the over usage of antibiotics, many bacteria have developed resistance against antibiotics. It is thus important to investigate traditional medicines for antimicrobial activities (Iwu, 1993., Eloff, 1998).

In the recent decades, antimicrobial resistance and sensitivity to bacteria have been considered as a major problem in Public Health (Bradley, 1999). Food and water can also be considered as an excellent way for introducing pathogenic microorganisms into the general population and into immunocompromised people and therefore it may transfer antibiotic resistant bacteria to the intestinal tract of consumers very efficiently. Transfer of resistant genes can occur in the intestine between non pathogenic bacteria and pathogenic or opportunistic bacteria. Because of the extended use of antibiotics, the number of bacteria that are resistant to antimicrobial agents is rapidly increasing (Bradley, 1999).

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise. In recent years, incidences of multi drug resistance in pathogenic and opportunistic bacteria have been

increasingly documented (Jones *et al.*, 2004). These multi-drug resistant bacteria have also created huge clinical problems in cancer and immune compromised patients such as HIV patients. Most important multi-drug resistant bacteria on the global scale include Gram-positive methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and Gram-negative bacteria *Escherichia coli* (Hopkins *et al.*, 2005).

Emergence of antibiotic resistance raises question about the future of drugs in chemotherapy, as the transmission of such resistant plasmid to other bacteria will help the fast distribution of resistance genes (Hopkins *et al.*, 2005). However, ß-lactamases continue to be the leading cause of resistance to ß-lactam antibiotics in Gram-negative bacteria. Thus a search for new antimicrobials to combat infectious diseases caused by multi-drug resistant bacteria (including fast spreading producing enteric bacteria) is urgently needed. Due to poor hygienic conditions in developing countries in both hospitals and communities, enteric bacterial infection caused by resistant strains of *E. coli* and other bacteria are more problematic and a major public problem (Bradford, 2001).

Screenings of traditional medicine against a problematic group of drug resistant bacteria are of great importance, as there is some reported broad spectrum activity of certain medicinal plants extracts against methicillin-resistant *Staphylococcus aureus* and a variety of other bacteria. Although such discussions of antibiotics resistance are still in an early phase, some scientists and physicians have raised the question of whether evaluation of new antibiotics should take into account human microbial flora (Casadevall and Pirofski, 2000).

3.2 Bacteria description

3.2.1 Description of ATCC micro-organism

ATCC micro-organisms are the American Type Culture Collection. These are the standard cultures. The American type culture collection is the key source for medical research and is the world premier biological culture repository. The ATCC gave medical and other biological scientists a reliable source of over 60,000 authenticated viable cultures.

3.2.2 *Escherichia coli* (ATCC No: 11775)

Escherichia coli (E. coli) are facultative anaerobic Gram-negative, non-sporing rods that ferment lactose. *Escherichia coli* is catalase positive, oxidase negative and indole positive and can grow at temperatures ranging from 37 to 46 °C at a minimum water activity of 0.95%. This bacteria is fairly acid tolerant and can grow at pH values ranging from 4.4 to 10 (Madigan *et al.*, 2003). *Escherichia coli* grow well on ordinary culture media (Nutrient broth and Nutrient agar) at 37 °C for 24 hours. In nutrient agar, colonies are large (2-3 mm diameter in 18 hours), thick, colourless and moist. The colonies are easily emulsifiable and seen on fresh isolation of smooth (S) form or on rough (R) form. On the MacConkey's agar, the colonies are rose pink due to lactose fermentation and on Blood agar some strains show ß haemolysis. However some strains are more heat resistant than other members of enterobacteriaceae and will survive at 60 °C for 15 minutes or at 55 °C for 60 minutes (Ballows *et al.*, 1991 and Chakraborty, 2005).

Pathogenesis

The antigenic structure of *E. coli* is based on the pressure of oxygen (O), hydrogen (H) and potassium (K) antigens detected by agglutination reactions; the polysaccharide O and K antigens protect the organism from bactericidal effect of complement and phagocytes in the absence of specific antibodies. However, phagocytosis is successful in the presence of antibody to K antigens alone, or both O and K antigens. K antigen acts as the virulent factor by impending phagocytosis and protects the bacilli from the killing action of antibody and complement. There are several different serotypes of *E. coli* and

most of them do not possess K antigens. Furthermore, haemolytic strains have been found to be more virulent than non-haemolytic ones in animal experiments. It is likely that haemolytic strains obtain iron from the erythrocytes of the host. Cytotoxic activity may also be important in some infections (Chakraborty, 2005).

Diseases

Escherichia coli are the predominant coliform bacteria, responsible for 60-80% of infections of the urinary tract. This infection often originates in the gut of the patient and the infection is thought to occur in an ascending manner. There is evidence that the ability of *E. coli* to infect the urinary tract is associated with fimbriae that specifically mediate adherence to the uroepithelial cells. *Escherichia coli* causes acute enteritis of young animals, including human infants, calves and lambs. The acute enteritis is subjected on human subjects of all ages the tropics and includes traveler's diarrhoea (Greenwood *et al.*, 1997).

Laboratory diagnosis

Specimens of *E. coli* are cultured on MacConkey agar or other suitable agar and may be stained by Gram staining methods for microscopically examination (Greenwood *et al.*, 1997).

Treatment

Escherichia coli are resistant to benzylpenicillin but sensitive to ampicillin and cephalosporin and usually to tetracycline, chloramphenicol, aminoglycosides and sulphonamides. Many strains have acquired plasmid conferring resistance to one or more of these drugs. The strains that are resistant to ampicillin, streptomycin, sulphonamides and tetracyclines are particularly common. Extra intestinal *E. coli* infections are treated with specific antibacterial therapy, preferably guided by results of laboratory tests for sensitivity. Urinary characterization and cytoscopy require rigorous aseptic techniques to minimize the introduction of bacteria to the bladder. Bladder irrigation and systematic treatment with antimicrobial agents have been used in catheter-associated infections. In particular, medical emergency and

vigorous early treatment should be required for meningitis (Greenwood *et al.*, 1997).

3.2.3 *Staphylococcus aureus* (ATCC No: 12600)

Staphylococcus aureus is a Gram-positive, cluster-forming, coccus, non-motile microorganism. This bacterium is also a non-spore forming, facultative anaerobe. The fermentation of glucose produces mainly lactic acid and ferments mannitol. *Staphylococcus aureus* is catalate positive, coagulase positive, and occurs in a golden yellow colony when grown on Nutrient agar and Blood agar for 24 hours at 37 °C. It is part of the normal flora of humans found in nasal passages, skin and mucous membranes. This pathogen mostly affects humans causing a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome (Ballows *et al.*, 1991, Madigan *et al.*, 2003., Chakraborty, 2005).

The ability of *S. aureus* to grow in a medium with high salt provides a selective means for isolation. For example, if an appropriate inoculums is spread on the agar with a rich medium containing 7.5% NaCl and the plate incubated aerobically, Gram positive often form predominant colonies (Madigan *et al.*, 2003). The main distinctive diagnostic features of *S. aureus* are: (i) production of enzyme coagulase which converts fibrinogen in citrated human rabbit plasma into fibrin, aided by an activator present in plasma; and (ii) production of themostable nucleases that break down DNA (Greenwood *et al.*, 1997).

Pathogenesis

Staphylococcal pathogenecity have been studied most extensively in the species of *S. aureus*. Certain strains of *S. aureus* can produce several pyrogenic exotoxins such as enterotoxins, leukocidin, exfoliatins or toxin responsible for toxic shock syndrome. Hemolysins are regarded as principal toxins, erythrocytes and alpha lysines are most important in pathogenicity. Protein A is a surface protein that is covalently bound to the peptidoglycan layer and found in more than 90% of *S. aureus* strains (Chakraborty, 2005).

Staphylococcus aureus is present in the nose of 30% of healthy people and may be found on the skin. It causes infection most commonly at site of lowered host resistance, for example, damaged skin or mucous membranes. *Staphylococcus aureus* causes a variety of diseases including acne, boils, pimples, impetigo, osteomyelitis and arthritis. Many of these diseases cause the production of pus, and are said to be suppurative or pus forming (Madigan *et al.*, 2003). Most habitats of *S. aureus* are in the upper respiratory tract, especially the nose, throat and the surface of the skin. Many healthy people are carriers and it does not cause disease. However, infants become infected during the first week of life from the mother or from another close human contact (Madigan *et al.*, 2003).

Diseases

The coagulase- positive species *S. aureus* and *S. dolphini* and the coagulase-variable species are regarded as serious pathogens. *Staphylococcus aureus* causes considerable morbidity and mortality as a nosocomial pathogen of hospitalized patients. Methicillin-resistant *S. aureus* strains (MRSA) emerged in the 1980s as a major clinical and epidemiological problem in hospitals (Chakraborty, 2005).

Laboratory diagnosis

Blood tests that show unusually high concentrations of white blood cells can suggest staphylococci infection, but diagnosis is based on laboratory analysis of material removed from pus-filled sores and on analysis of normally uninfected body fluids, such as blood and urine. Also X-rays can be used to locate internal abscesses and estimate the severity of infection. Removing tissue with a needle and examining it under a microscope may show bone involvement (Sriskandan and Cohen, 1999).

Treatment

Staphylococci infections can generally be cured by keeping the area clean, using soaps that leave a germ free film on the skin and applying moist compresses on affected areas for 20-30 minutes three or four times a day. In case of more serious infection, antibiotics may be administered intravenously

for as long as six weeks. Intravenous antibiotics are also used to treat staphylococci infections around the eyes or on the other part of the face (Sriskandan and Cohen, 1999).

3.2.4 *Bacillus subtilis* (ATCC No: 6051)

One of the earliest bacteria to be described was "Vibrio subtilis". In 1872 the organism was renamed *Bacillus subtilis*. That organism was a charter member of a large and diverse genus that is part of the Bacillaceae family. The family's distinguishing feature is the production of endospores, which are round, oval or highly cylindrical refractile structures formed within bacterial cells. Bacteriophages that infect *Bacillus* are common in soil. The most extensively studied *Bacillus* phages are those associated with *B. subtilis* and can be grown in minimal salt media with glucose as a carbon source. *Bacillus subtilis* is a Gram-positive, rod-shaped and endospore-forming aerobic bacterium. It is one of the most studied Gram-positive bacteria (Madigan *et al.*, 2003).

Pathogenesis

Bacillus subtilis produces the proteolytic enzyme subtilism which is an extracellular enzyme that catalyzes the breakdown of proteins into polypeptides and resembles trypsin in its action and has been shown to be a potent occupational allergen. *Bacillus subtilis* is not regarded as a pathogen but can contaminate food to cause food poisoning because its spores can survive the extreme heating that is often used to cook food and it may also be responsible for causing ropiness in spoiled bread (Nakano, 1998).

Laboratory diagnosis

Bacillus species are easily isolated and readily grown in the bacteriology laboratory. The simplest technique to enrich aerobic formers is to pasteurize a diluted soil sample at 80 °C for 15 minutes, then place it onto nutrient agar and incubates at 37 °C for 24 hours up to several days. The plates are examined after 24 hours for typical *Bacillus* colonies and identified as catalase-positive, Gram-positive, endospore forming rods. Some cultures

must be incubated 5-7 days before mature sporangia, the size and the shape of endospore contained therein, can be observed (Nakano, 1998).

Treatment

Bacillus species are rarely a pulmonary pathogen but may cause pneumonia in immunocompromised patients. There were two cases where a patient with bronchictasis and no recognizable immunodeficiency had this organism isolated during two infective exacerbations, one from respiratory secretions and the other by blood culture. Ciprofloxacin treatment was effective on both occasions (Madigan *et al.*, 2003).

3.2.5 *Klebsiella pneumoniae* (ATCC No: 13883)

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting bacteria. This bacterium is also facultative anaerobic, rod shaped and found in the normal flora of the mouth, skin and intestine. It is clinically the most important member of the Enterobacteriaceae and is closely related to *Klebsiella oxytoca* from which it is distinguished by being indole-negative. It has the ability to grow on both melezitose and 3-hydroxybutyrate. *Klebsiella oxytoca* occurs naturally in the soil and about 30% can fix nitrogen in anaerobic conditions (Madigan *et al.*, 2003).

Pathogenesis

Klebsiella pneumoniae is found in 10% of normal individuals as normal flora of the respiratory tract. It can cause pneumonia in diabetics, alcoholics and immunocompromised patients and may also produce lung abscesses (Chakraborty, 2005). The main importance of *K. pneumoniae* as a human pathogen is that it causes nosocomial infections. *Klebsiella pneumoniae can* develop in surgical wounds and in the urinary tract, and can cause bacteraemic infections. *Klebsiella* can cause lobar pneumoniae resulting in necrotic destruction of alveolar spaces, cavity formation and production of thick blood-tinged viscuous sputum (Greenwood *et al.*, 1997). *Klebsiella* can also cause less serious respiratory infections, such as bronchitis which is usually a hospital acquired infection (Cohen and Powderly, 2004).

Treatment

Many strains of *K. pneumoniae* have acquired an extended spectrum beta lactamase with additional resistance to carbenicillin, ampicillin, quinolones and increasingly to ceftazidime. The bacteria remain largely susceptible to aminoglycosides and cephalosporins. Strains of *K. pneumoniae* and *K. oxytoca* are naturally resistant to aminopenicillins and carboxypenicillins and are 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).

3.3 Description of micro-organisms collected at Lancet laboratory

The common enteric bacteria pathogens are *Salmonella spp*, *Shigella spp*, enteropathogenic *Escherichia coli*, *Vibrio cholerae*, *Campylobacter spp*, *Yersinia enterocolitica* and *Staphylococcus aureus*. Fecal matter contains numerous non pathogenic microorganisms. It is necessary to employ selective and differential media for the isolation of intestinal tract pathogens. Usually selective media contains some inhibiting compound that selectively inhibits mostly Gram-positive bacteria. *Escherichia coli* typically produce test positive in indole, lysine decarboxylase and mannitol fermentation and produce gas from glucose. An isolate from urine can be quickly identified as *E. coli* by it hemolysis on blood agar. The *Salmonellae* species are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose.

Most *Salmonellae* produce H_2S and are often pathogenic for humans or animals when ingested. The *Shigellae* species are non-motile and usually do not ferment lactose but do ferment other carbohydrates, producing acid but not gas. This bacterium does not produce H_2S. *Shigella* species are closely related to *E. coli*. Many share common antigens with one another and with other enteric bacteria. *Yersinia* organisms are short, pleomorphic, Gram-negative rods that can inhibit bipolar staining and do not form spores and are catalase-positive, oxidase-negative and micro-aerophilic or facultative anaerobic. Most have animals as their natural hosts, but can produce serious diseases in humans. The genus *Yersinia* includes *Yersinia pestis*, the cause

of plaque, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, important causes of human diarrhoea diseases (Jawetz *et al.*, 1987).

Vibrio and *Campylobacter* species are Gram-negative rod. Vibrios are widely distributed in marine environments and *Campylobacters* in animals and birds. These bacteria are important causes of enteritis. Some strains of *Vibrio chorelae* produce an enterotoxin that causes cholera, a profuse watery diarrhoea that can rapidly lead to dehydration and death of the patient. *Campylobacter jejuni* is one of the common causes of enteritis in humans. Vibrios are among the most common bacteria in surface waters worldwide, are curved, Gram-negative, non-endospore forming, aerobic rods and are motile by means of a flagellum. The epidemiology of cholera closely parallels the recognition of its transmission in water and the development of sanitary water systems (Jawetz *et al.*, 1987).

3.4 Pathogens used

The following four ATCC bacteria strains were used: *Bacillus subtilis (*6051), *Escherichia coli* (7751), *Klebsiella pneumoniae* (13883) and *Staphylococcus aureus* (12600). At Lancet Laboratory in Durban the following bacteria cultures were collected: *Staphylococcus aureus* (P5020, P4790, T1266); *Escherichia coli* (U1405s, U16406, U16403); *Salmonella* spp, *Shigella flexneri and Shigella sonnei* (Table 3.1 and 3.2).

Table 3.1 Sensitivity pattern against different antibiotics of *Escherichia coli.*

ANTIBIOTICS	EC U1505s	EC U16406	EC U16403
Amikacin	S	S	S
Ampicillin	R	R	S
Cephalothin	S	R	S
Cefpodoxime	S	R	S
Cefuroxime	S	R	S
OFX : Ciproflox	S	R	S
Augmentin	S	R	S
Cotrimoxazole	R	R	S
Fosfomycin	S	S	S
Gentamicin	S	R	S
OFX : Levoflox	S	R	S
Loracarbeb	S	R	S
Nitrofurantoin	S	S	S

Ec – *Escherichia coli*, **S** – Sensitive, **R** - Resistant

Table 3.2 Sensitivity pattern against different antibiotics of *Staphylococcus aureus*.

ANTIBIOTICS	SA P5020	SA T1266	SA P4790
Cephaloth	S	S	S
Cip : GATI	S	S	S
Cip : LEVO	S	S	S
Cip : MOXI	S	S	S
Clindamycin	S	R	S
Oxa : Clox	S	S	S
Augmentin	S	S	S
Cotrimoxozole	S	S	R
Erythromycin	R	R	S
Fucid : Acid	S	S	S
Gentamicin	R	R	R
Linezolid	S	S	S
Oxa : methl	S	S	S
Penicillin	_	R	R
Rifampicin	R	S	S
Vancomycin	S	S	S
Teicoplanin (TEI)	S	S	S
Cefoxitin (FOX)	S	S	S

Sa – *Staphylococcus aureus*, **S** – Sensitive, **R** – Resistance

3.5 Standard experimental procedures

3.5.1 Plant extraction

The plants collected (Tables 2.1 and 2.2) were dried at room temperature and ground into a fine powder which was then stored in airtight containers at room temperature for further use in the screening for antimicrobial activity. These plant powders were extracted with different solvents: methanol, acetone, cold and hot water for comparative analyses. Five grams of powder material were mixed with 50 ml of each solvent. The mixtures were left overnight on a mechanical shaker at 150 rpm for 24 hours at room temperature. The extracts were then filtered through a Whatman No 1 filter using a Buchner funnel. The extracts were further concentrated to dryness in a fume cupboard for the solvents to evaporate. The yields from the different extracts were weighed, calculated (Table 3.3), recorded and dissolved in dimethly sulfoxide (DMSO-dissolves both polar and non polar solvents) to a final concentration of 100 mg/ml. The samples were then stored at 4 °C until further use (Mathabe *et al.*, 2006).

Table 3.3 Yield of the different plant extracts.

Plant species	Extract yield per 100 mg/ml			
	Acetone	**Methanol**	**Cold water**	**Hot water**
Barks				
Ihlaza	6.02	5.66	7.67	9.36
Ungazini	2.17	4.2	1.64	1.46
Schotia brachypetala	7.86	8.09	4.29	8.78
Sclerocarya birrea	8.89	9.0	13.21	7.17
Syzygium cordatum	3.57	5.84	7.18	4.19
Flowers				
Vernonia tigna	10.57	5.79	0.53	0.35
Leaves				
Acacia karoo	3.13	2.32	7.23	3.50
Acacia robusta	5.24	18.77	11.78	15.39
Aloe arborescens	1.07	7.2	13.3	3.91
Acanthospermum australe	1.29	1.04	6.50	1.72
Catharanthus roseus	3.29	12.16	10.64	9.43
Chromolaena odarata	2.67	7.02	14.49	5.42
Dichrostachys cinerca	3.36	14.46	8.76	8.47
Faurea macnaughton	3.83	3.53	1.74	7.77
Hypoxis hemerocallidea	0.75	7.25	12.50	2.38
Lippia javanica	8.06	7.02	2.61	1.63
Maytenus heterophylla	2.11	3.59	10.95	1.42
Melia azedarach	2.06	5.58	5.15	4.91
Psidium guajava	4.33	11.56	5.52	2.22
Schotia brachypetala	3.35	4.36	2.59	6.37
Syzygium cordatum	5.11	8.24	3.4	3.75
Tetradenia riparia	7.18	4.96	9.36	2.12
Trichilia dregeana	7.25	5.14	3.57	3.73
Vernonia oligocephala	4.12	1.51	7.34	6.22
Vernonia tigna	4.67	3.07	10.87	1.7

Table 3.3 Continued

Plant species	Extract yield per 100 mg/ml			
	Acetone	**Methanol**	**Cold water**	**Hot water**
Plants				
Chenopodium ambrosioids	4.26	4.42	2.89	7.67
Baccharoides adoensis	1.61	1.32	5.21	1.50
Hewittia malambarica	1.35	2.0	0.74	1.48
Stems				
Acacia robusta	4.04	4.57	1.51	2.91
Acanthospermum australe	1.29	0.99	7.06	3.31
Acridocarpus natalitius	1.7	15.91	9.76	6.88
Catharanthus roseus	1.8	6.5	3.11	1.58
Chromolaena odarata	0.46	3.32	3.82	1.33
Dichrostachys cinerca	0.79	4.49	0.6	0.87
Faurea macnaughton	1.54	10.05	9.65	7.06
Hypoxis hemerocallidea	10.9	2.02	2.14	7.13
Lippia javanica	0.87	3.66	1.88	1.52
Maytenus heterophylla	1.98	3.14	1,01	7.09
Melia azedarach	0.89	2.74	2.42	1.31
Psidium guajava	1.03	5.22	0.95	1.17
Schotia brachypetala	4.06	4.12	1.5	1.22
Tetradenia riparia	0.66	2.55	1.74	3.65
Trichilia dregeana	8.08	6.51	6.16	2.79
Vernonia oligocephala	2.33	4.10	0.89	6.11
Vernonia tigna	0.98	2.32	1.11	4.80
Tubers				
Callilepis laureola	2.26	5.76	7.84	15.08

3.6 Antibacterial assays

All the media was prepared according to the instructions provided by the supplier (Oxoid and Merck) i.e. weighed, dissolved in distilled water and autoclaved at 121 °C for 15 minutes. Different bacterial strains were maintained on the agar plates and restreaked every two to three weeks to keep the cultures alive. Bacterial cultures were prepared by transferring one colony into a flask containing 200 ml sterile Mueller-Hinton (MH) broth and incubated to a 0,5 McFarland standard (approximately 1×10^6 CFU/ml). The density of the turbidity was measured by using a spectrophotometer (Thermo spectromic Merck) with 1 cm light path at a wavelength of 640 nm. An absorbance of 0.008 to 0.10 was accepted as a 0.5 McFarland standard (Mathabe *et al.*, 2006).

3.6.1 Culture maintenance

Aseptic technique was used in the process of streaking out the bacteria pathogens. The bacteria had to be streaked one at a time. *Staphylococcus aureus* were streaked on the plates with 7.5% NaCl (Lalitha, 1997). Firstly the loop was flamed and the initial inoculum was streaked several times over an area corresponding to area 1 (Figure 3.1). Futhermore the bacteria were streaked several times in area 2 and the process was repeated for area 3 and area 4. The inoculated plates were incubated at 37 °C for 24 hours; the plates were observed for single colonies and stored at 4 °C for later use (Chan *et al.*, 1993).

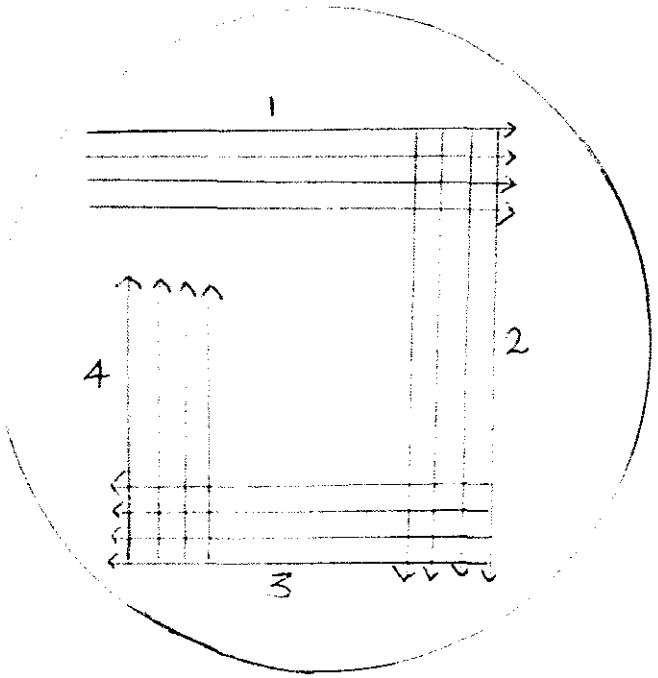


Figure 3.1 The four-way streaking method.

3.6.2 Disk-diffusion technique

The disc-diffusion technique was used for testing for antibacterial activity. The bacteria was maintained on Mueller-Hinton (MH) agar and stored in the refrigerator at 4 °C for further use. Mueller-Hinton broth cultures were prepared by transferring 1 colony to 200 ml Mueller-Hinton broth. Respective bacterial strains (50 µl) were aseptic on 10 ml Mueller Hinton agar in sterile Petri dishes (9 cm). Different plant extract (30 µl) was applied to a sterile filter paper disk (Whatman No 1). The disks were allowed to dry before being placed onto the seeded top layer of the agar plates. Dimethly sulfoxide (DMSO) was used as a negative control and prepared in the same manner as the extracts (Basch, 1968). Each plate contained 4 paper discs with different plant extracts. A disc with 30 µl neomycin (0.1 mg/ml) (antibiotic) was placed on the agar and used as a positive control. The plates were incubated at 37 °C for 12 hours and the inhibition zones were measured in millimeters, antibacterial activity was expressed in terms of the average diameter of the zone of inhibition (Vlietinck *et al.*, 1995).

3.6.3 Agar-well diffusion technique

An alternative method of agar-well diffusion technique was used. Sterile Mueller-Hinton (MH) agar was prepared. Bacterial strains of standardized cultures were spread evenly using a sterile glassrod. Four wells (5 mm diameter) were made in each plate using sterile Pasteur pipettes, 30 µl of methanol, ethanol, acetone and distilled water plant extracts (100 mg/ml) were then added in each well. DMSO (30 µl) was used as the negative control and Neomycin (30 µl) of concentration 0.1 mg/ml was used as the positive control. Diffusion of the extracts was done at room temperature for 1 hour. The plates were covered with lids and incubated at 37 °C for 24 hours. The plates were observed for the presence of clear zones of inhibition. The zones of inhibition were measured in millimeters and antibacterial activity was expressed in terms of the average diameter of the zone of inhibition. The absence of a zone of inhibition interpreted the absence of activity (Mathabe *et al*, 2006).

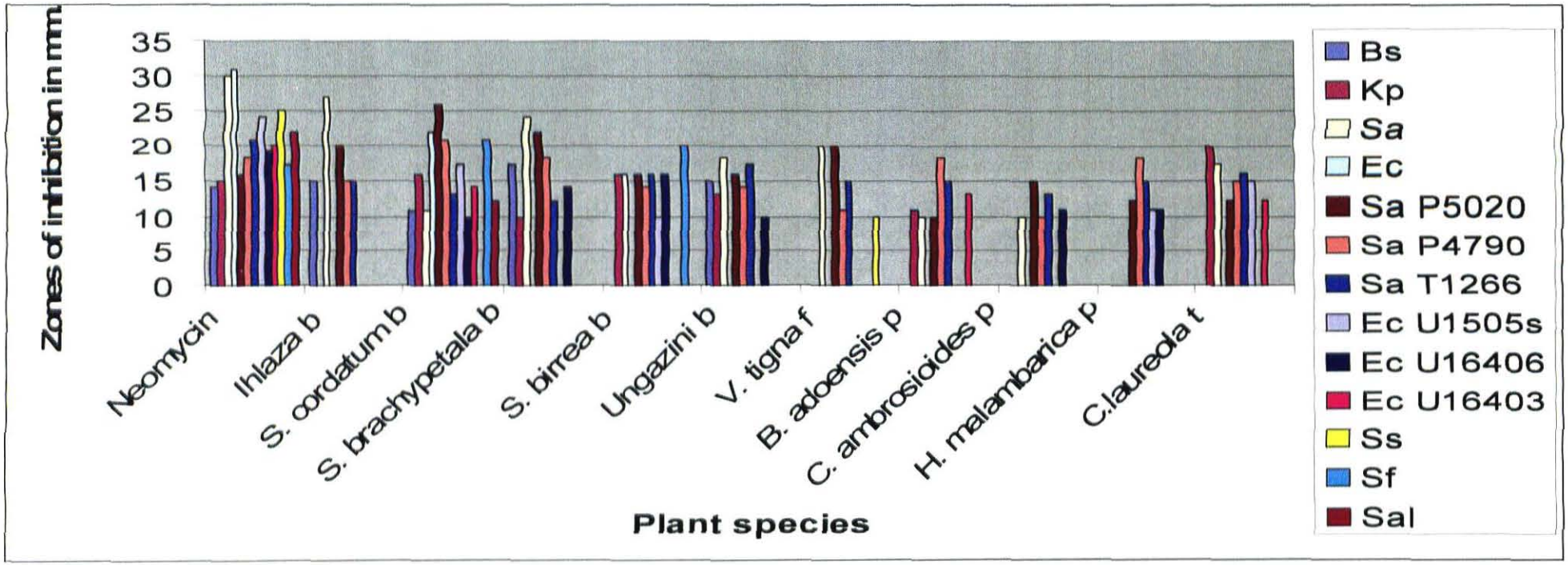
3.6.4 Minimum inhibition concentration (MIC)

Serial dilution assay was used for the determination of the minimum inhibitory concentration (MIC). A micro-dilution technique using 96 well micro-plates, as described by Eloff (1998) was used to obtain MIC values of the crude plant extracts. An equal volume of 25 µl distilled water, different plant extract and fresh bacterial cultures were added to the wells. Similar serial dilution was performed for neomycin (0.1 mg/ml) as a positive control and a DMSO was used as a negative control. Micro–plates were then covered with lids and incubated at 37 °C overnight. *P-iodonitrotetrazolium* violet (*Sigma*) (25 µl) reagent (0.2 mg/ml) was used to indicate the presence of inhibition of bacterial growth, a pink or purple color was an indication of the growth of bacteria and a colourless appearance was an indication of the inhibition of bacterial growth in each well. The lowest concentration of the extract that inhibited the bacterial growth after incubation was taken as the MIC of a crude extract.

3.7 Results

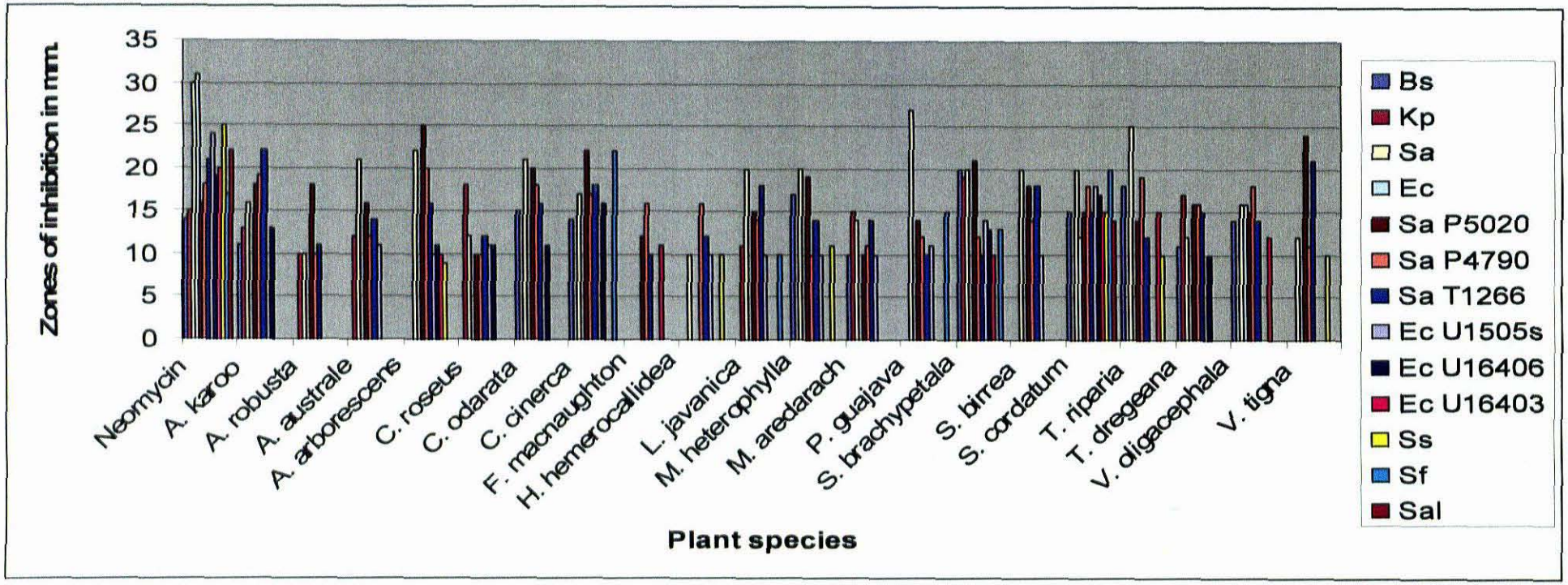
3.7.1 Graphs showing the antibacterial activities obtained using the agar-well diffusion assay.

Figure 3.2 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with acetone (Table representation - appendix C, Table 1).



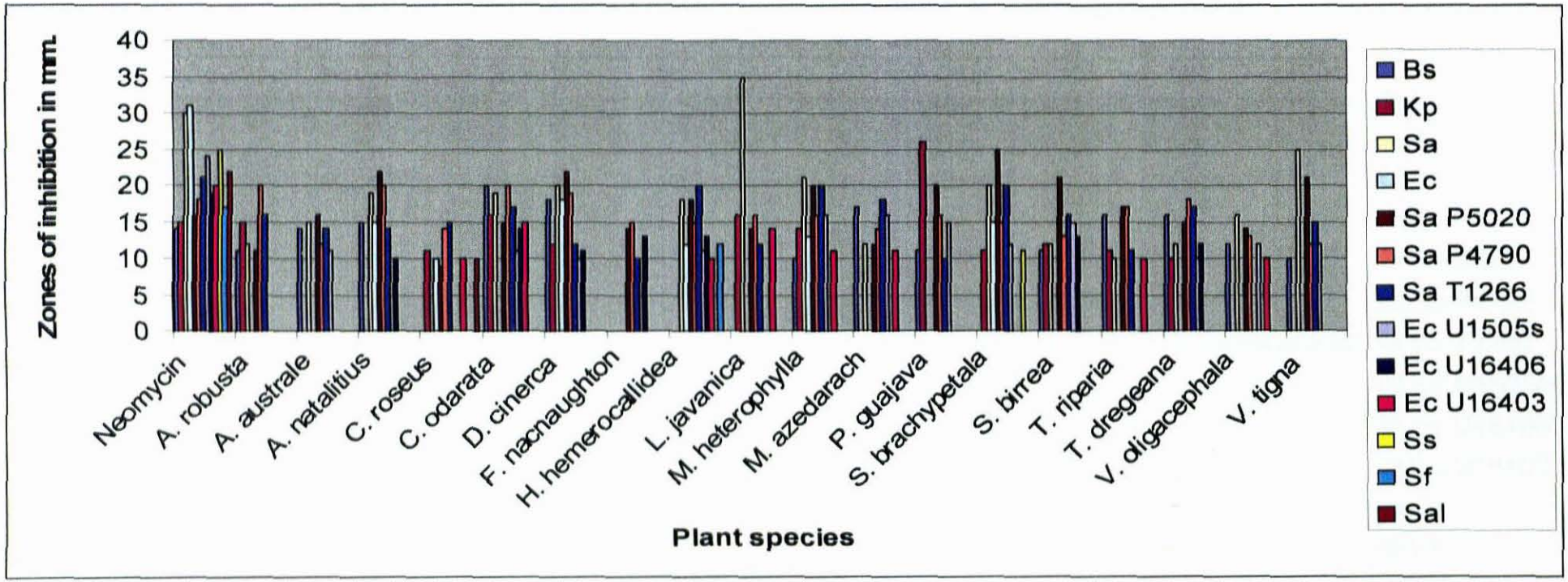
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf– *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.3 Antibacterial activities of leaves extracted with acetone (Table representation - appendix B, Table 1).



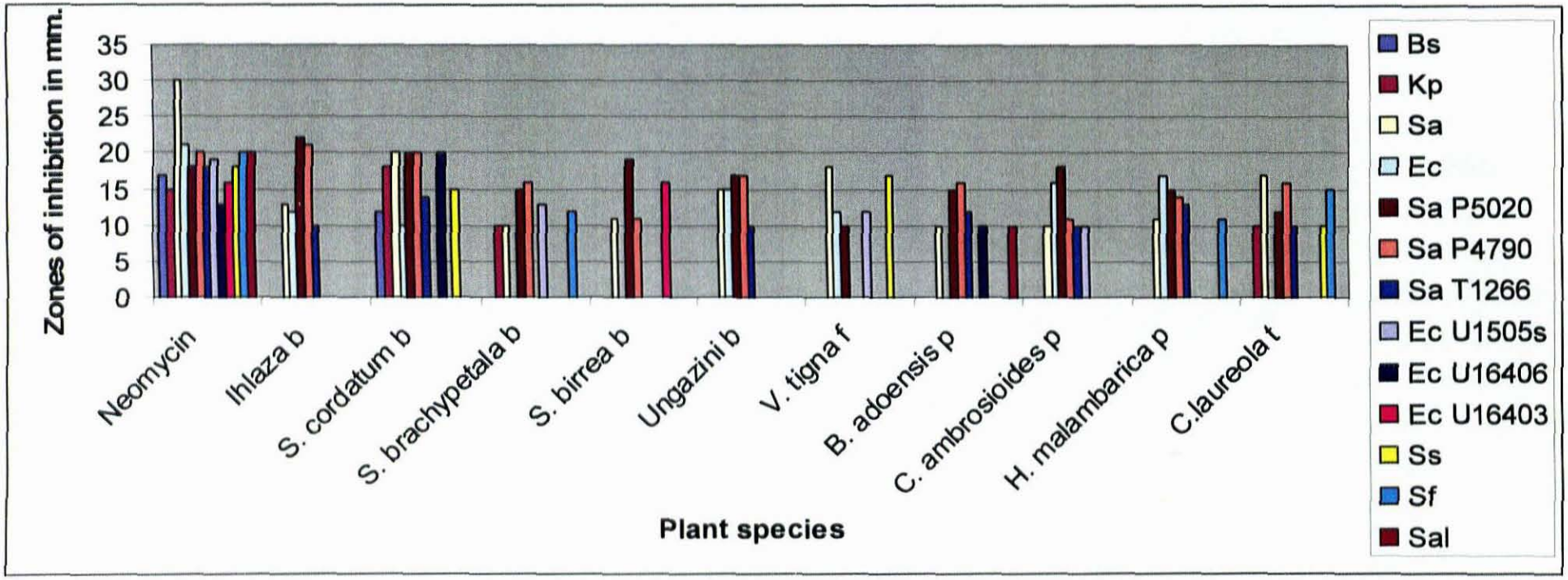
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.4 Antibacterial activities of stems extracted with acetone (Table representation - appendix B, Table 1).



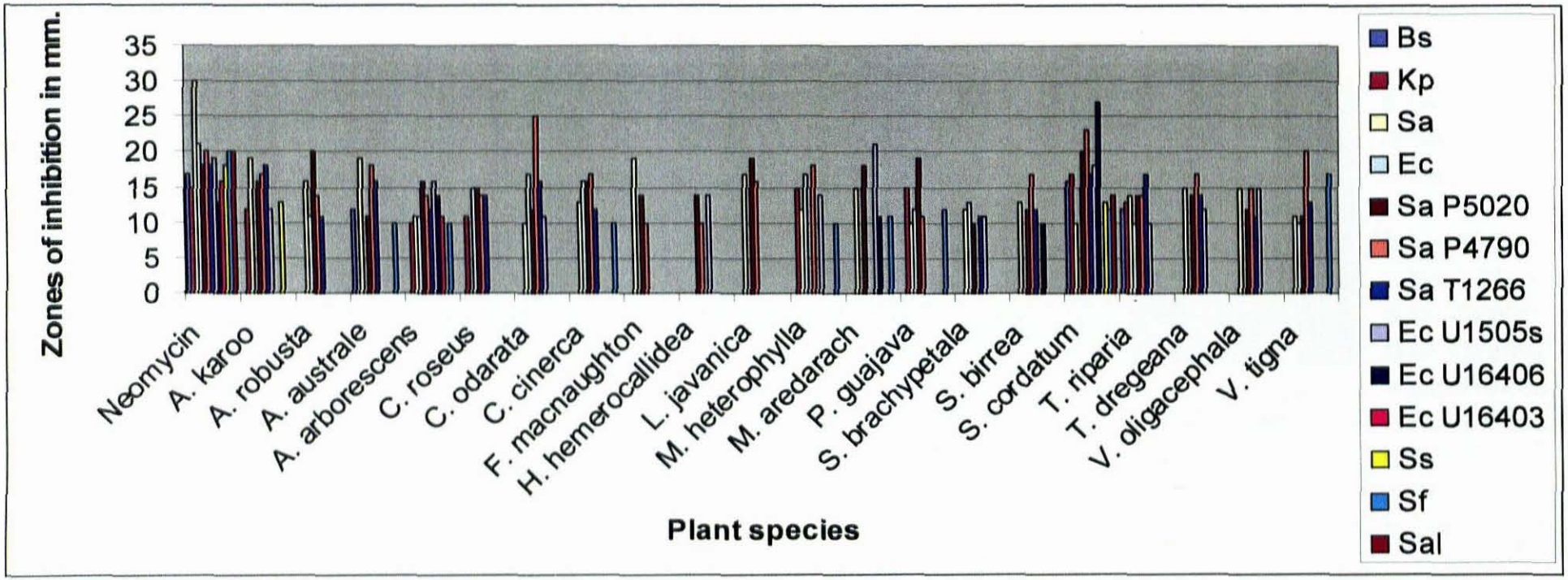
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.5 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with methanol (Table representation - appendix B, Table 2).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonie*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii, Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

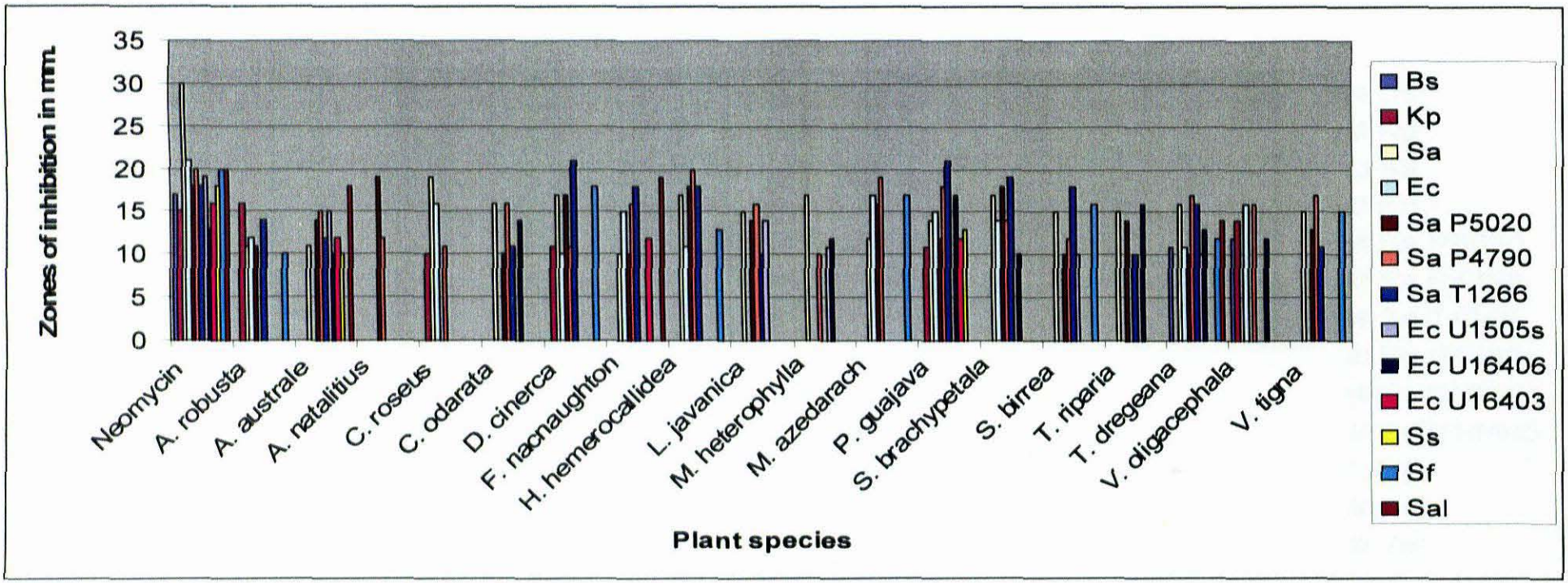
Figure 3.6 Antibacterial activities of leaves extracted with methanol (Table representation - appendix B, Table 2).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*.

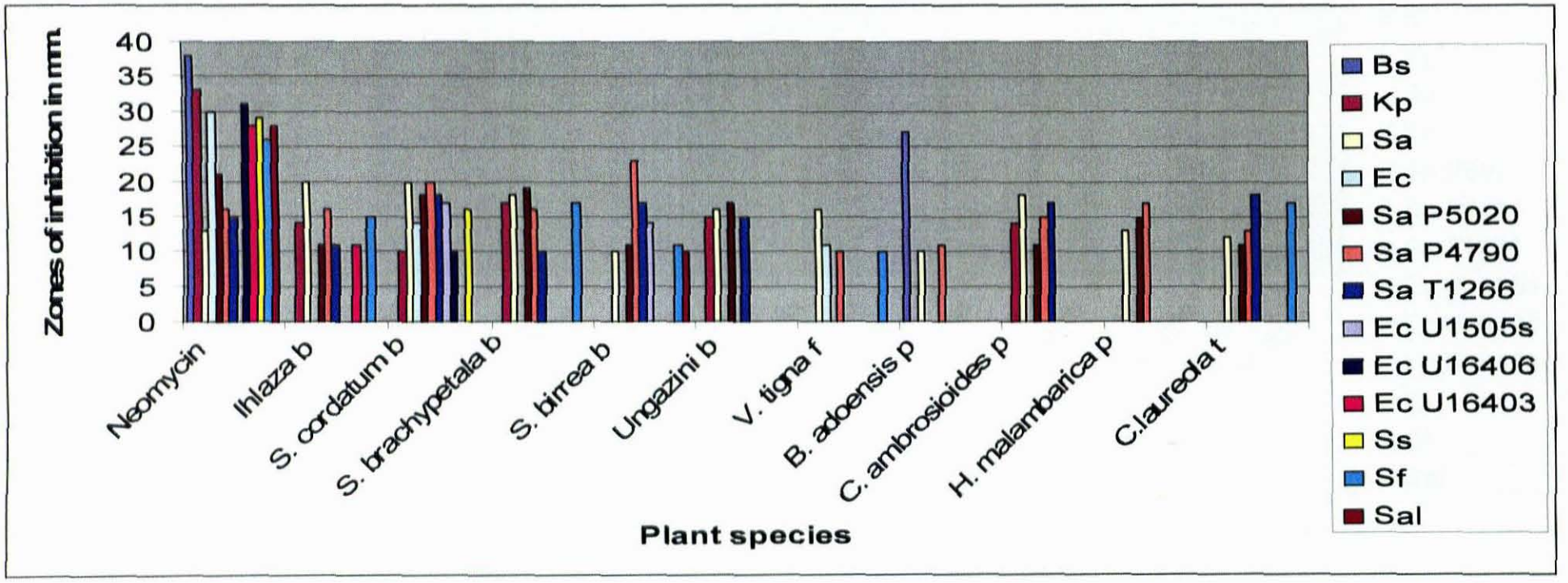
Ss – *Shigella sonnie*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.7 Antibacterial activities of stems extracted with methanol (Table representation - appendix B, Table 2).



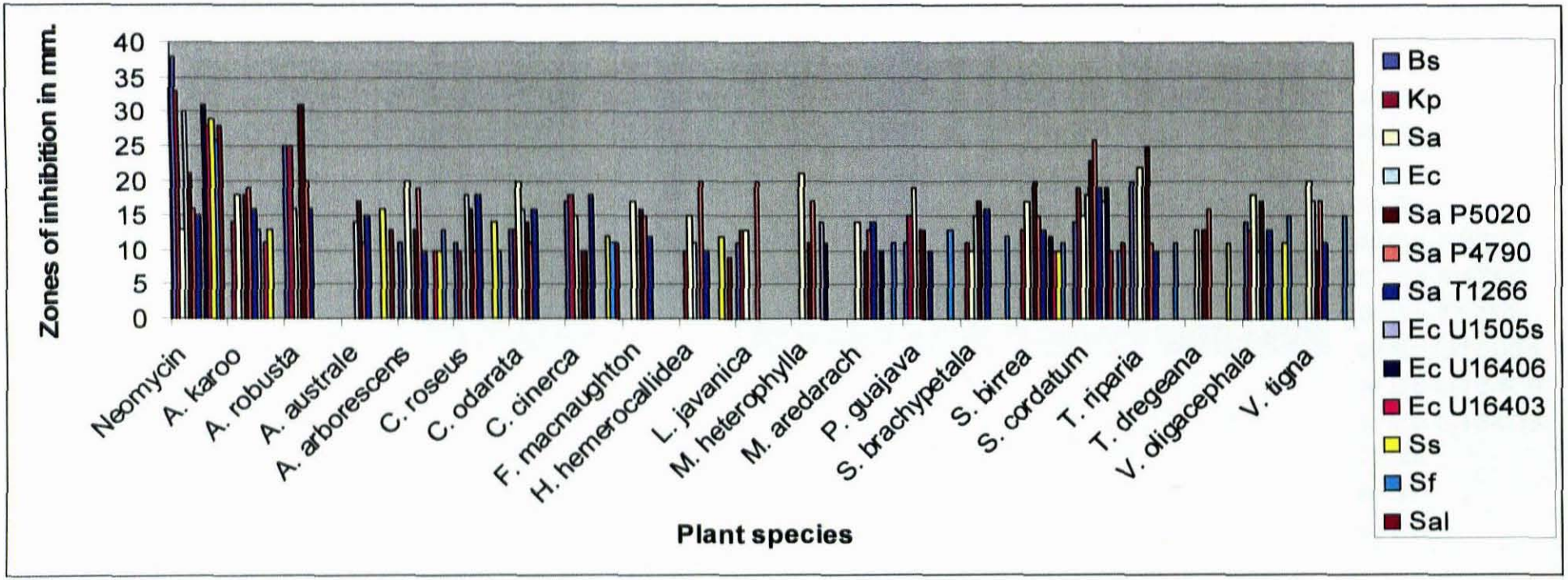
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.8 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with cold distilled water (Table representation - appendix B, Table 3).



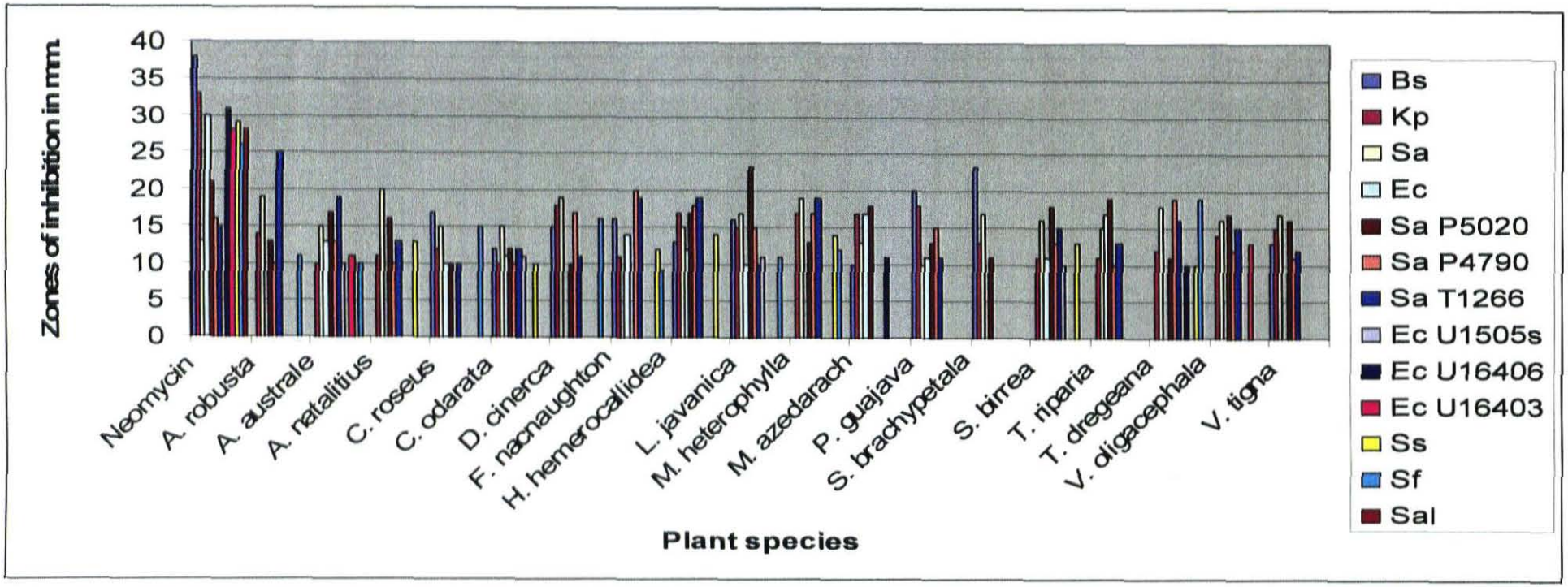
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.9 Antibacterial activities of leaves extracted with cold distilled water (Table representation - appendix B, Table 3).



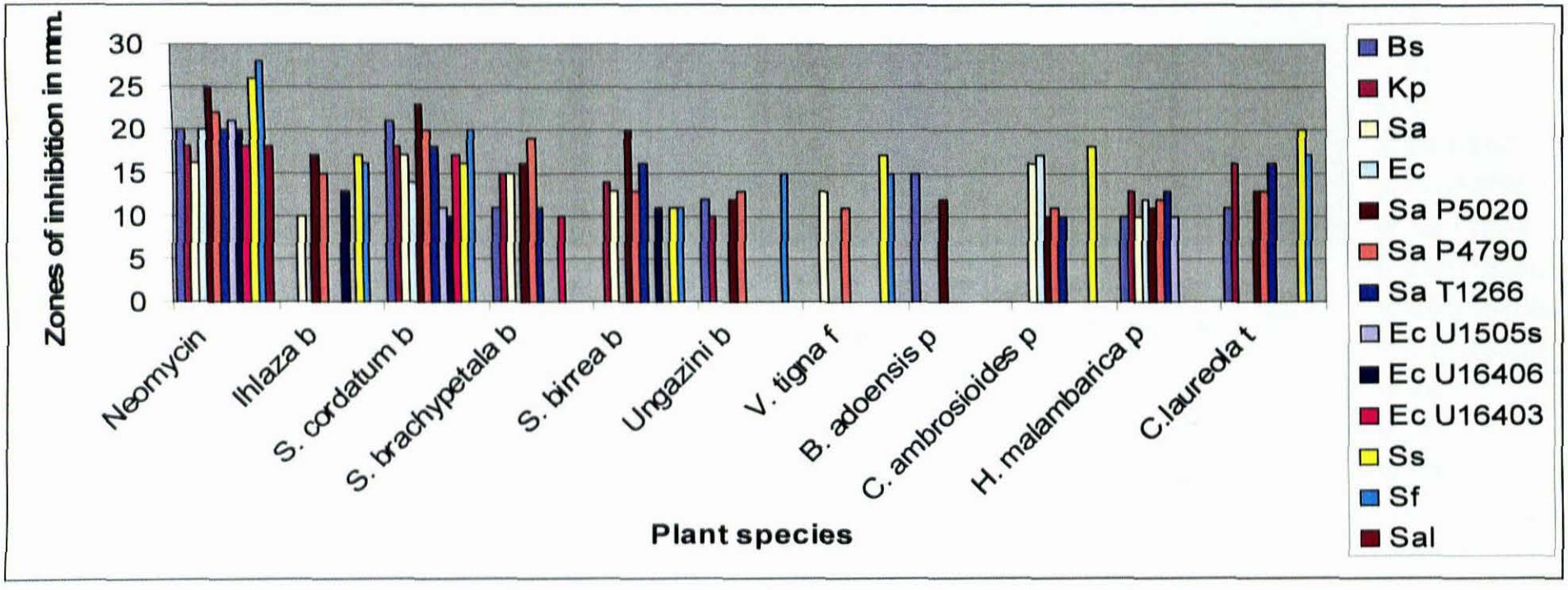
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.10 Antibacterial activities of stems extracted using cold distilled water (Table representation - appendix B, Table 3).



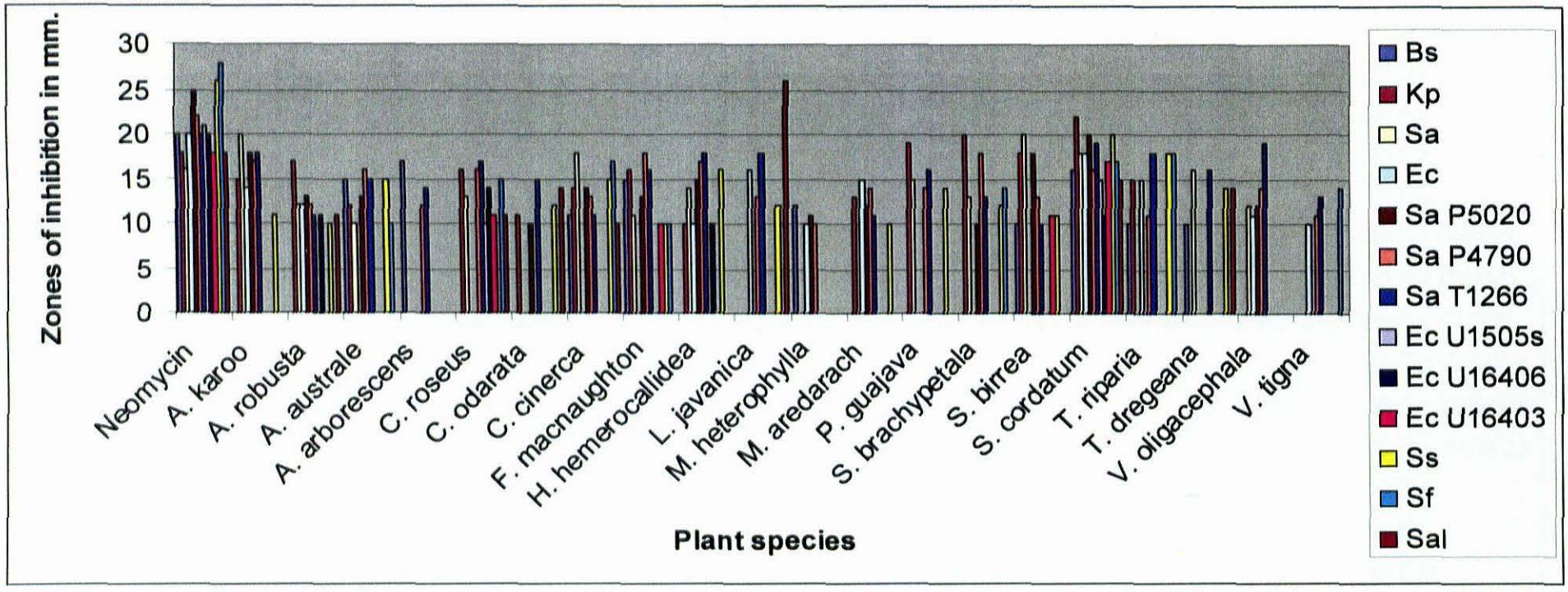
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal -*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.11 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with hot distilled water (Table representation - appendix B, Table 4).



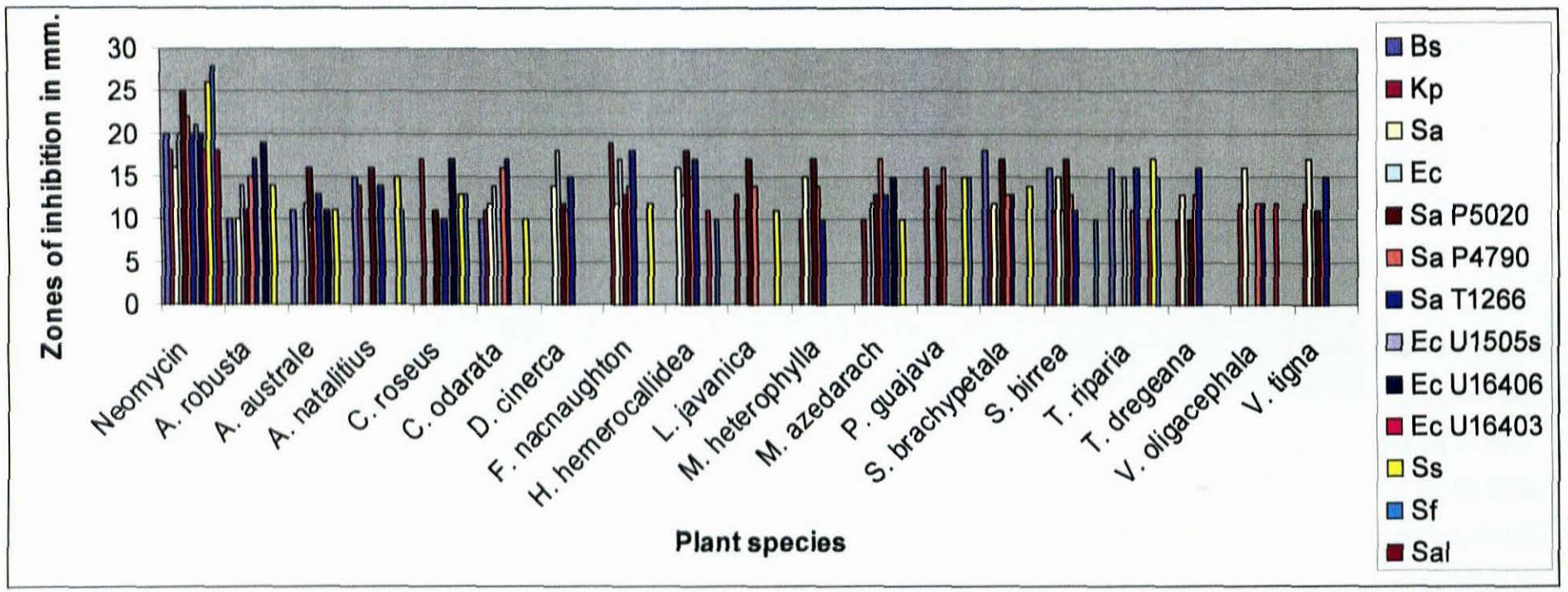
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.12 Antibacterial activities of leaves extracted with hot distilled water (Table representation - appendix B, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

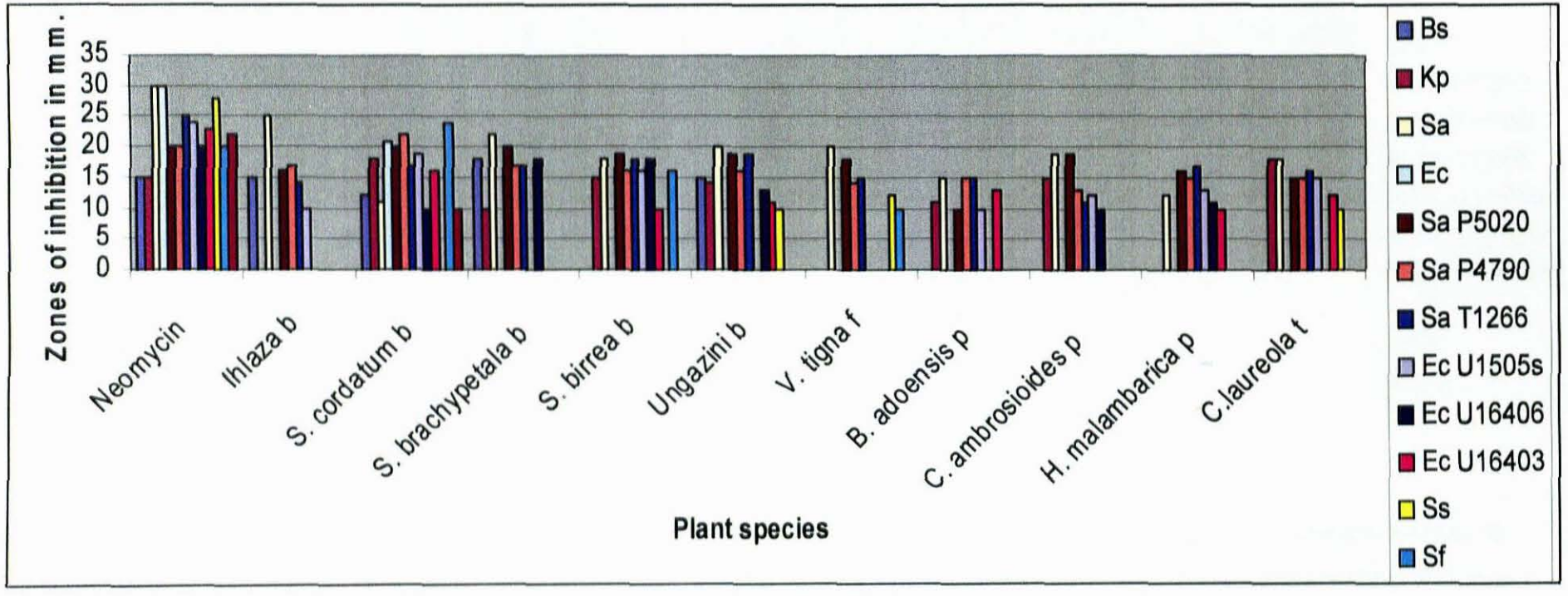
Figure 3.13 Antibacterial activities of stems extracted with hot distilled water (Table representation - appendix B, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

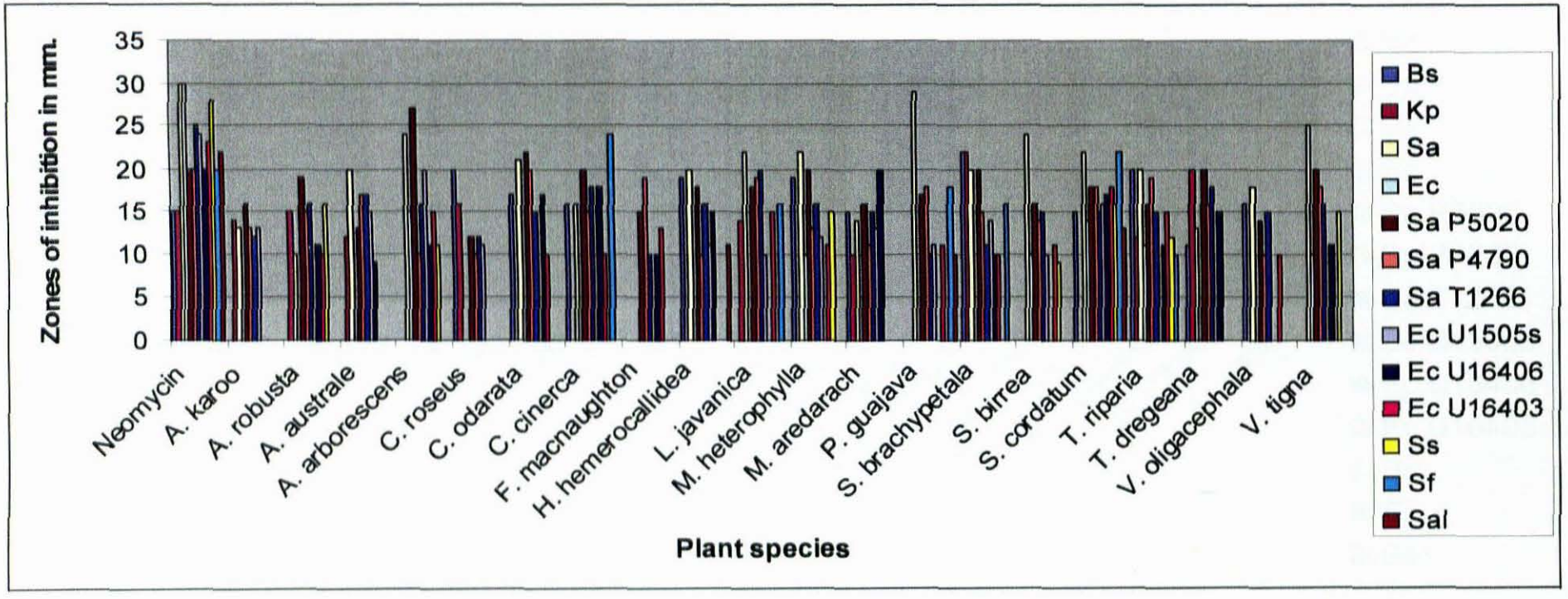
3.7.2 Graphs showing the antibacterial activities obtained using the disk-diffusion assay.

Figure 3.14 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with acetone (Table representation - appendix C, Table 1).



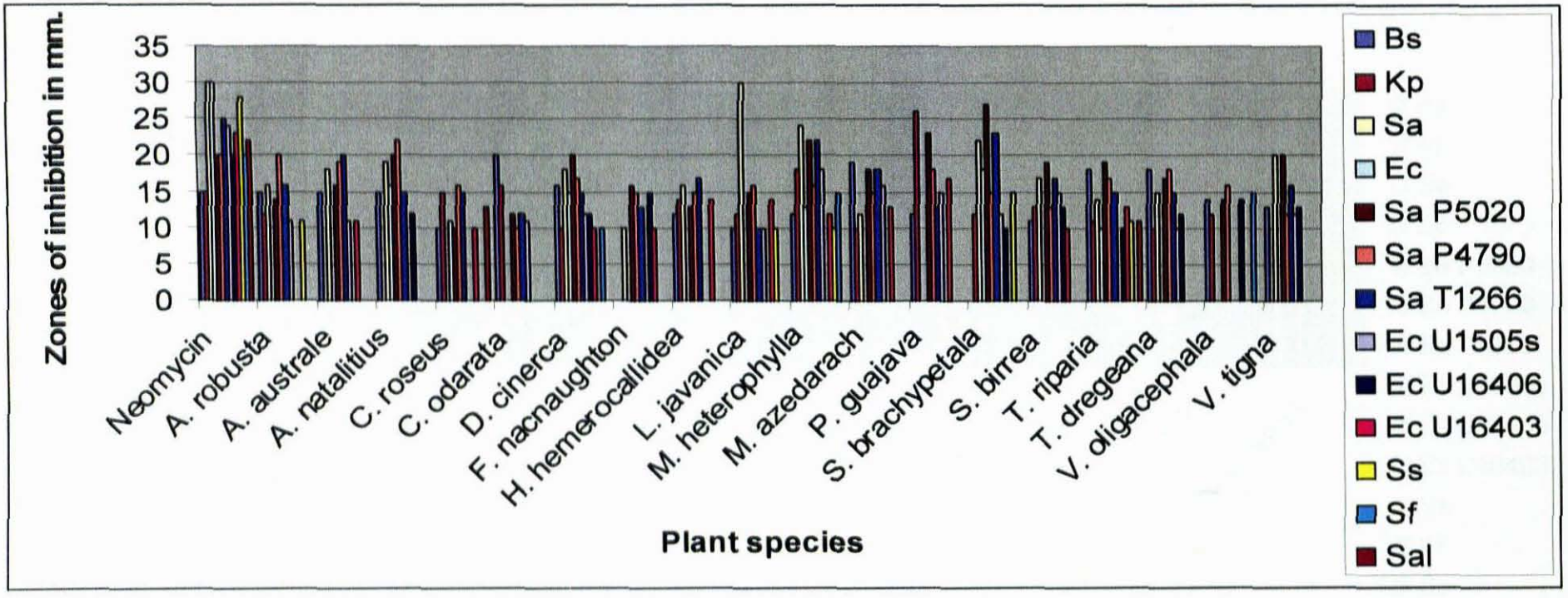
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.15 Antibacterial activities of leaves extracted with acetone (Table representation - appendix C, Table 1).



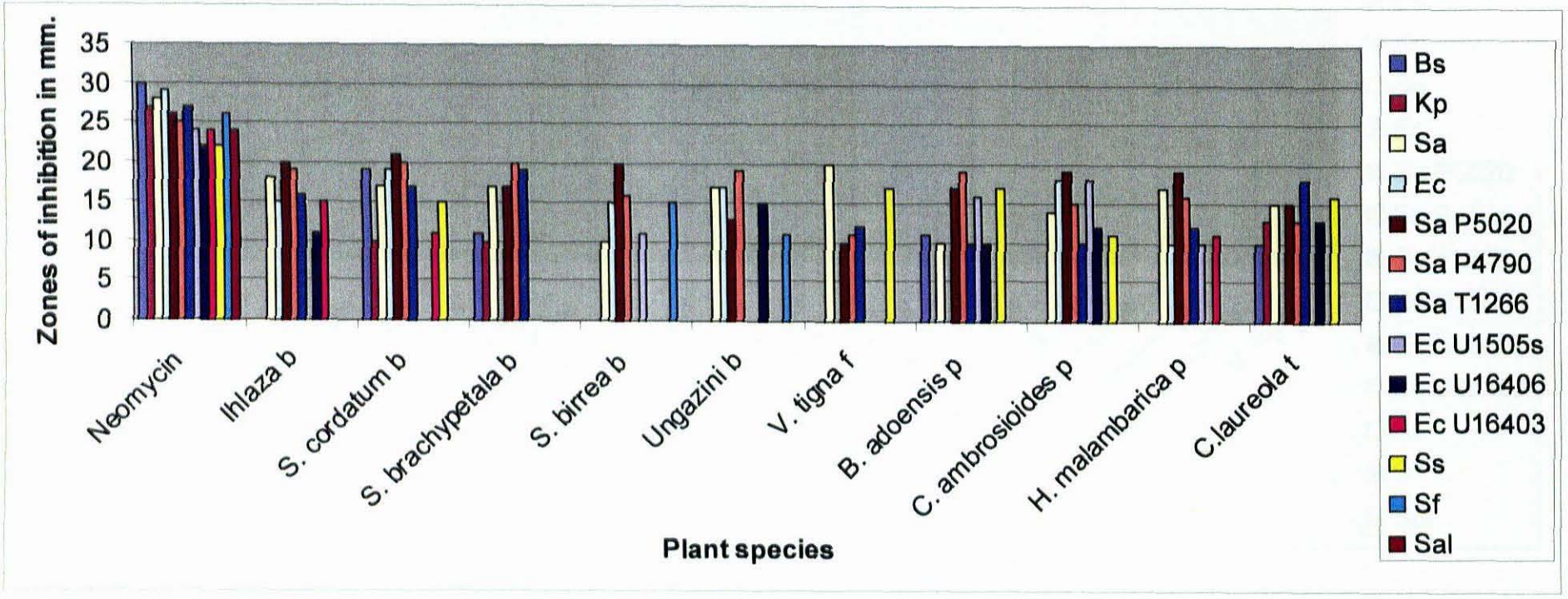
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.16 Antibacterial activities of stems extracted with acetone (Table representation - appendix C, Table 1).



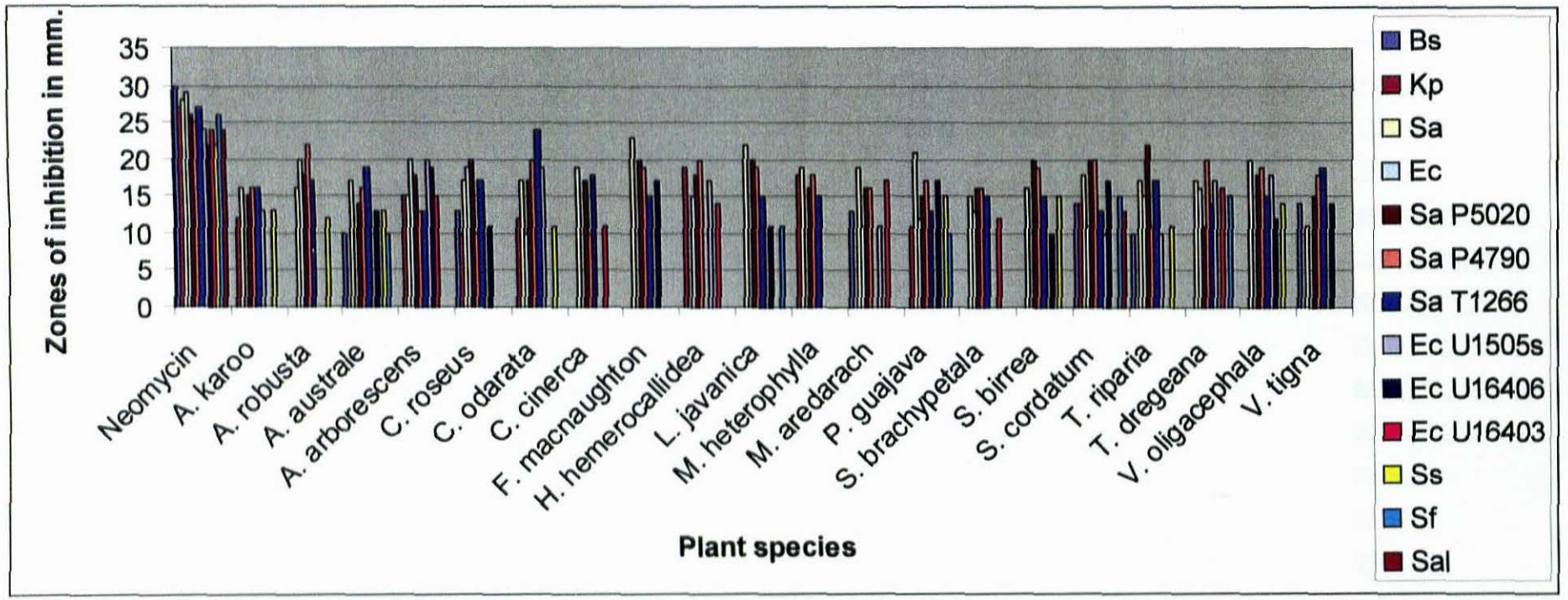
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.17 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with methanol (Table representation - appendix C, Table 2).



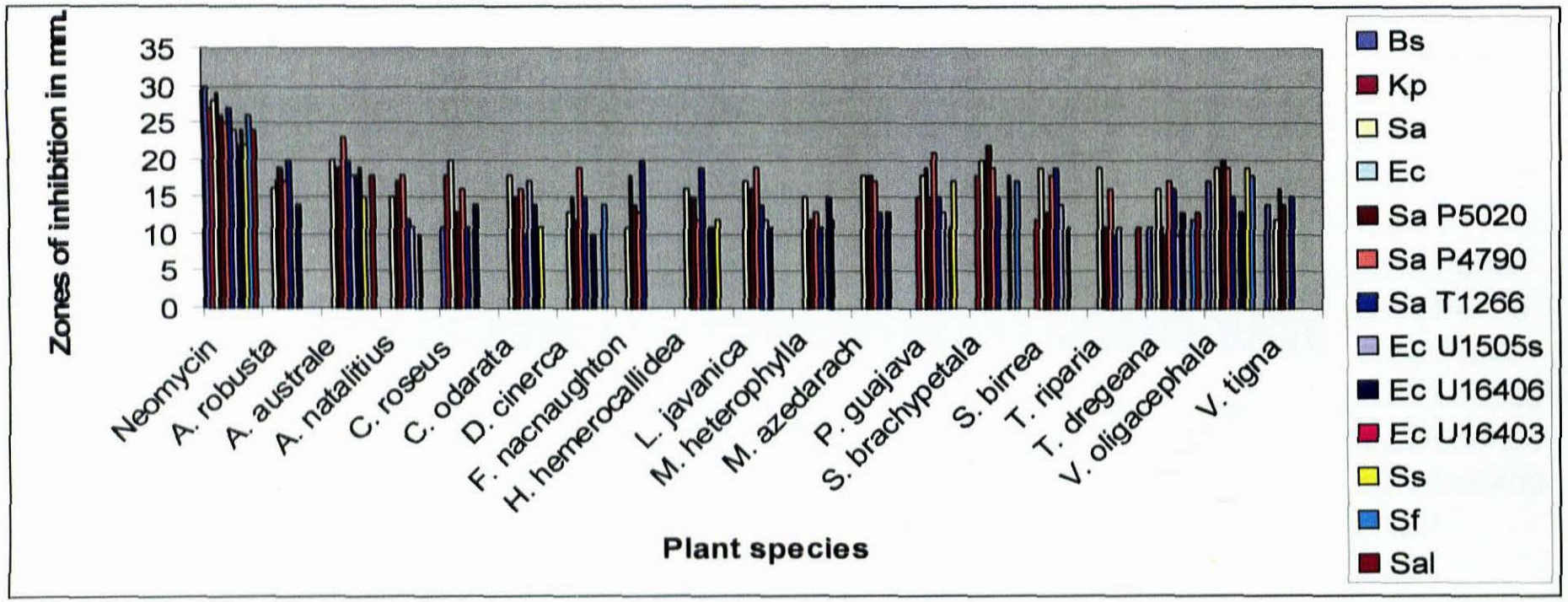
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.18 Antibacterial activities of leaves extracted with methanol (Table representation - appendix C, Table 2).



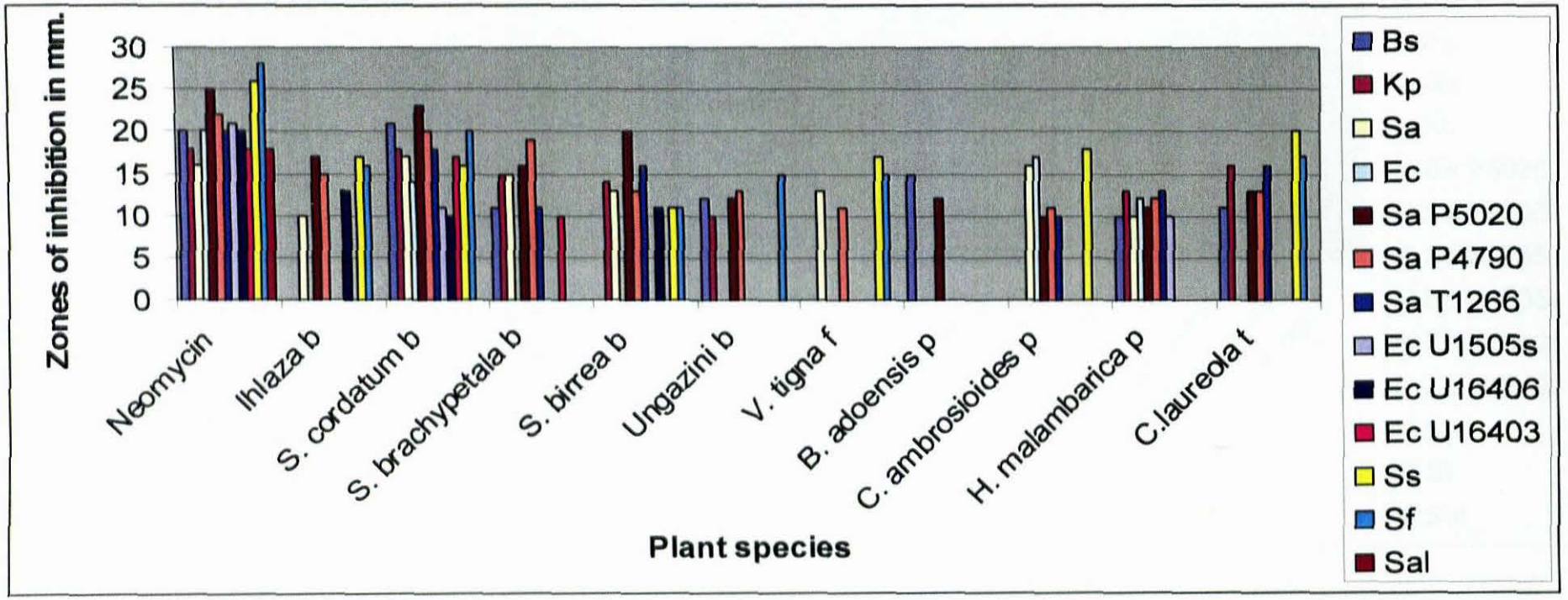
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.19 Antibacterial activities of stems extracted with methanol (Table representation - appendix C, Table 2).



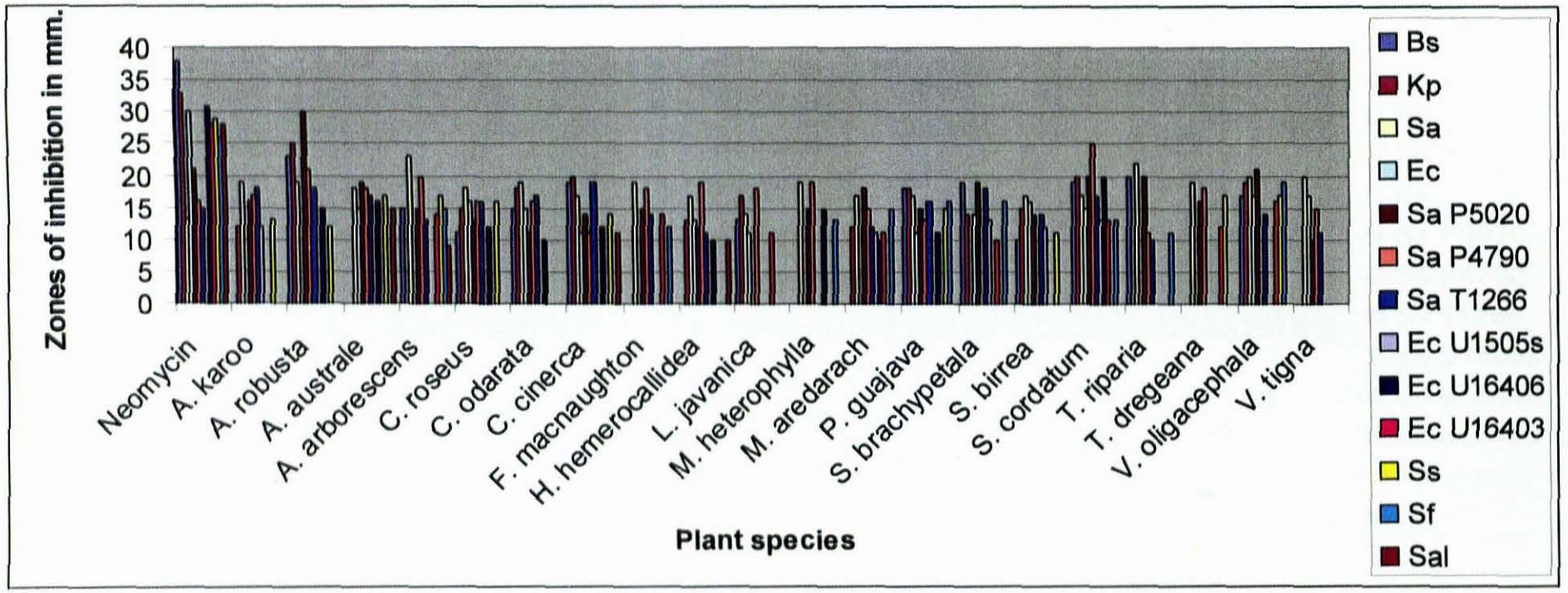
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.20 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with cold distilled water (Table representation - appendix C, Table 3).



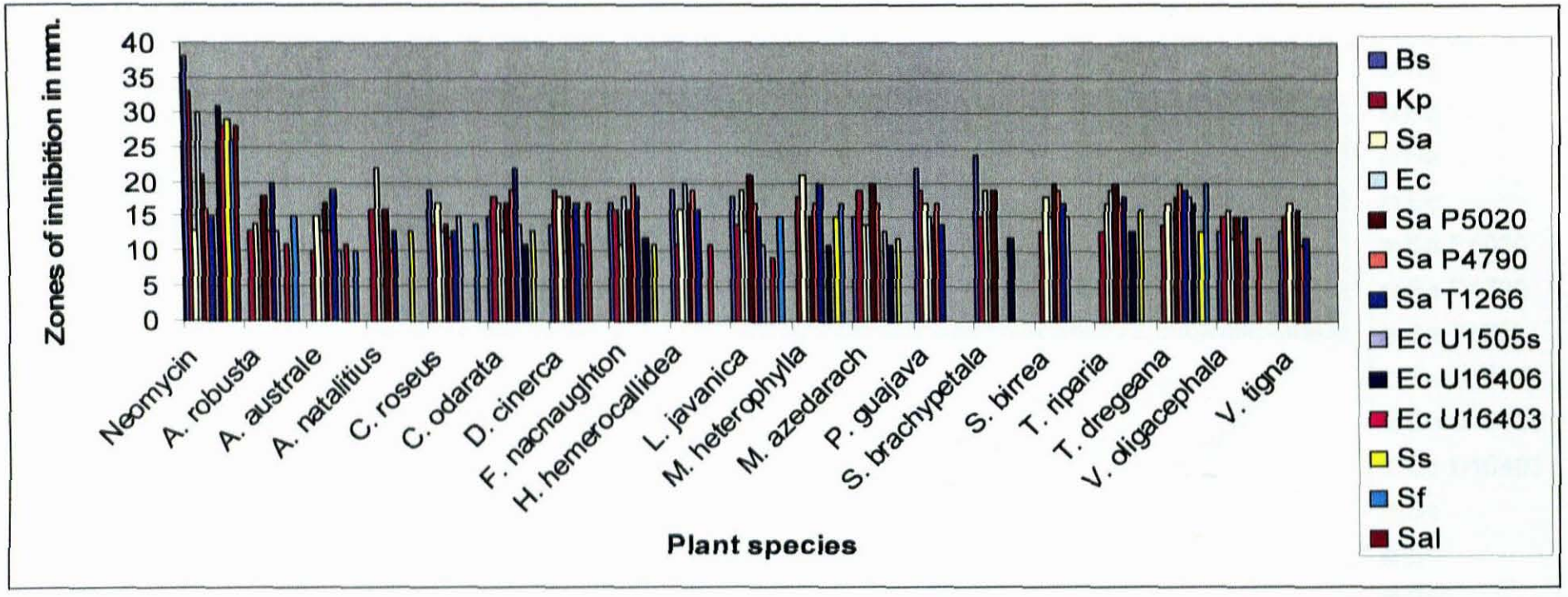
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.21 Antibacterial activities of leaves extracted with cold distilled water (Table representation - appendix C, Table 3).



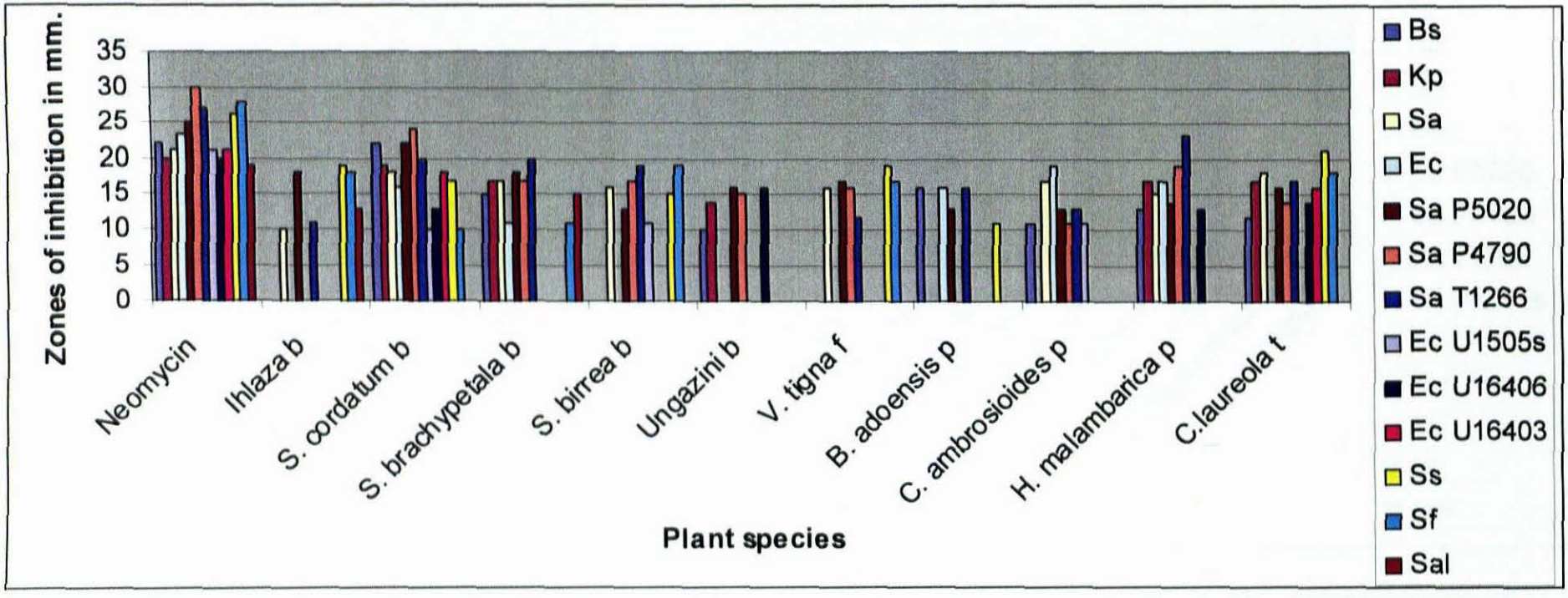
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumonie*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.22 Antibacterial activities of stems extracted with cold distilled water (Table representation - appendix C, Table 3).



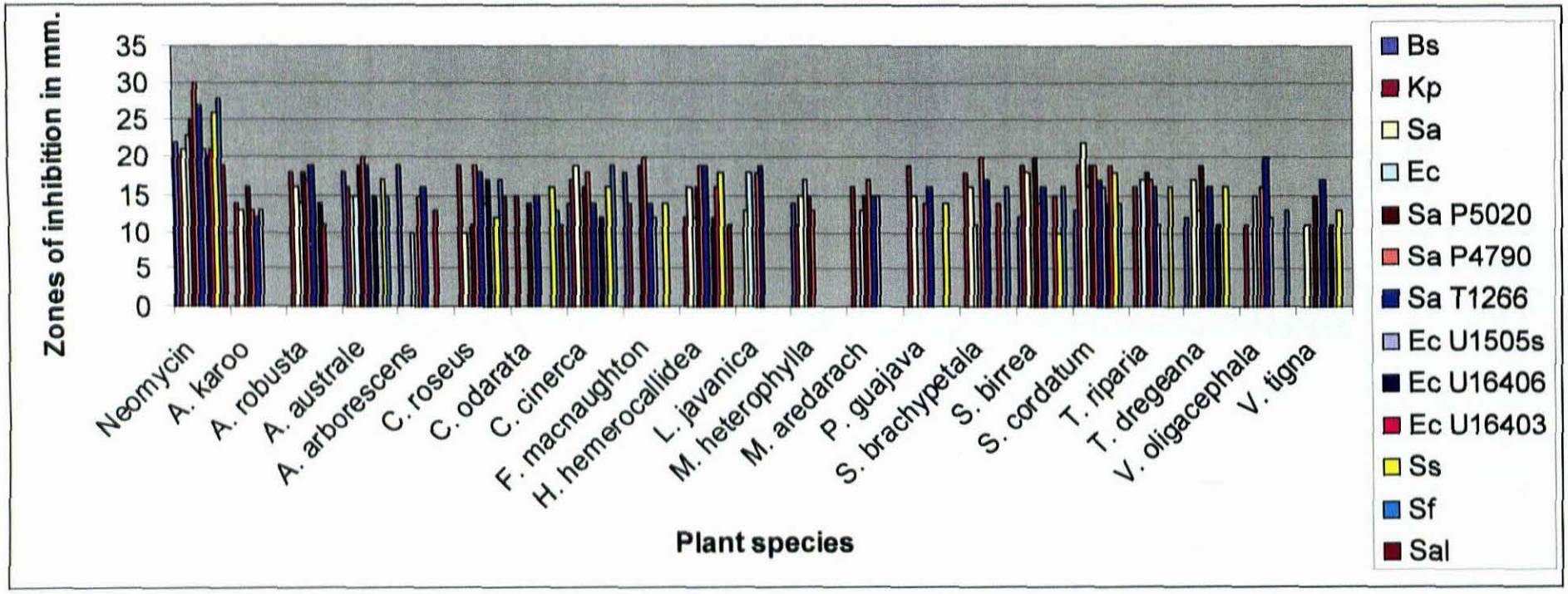
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.23 Antibacterial activities of bark (b), tubers (t) and plants (p) extracted with hot distilled water (Table representation - appendix C, Table 4).



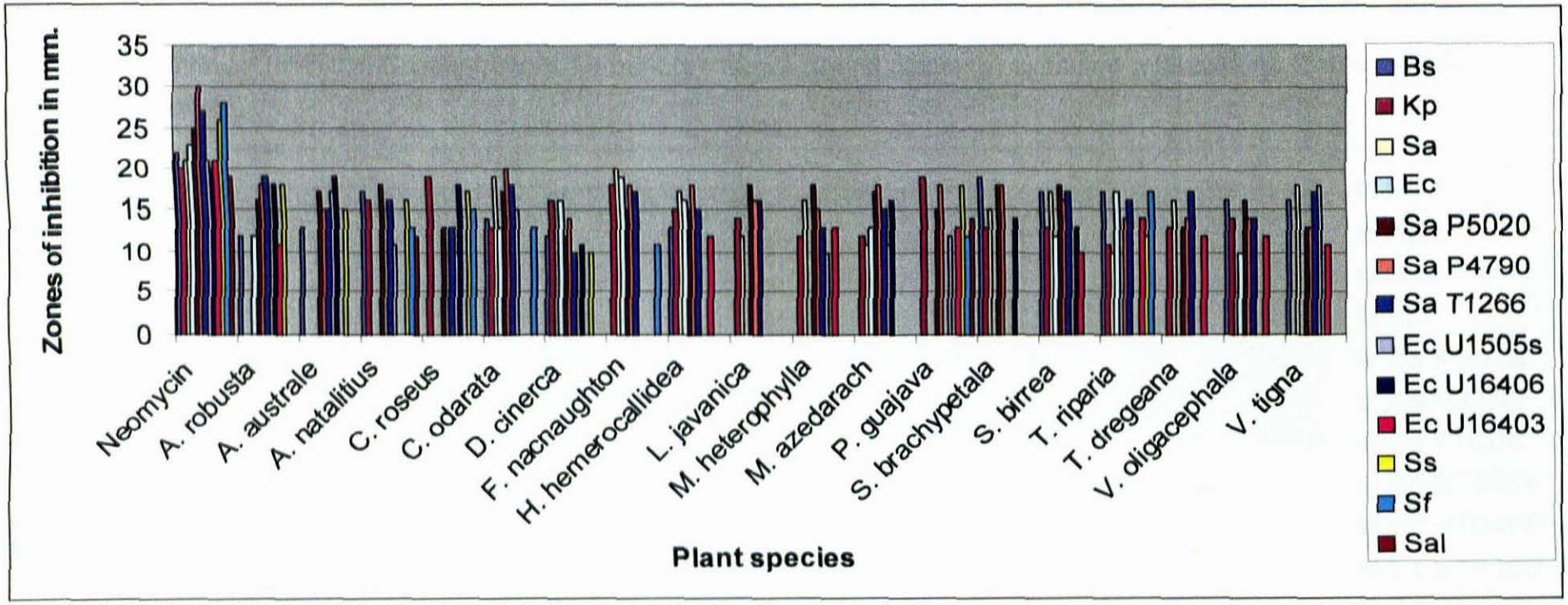
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.24 Antibacterial activities of leaves extracted with hot distilled water (Table representation - appendix C, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

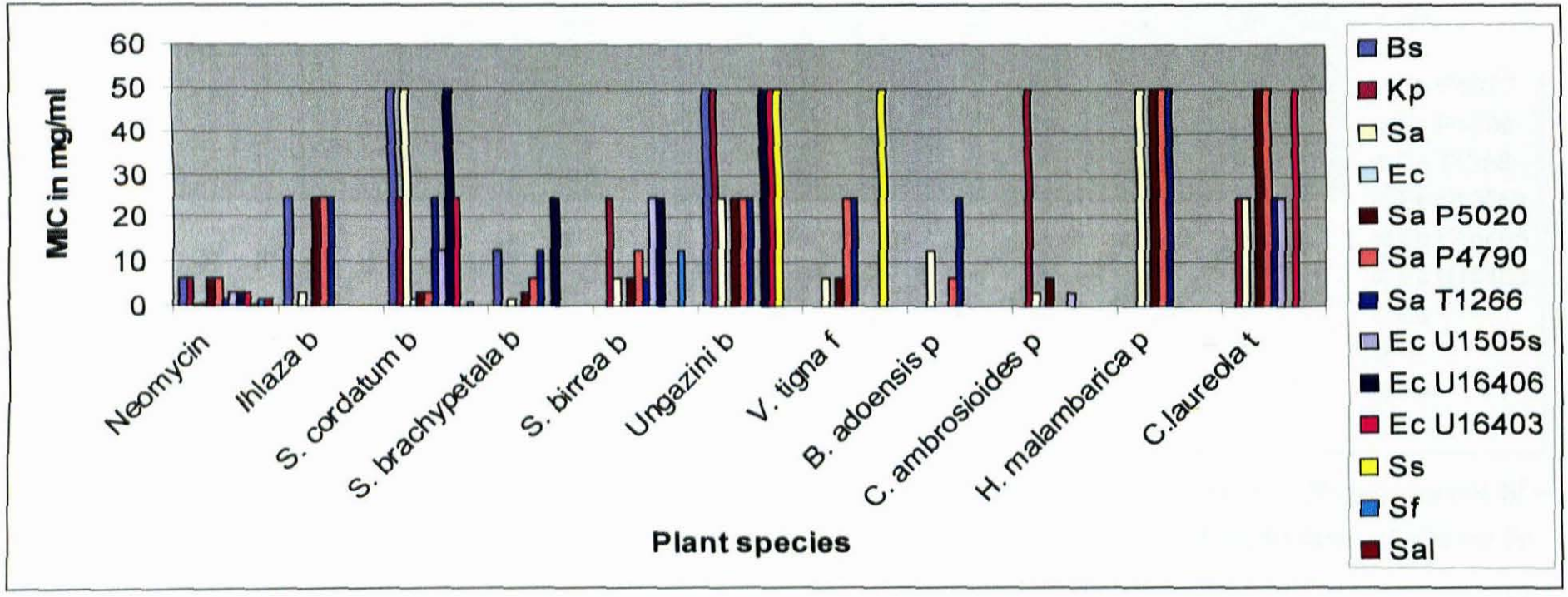
Figure 3.25 Antibacterial activities of stems extracted with hot distilled water (Table representation - appendix C, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

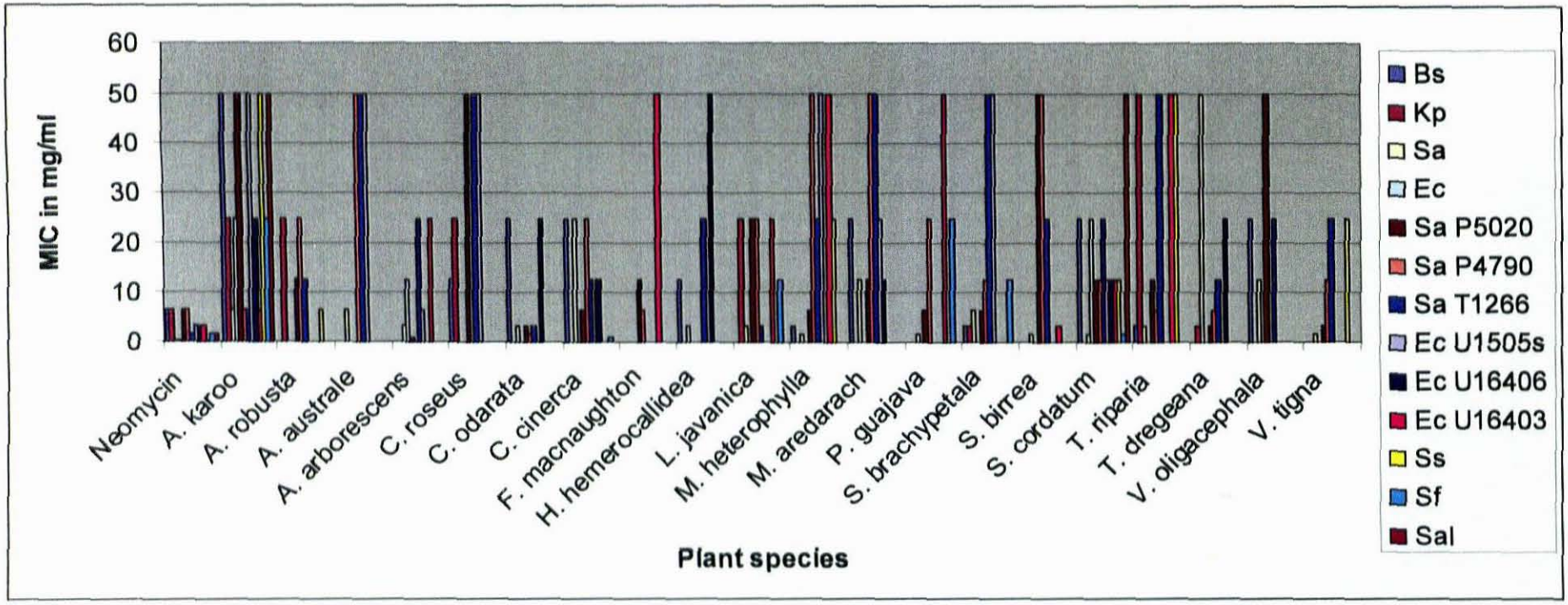
7.3 Graphs showing the Minimum Inhibition Concentrations for the different plant extracts.

Figure 3.26 Minimum Inhibition Concentrations for bark (b), tubers (t) and plants (p) extracted with acetone (Table representation - appendix D, Table 1).



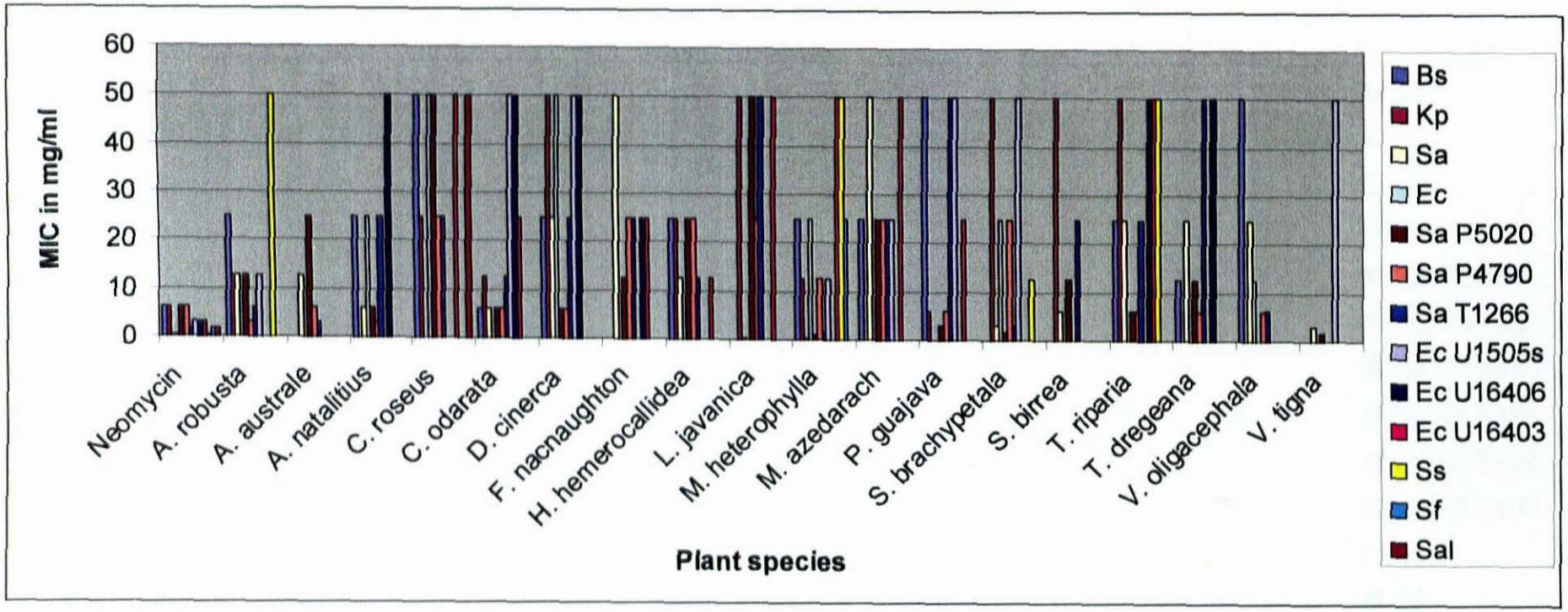
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.27 Minimum Inhibition Concentrations for leaves extracted with acetone (Table representation - appendix D, Table 1).



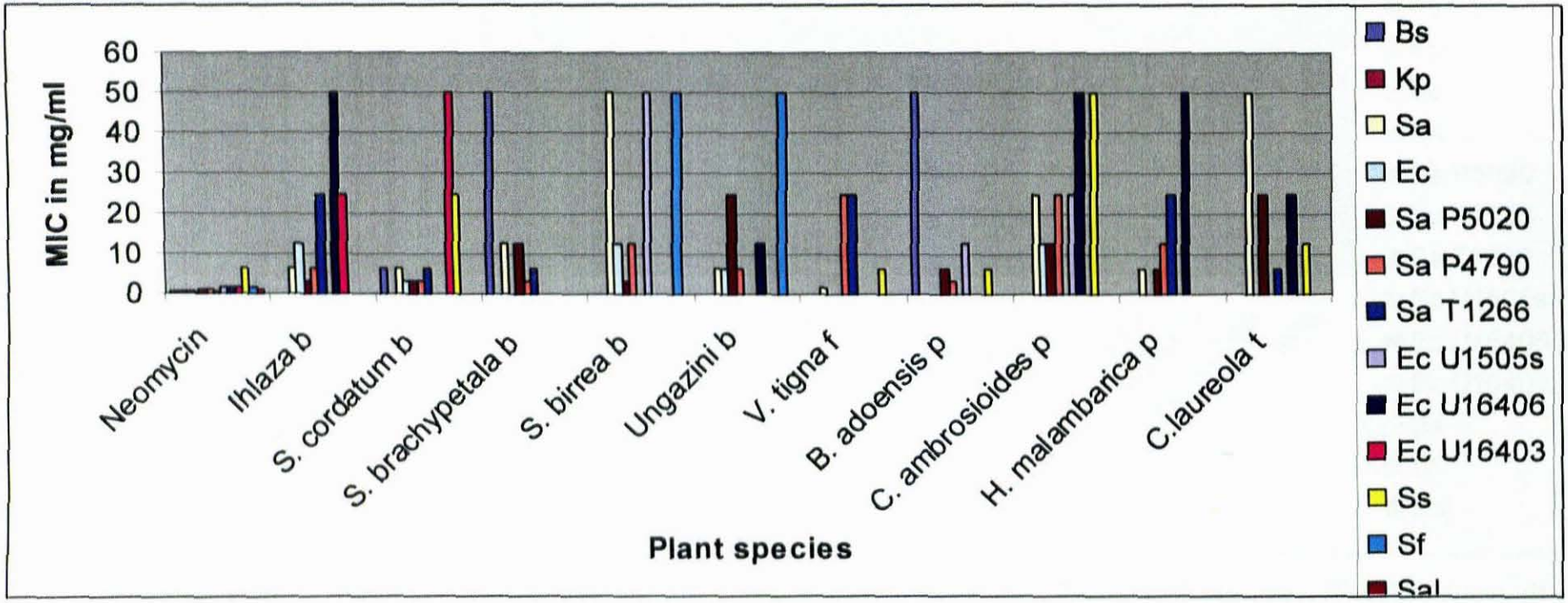
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.28 Minimum Inhibition Concentrations for stems extracted with acetone (Table representation - appendix D, Table 1).



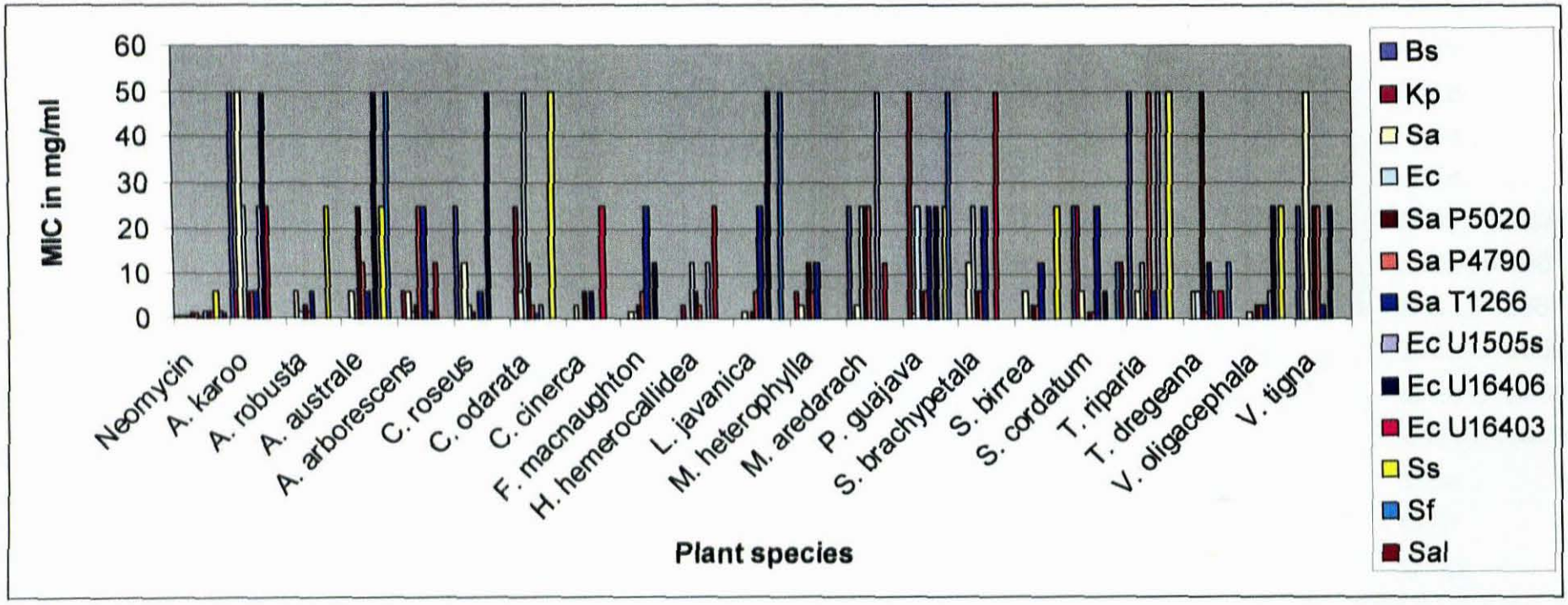
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.29 Minimum Inhibition Concentrations for bark (b) tubers (t) and plants (p) extracted with methanol (Table representation – appendix D, Table 2).



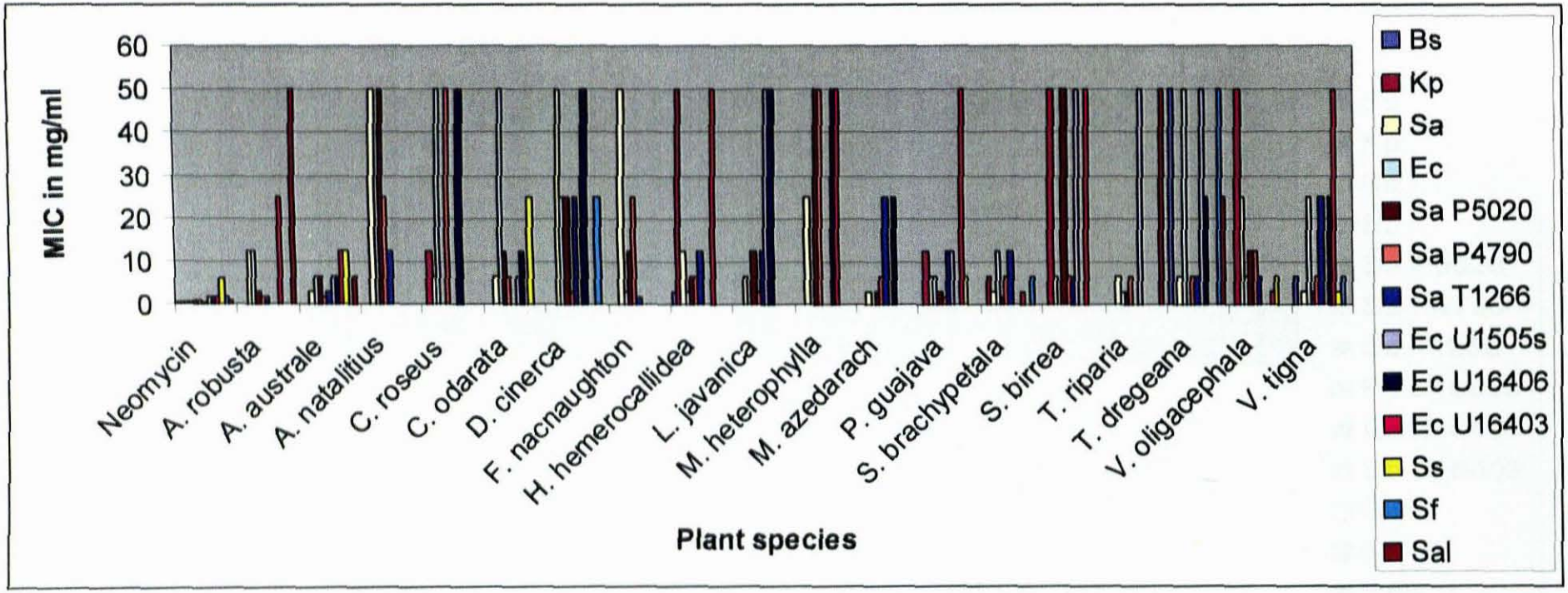
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.30 Minimum Inhibition Concentrations for leaves extracted with methanol (Table representation - appendix D, Table 2).



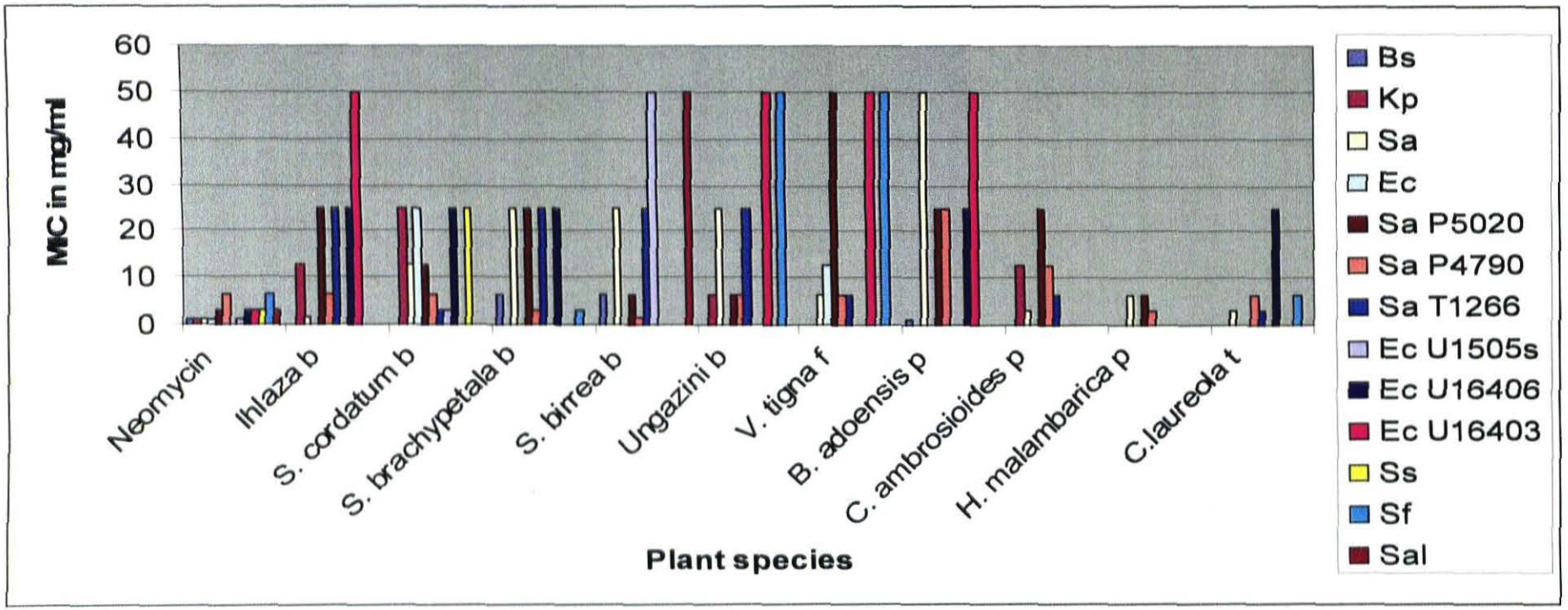
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.31 Minimum Inhibition Concentrations for stems extracted with methanol (Table representation - appendix D, Table 2).



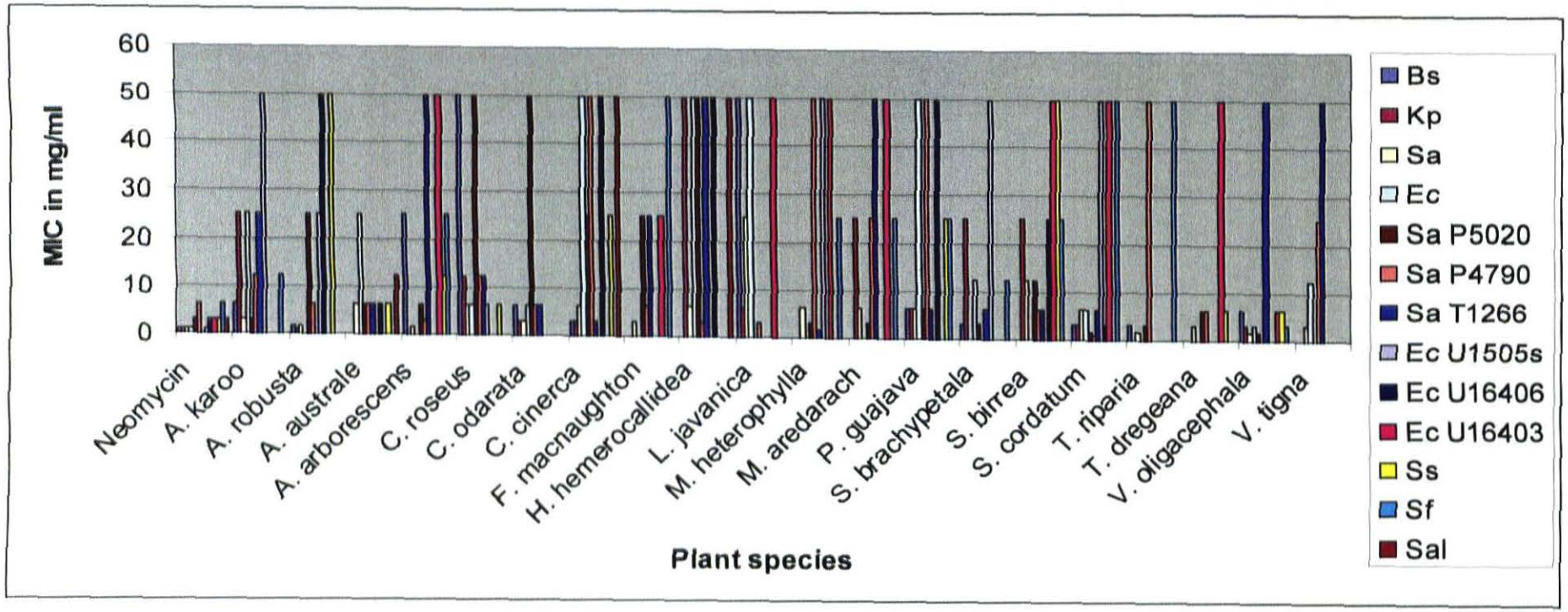
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strain - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.32 Minimum Inhibition Concentrations for bark (b), tubers (t) and plants (p) extracted with cold distilled water (Table representation - appendix D, Table 3).



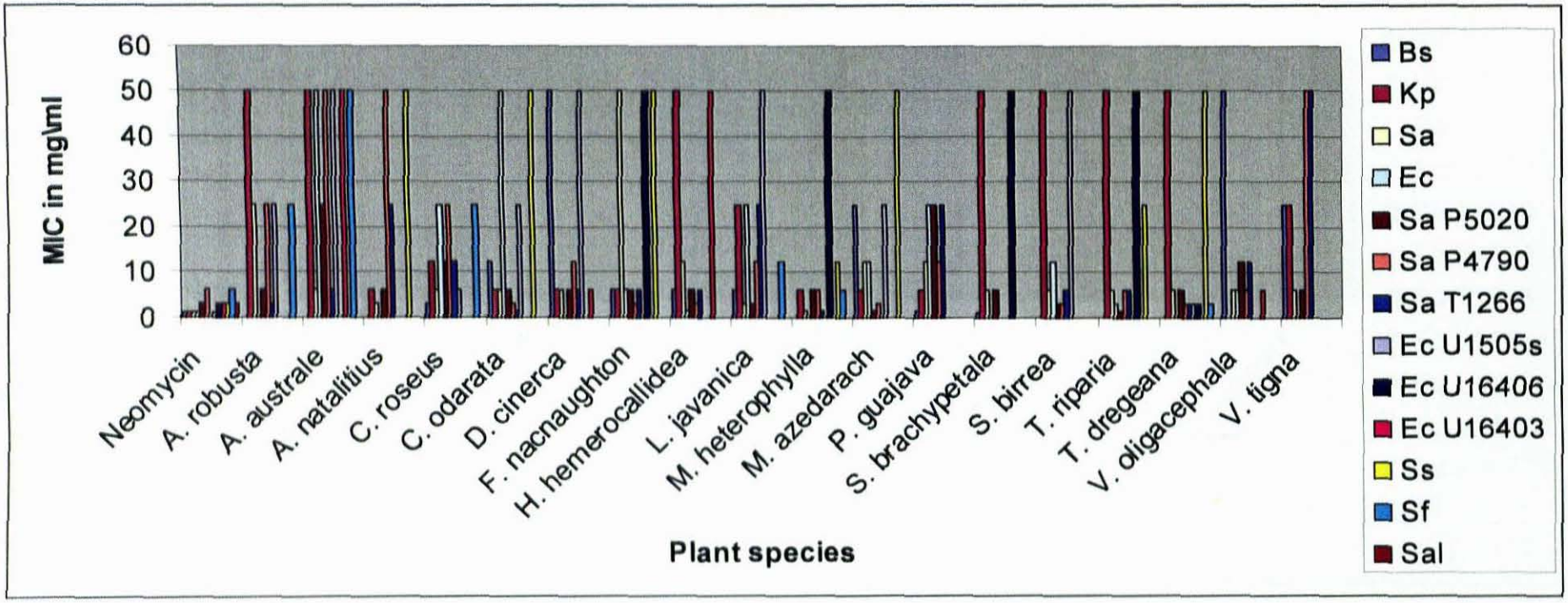
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.33 Minimum Inhibition Concentrations for leaves extracted with cold distilled water (Table representation - appendix D, Table 3).



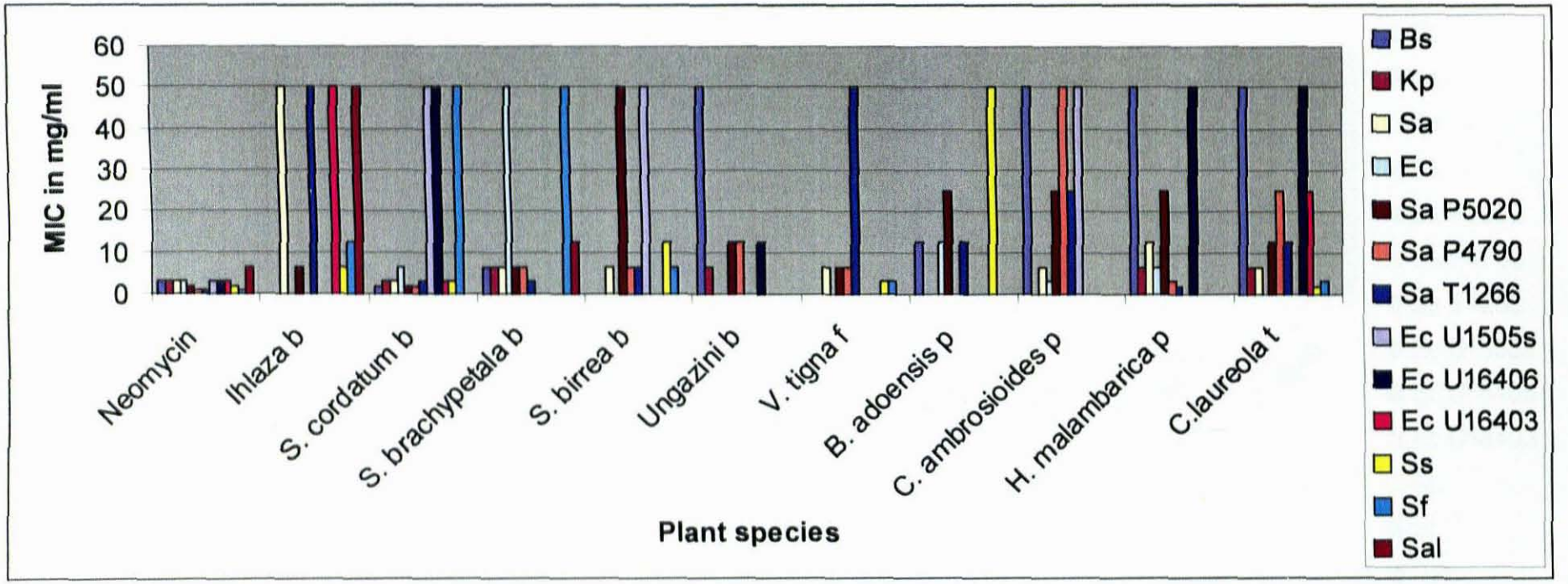
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.34 Minimum Inhibition Concentrations for stems extracted with cold distilled water (Table representation - appendix D, Table 3).



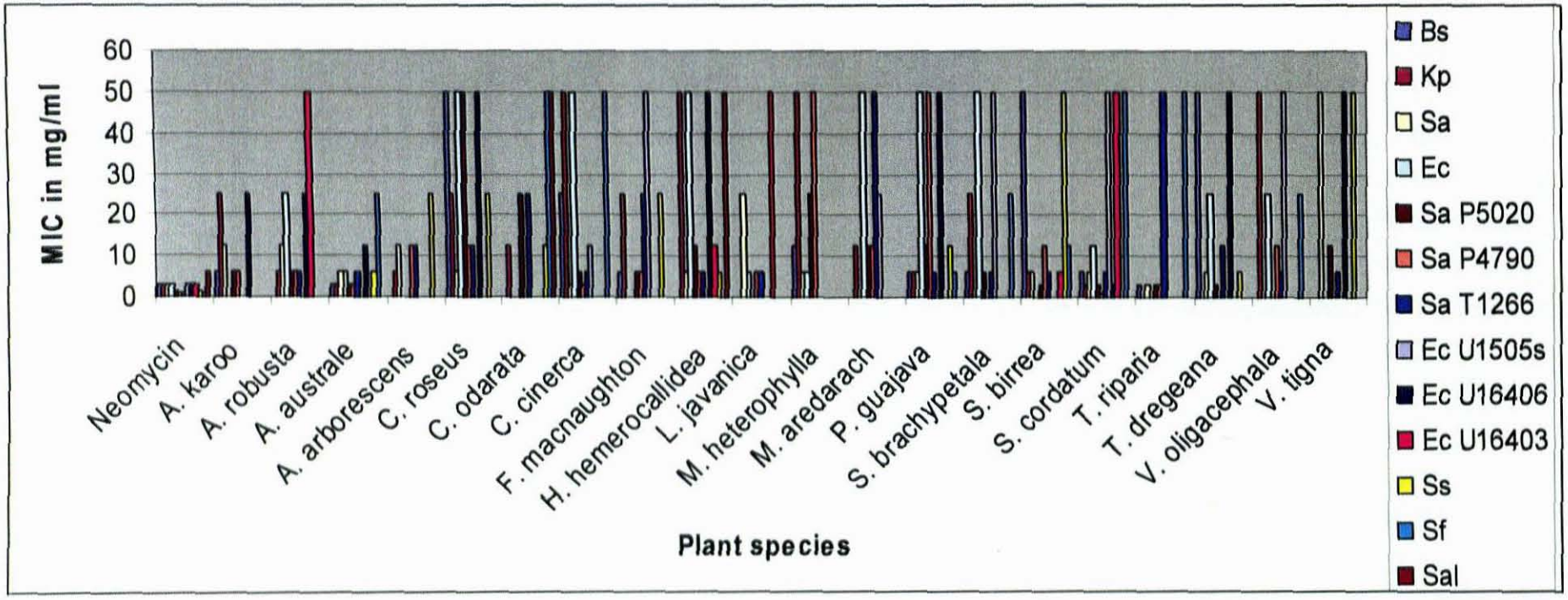
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

Figure 3.35 Minimum Inhibition Concentrations for bark (b), tubers (t) and plants (p) extracted with hot distilled water (Table representation - appendix D, Table 4).



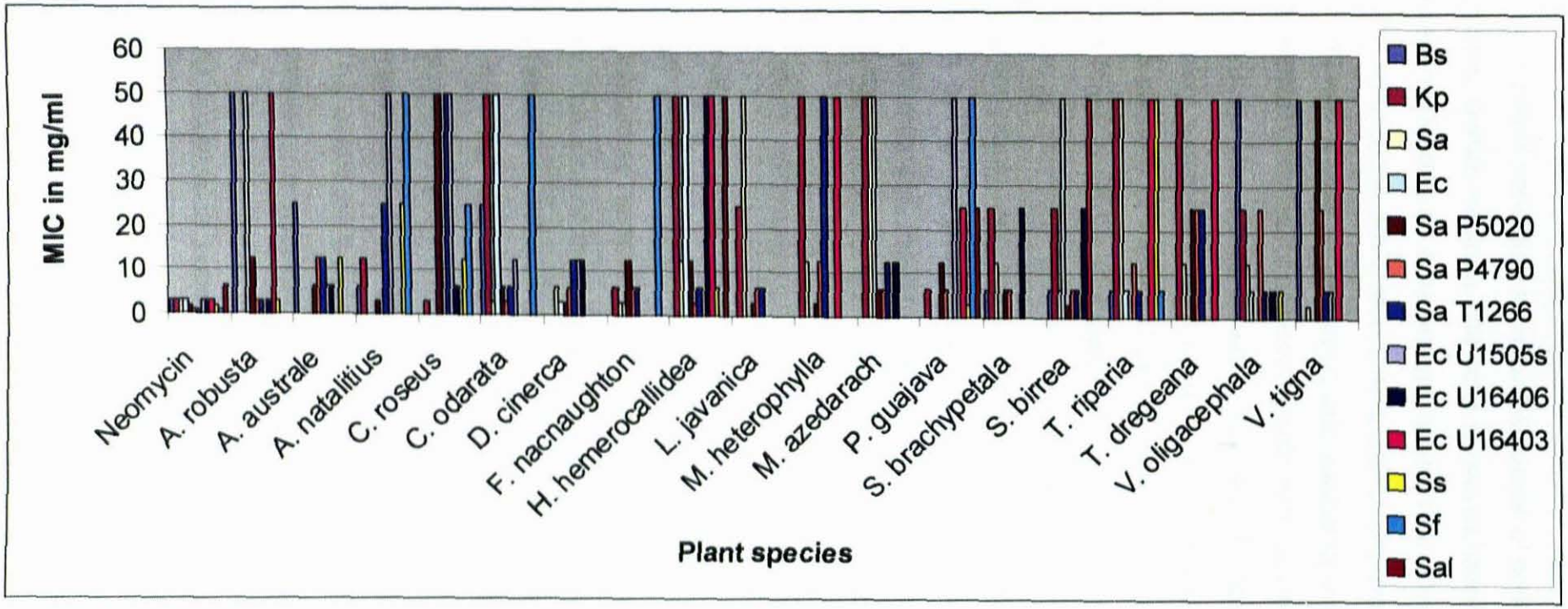
Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii, Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.36 Minimum Inhibition Concentrations for leaves extracted with hot distilled water (Table representation - appendix D, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

Figure 3.37 Minimum Inhibition Concentrations for stems extracted with hot distilled water (Table representation - appendix D, Table 4).



Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec - *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403.

3.8 Discussion

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and rural populations depend on it as primary health care. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (WHO, 2003). The results obtained from the ethnobotanical survey showed that 35 plant species are being used by people at and around Ongoye forest. The high diversity of medicinal plants used in the treatment of diarrhoea in Ongoye forest could be attributed to different interpretations of what may be the cause of diarrhoea and the abundance of plants in a specific locality. Some of the plants used in this study were reported to be used for diarrhoea treatment by Venda people (Ngobeli, 2002) and Zulu speaking traditional healers, for example, *Psidium guajava* having the antibacterial activity when extracted using methanol and when extracted using distilled water (Lin *et al.*, 2002).

The disk-diffusion assay procedure entails inoculating the organism of interest on an agar plate, placing the disk impregnated with the different plant extracts on the inoculated surface and, after incubation, measuring the zone of inhibition. The size of the inhibition zone is influenced by complex factors, such as rate of the diffusion of the plant extract through the agar, the size of the inoculum, the rate of the growth of the bacterium and the bacterium's susceptibility to the plant extracts. For this experiment the amount of the plant extract impregnated was measured out in equal amounts in micro liters in order to be aware of the actual concentration effective against particular bacteria strains.

Successful isolation of botanical compounds from plant material is largely dependant on the type of solvent used in the extracting procedure. The traditional healers use primary water as the solvent. In this study different solvents were used such as methanol, acetone, hot and cold distilled water. The plant materials extracted by cold and hot distilled water were of interest

because these plants provided consistent antibacterial activity comparing to those extracted by acetone and methanol. The choice of solvent depends on what is intended with the extract. If the extraction is to screen plants for antimicrobial components, the effect of the extractant on subsequent separation procedures is not important, but the extractant should not inhibit the bioassay procedures. The growth media seemed to play an important role in the determination of the antibacterial activity. Mueller-Hinton agar was found to be the best media to explicate the antibacterial activity.

In Agar diffusion assays, the results were found to be good, similar to those obtained in the disk diffusion assay. Several of the plants tested in this study have been previously investigated, for instance, *Tetradenia riparia* have been tested for colds and flu (Jager, 2003); *Aleppidea amatymbica* plant have been tested for the treatment of infections (Jager, 2003); *Acacia karoo* for the treatment of pathogenic bacteria and fungi (Pretorius *et al.*, 2003); *Vernonia oligocephala* for the treatment of pathogenic bacteria and fungi (Pretorius *et al.*, 2003) and *Hypoxic hemerocallidea* has been tested against urinary tract infections (Buwa and Van Staden, 2006). *Syzygium cordatum, Schotia brachypetala* (Mathabe *et al.*, 2006) and *Psidium guajava* (Lin *et al.*, 2002) have previously been investigated against diarrhoea. The results of *Syzygium cordatum* and *Schotia brachypetala* antimicrobial activity are correlating to antimicrobial activities previously investigated. Results from literature showed that *Syzygium cordatum* had zones of inhibition ranging from 13 mm to 25 mm (Mathabe *et al.*, 2006) and in the present study zones of inhibition ranging from 14 mm to 20 mm were observed (Figure 3.7.1; Tables 1 - 4 and Figure 3.7.2 ;Table 1 - 4). *Schotia brachypetala* was previously investigated with zones of inhibition ranging from 13 mm to 23 mm (Mathabe *et al.*, 2006), and in the present study zones of inhibition ranging from 10 mm to 21 mm were observed (Figure 3.7.1; Tables1 - 4 and Figure 3.7.2 ; Tables 1 - 4).

The inhibition potential of all the plants tested using either the disk diffusion or the agar diffusion methods ranged from 10 mm to a maximum of 35 mm. *Staphylococcus aureus* was the bacteria that was the most effectively inhibited by almost all the plant extracts, followed by *Klebsiella pneumoniae,*

Escherichia coli, Bacillus subtilis and the least inhibited bacteria strains were the *Shigella sonnei, Shigella flexneri* and *Salmonella typhii* as shown in Tables 1-4; Appendix B & C. Almost all the plant extracts investigated gave positive results; only a few gave negative results. In the disk diffusion assay, plant materials extracted with acetone with an inhibition pontential of more than 25 mm in diameter were *ihlaza* (bark) (Figure 3.14; Appendix C,Table 1) *Aloe arborescens* (Figure 3.15; Appendix C, Table 1); *Psidium guajava* (Figure 3.15; Appendix C, Table 1)., *Vernonia tigna* (leaves) (Figure 3.15; Appendix C, Table 1).

The plants extracted with cold distilled water with an inhibition potential of more than 25 mm in diameter were: *Baccharoides adoensis* (plant) (Figure 3.17; Appendix C, Table 2; *Syzygium cordatum* (Figure 3.18; Appendix C, Table 2) and *Acacia robusta* (leaves) (Figure 3.14; Appendix C, Table 1). In the agar well diffusion assay, materials extracted with acetone that had the highest potential to inhibit bacteria pathogens were: *ihlaza* (Figure 3.2; Appendix B, Table 1); *Syzygium cordatum* (bark) (Figure 3.2; Appendix 3,Table 1); *Aloe arborescens* (Figure 3.3; Appendix B, Table 1); *Psidium guajava* (Figure 3.2; Appendix B,Table 1); *Tetradenia riparia* (Figure 3.3; Appendix B, Table 1) (leaves); *Lippia javanica* (Figure 3.4; Appendix B, Table 1) , *Psidium guajava* (Figure 3.4; Appendix B, Table 1), *Syzygium cordatum* (Figure 3.6; Appendix B, Table 2) and *Vernonia tigna* (leaves) (Figure 3.3; Appendix B, Table 1). The plants extracted with methanol with an inhibition potential of more than 25 mm in diameter were: *Chromolaena odarata* (Figure 3.6; Appendix B, Table 2) and *Syzygium cordatum* (leaves) (Figure 3.6; Appendix B, Table 2). The plants extracted using cold distilled water with an inhibition potential of more than 25 mm in diameter were: *Acacia robusta* (Figure 3.9; Appendix B, Table 3); *Tetradenia riparia* (Figure 3.9; Appendix B, Table 3); *Syzygium cordatum* (leaves) (Figure 3.9; Appendix B, Table 3); *Baccharoides adoensis* (plant) (Figure 3.8; Appendix B, Table 3) and *Acacia robusta* (stem) (Figure 3.9; Appendix B, Table 3). The only plant extracted using hot distilled water, having the inhibition potential of more 25 mm in diameter was *Lippia javanica* (leaves) (Figure 3.10; Appendix 3, Table 3).

From these findings the solvent that was most capable to extract most of the chemical components was acetone, followed by cold water, methanol and lastly hot water. This study contradicts results of other researchers who have reported that water extracts have low activities when compared to organic extracts (Shale *et al.*, 1999, Lall and Meyer, 2000., Matu and Van Staden, 2003). The results obtained from the plants extracted with distilled water were of interest in this study. The plant part with the highest inhibition potential for most of the bacterial pathogens was the leaves, followed by the stems, the bark and then the whole plant. These results contradict those obtained by Kelmanson *et al.*, 2000, who reported that bark should contain the highest antibacterial activity as it is the part of the plant that is in constant contact with soil. The minimum inhibition concentration was 1 mg\ml for plants tested against different bacteria strains such as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Shigella sonnie, Shigella flexneri* and *Salmonella typhii.* Some of the plant parts had no minimum inhibition concentration at highest concentration tested found to be resistant, due to the fact that all the plant parts were tested for minimum inhibition concentration regardless of whether the plant showed any antibacterial activity.

The acetone plant extracts with a MIC of 0.4 mg\ml were *Lippia javanica* and *Maytenus heterophylla* (stems) against *Staphylococcus aureus* (Figure 3.27; Appendix D, Table 1) The methanol plant extracts with a MIC of 1 mg\ml was *Chromolaena odarata* and *Psidium guajava* (leaves) against *Staphylococcus aureus* (Figure 3.28; Appendix D, Table 3). The cold distilled water plant extracts with a MIC of 1 mg\ml were: *Acacia robusta* (leaves) against ATCC strain: *Klebsiella pneumonia*; *Baccharoides adoensis* (plant) against *Bacillus subtilis*; *Schotia brachypetala* (stem) against *Bacillus subtilis* and *Syzygium cordatum* (leaves) against *Staphylococcus aureus* (Figure 3.32 and 3.33; Appendix D, Table 3). The hot distilled water plant extracts with a MIC of 1 mg\ml were *Syzygium cordatum* (leaves) against *Staphylococcus aureus* (Figure 3.35; Appendix D, Table 4). The plant extracts listed above were nearly as effective as neomycin antibiotic against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Bacillus subtilis* bacteria strains.

The reason for the high MIC values could be that the extracts are mixtures of a larger number of compounds, and might suppress the biological activities of each other, or that the active compounds are present in very low concentrations. Plant extracts containing secondary metabolites like saponins, phenolics in a physiologically inactive glycosidic form, may explain why some of the extracts did not produce marked inhibitions. The other reason could be the slow diffusion of larger molecules into the agar, thus blocking detention of the antimicrobial potential of the plant extract.

From the MIC results obtained in the present study, it can be concluded that plants that showed low MIC (1 mg/ml and 0.4 mg/ml), could be a good source of bioactive components with antimicrobial potency. MIC values below 1 mg/ml are considered noteworthy (Van Vuuren, 2008). The plants listed above can be recommended for further investigation for the development of potential drugs against microbial infections. According to Rios *et al.*, 2005, plants extracts that are active at a concentration of 1 mg/ml using the micro-plate dilution method could be considered to have a good antimicrobial potency level. The results obtained in this study appear to confirm the antibacterial potential of the plants investigated, for treatment of diarrhoea that may be as a result of bacterial infections.

CHAPTER FOUR

Phytochemical screening

4.1 Introduction

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. Several phytochemical surveys have been published, including the random sampling approach, which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins). However, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported (Mojab *et al.*, 2003).

Primary metabolites can be considered as those metabolites essential to the life of plants. Most plants synthesize sugar, amino acids and nucleotides. These simple molecules are used to produce polymers essential to the life of the plant. This aspect of the plant biochemistry can be considered as distinct from the production of more complex molecules produced by more diverse pathways. Secondary metabolites are molecules produced by plants, which are important as fine chemicals and biologically active dietary requirements and are useful for the plant for the development of flower pigments, scents or stabilizing elements and are basically used for characterization and control of secondary metabolism. Secondary metabolites are also used as food, flavour, colour dye, poisons, perfumes, scented oils in aromatherapy and industrial products such as rubber and oils. It is estimated that one quarter of prescription drugs contain at least one chemical originally identified and extracted from a plant (Walton and Brown, 1999).

4.2 Phytochemical screening assays

4.2.1 Alkaloids

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

(+) Slight opaqueness

(++) Definite turbidity but no flocculation

4.2.2 Flavonoids

Dried plant material (1 g) was extracted with 5 ml ethanol, evaporated and treated with a few drops of concentrated hydrochloric acid and 0.5 g Magnesium turnings. The presence of the flavonoids was indicated by the development of a pink or magneta-red color within three minutes (Harborne, 1973).

4.2.3 Saponins

Dried Plant material (2, 5 g) was extracted with boiling water (5 ml) and then allowed to cool. The extract was shaken vigorously to froth and then allowed to stand for 15-20 minutes (Brain and Turner, 1975). The saponin content was classified as follows:

(-) No froth

(±) Froth less than 1 cm

(+) Froth 1.2 cm high

(++) Froth higher than 2 cm

4.2.4 Anthraquinones

Dried plant material (1 g) was extracted with 5 ml ethanol, evaporated and the residue re-dissolved in 10 ml of benzene. The extract was shaken and filtered through Whatman's Filter paper. An ammonia solution of 5 ml was added to

the filtrate and the mixture was shaken and allowed to settle. The presence of a pink, red or violet colour in ammonia solution (lower phase) was indicative of the presence of anthraquinones (Odebiyi and Sofowora, 1978).

4.2.5 *Cardiac Glycosides*

Dried plant material (1 g) was extracted with 5 ml ethanol. Two 5 ml alcoholic plant extracts were evaporated to which 2 ml of acetic anhydride, 2 ml of chloroform and 2 ml of glacial acetic acid containing 1 drop 10% iron chloride were added to extracts 1 and 2 respectively. Extract 1 was mixed and cooled in ice and 1 ml of sulphuric acid was added carefully down the sides. A colour change from violet to blue to green was indicative of the presence of cardiac glycosides. Sulphuric acid (1 ml) was added to extract 2. A brown ring at the interface, and/or violet ring below the interface and/or greenish ring above the interface that gradually spreads throughout the layer was indicative of the presence of cardiac glycosides (Sofowora, 1993).

4.2.6 Tannins

Dried plant material (1 g) was extracted with 5 ml ethanol, evaporated and the residue was extracted with 10 ml of a hot 0.9% sodium chloride solution, filtered and divided into 3 equal halves. Sodium chloride solution was added to the first extract, 1% gelatine to the second extract and a gelatine- salt reagent to the third extract. Precipitation with the gelatine- salt solution or gelatine was an indication of the presence of tannins. Positive tests were confirmed with the addition of iron chloride 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).

4.3 Results

Table 4.1 Screening of crude plant extracts for the presence of six secondary metabolites.

Plant names	Chemical Components					
	Alkaloids	Anthra - quinones	Cardiac - glycosides	Flavonoids	Saponins	Tannins
Acacia karoo l	-	+	-	-	-	+
Acacia robusta l	-	-	-	-	-	-
Acacia robusta s	-	-	-	-	-	+
Acanthospermum australe l	+	-	+	+	-	-
Acanthospermum australe s	+	-	+	+	-	+
Acridocarpus natalitius s	-	+	-	+	+	-
Aloe arborescens l	-	-	+	-	+	-
Azima tetracantha b	+	-	-	-	-	-
Baccharoides adoensis p	+	+	-	+	-	-
Catharanthus roseus l	+	-	-	-	-	+
Catharanthus roseus s	+	-	+	-	-	-
Chenopodium ambrosioids p	-	-	+	-	+	-
Chromolaena odarata l	-	-	-	-	+	-
Chromolaena odarata s	-	-	+	-	+	-
Dichrostachys cinerca l	+	+	-	+	+	-
Dichrostachys cinerca s	+	+	-	-	+	-
Faurea macnaughton l	-	+	+	+	-	-
Faurea macnaughton s	-	-	-	+	-	-
Hewittia malambarica p	-	-	-	-	+	-
Hypoxis hemerocallidea l	-	-	+	-	-	+
Hypoxis hemerocallidea s	-	-	-	-	-	-
Lippia javanica l	+	-	-	-	+	-
Lippia javanica s	-	-	-	+	+	-
Maytenus heterophylla l	-	-	-	+	-	-
Maytenus heterophylla s	-	-	-	+	-	+
Melia azedarach l	+	-	-	-	+	+
Melia azedarach s	+	-	+	-	+	-
Psidium guajava l	-	-	+	-	+	+
Psidium guajava s	-	+	+	+	+	+
Schotia brachypetala b	-	-	-	-	+	+
Schotia brachypetala l	-	+	+	-	+	+
Schotia brachypetala s	-	-	-	+	+	+
Sclerocarya birrea b	+	+	+	+	-	+
Syzygium cordatum	-	-	-	+	-	+
Syzygium cordatum l	-	+	-	+	-	+
Tetradenia riparia l	+	+	-	+	-	-
Tetradenia riparia s	+	-	-	-	-	-

Table 4.1 (continued)

Plant names	Chemical Components					
	Alkaloids	Anthra - quinones	Cardiac - glycosides	Flavonoids	Saponins	Tannins
Trichilia dregeana l	-	-	-	-	-	-
Trichilia dregeana s	-	-	-	-	-	-
Ungazini b	+	+	-	-	-	-
Vernonia oligocephala l	-	-	+	-	-	+
Vernonia oligocephala s	-	-	-	-	-	+
Vernonia tigna f	-	-	+	+	+	+
Vernonia tigna l	-	+	+	+	+	-
Vernonia tigna s	-	+	-	+	+	-

+ = Positive; - = Negative; b - bark, l - leaves, s - stem and p - plant; Ungazini - not identified.

4.4 Discussion

In the present study, we try to establish the rational usage of medicinal plants by investigating plants for pharmacological activities, which healers and ordinary people claim it to have. It is possible to isolate chemical compounds from the plants which are pharmacologically active and give scientific credibility to what healers have known for centuries. The activity of some of the plant extracts on different micro-organisms explains their broad spectrum nature. Most of the plant extracts have an effect on only a few micro-organisms and this may be due to their narrow spectrum of activity. This difference of activity appears to be directly related to the qualitative or quantitative diversity of the compounds that are accumulated by the plants investigated.

Alkaloids have been detected in 10 of the 33 plant species namely: *Azima tetracantha, Acanthospermum australe, Baccharoides adoensis, Catharanthus roseus, Dichrostachys cinerca, Lippia javanica, Melia azedarach, Sclerocarya birrea, Tetradenia riparia* and *Ungazini* (Table 4.1). Anthraquinones have been detected in 12 of the 33 plant species namely: *Acacia karoo, Acridocarpus natalitius, Baccharoides adoensis, Dichrostachys cinerca, Faurea macnaughton, Psidium guajava, Sclerocarya birrea, Schotia brachypetala, Syzygium cordatum, Tetradenia riparia, Ungazini and Vernonia*

tigna (Table 4.1). Cardiac-glycosides have been detected in 13 of the 33 plant species namely: *Acanthospermum australe, Aloe arborescens, Catharanthus roseus, Chenopodium ambrosioids, Chromolaena odarata, Faurea macnaughton, Hypoxis hemerocallidea, Melia azedarach, Psidium guajava, Schotia brachypetala, Sclerocarya birrea, Vernonia oligocephala and Vernonia tigna* (Table 4.1). Flavonoids have been detected in 13 of the 33 plant species namely: *Acanthospermum australe, Acridocarpus natalitius, Baccharoides adoensis, Dichrostachys cinerca, Faurea macnaughton, Lippia javanica, Maytenus heterophylla, Psidium guajava, Sclerocarya birrea, Syzygium cordatum, Schotia brachypetala, Tetradenia riparia* and *Vernonia tigna* (Table 4.1). Saponins have been detected in 11 of the 33 plant species namely: *Acridocarpus natalitius, Aloe arborescens, Chenopodium ambrosioids, Chromolaena odarata, Dichrostachys cinerca, Hewittia malambarica, Lippia javanica, Melia azedarach, Psidium guajava and Vernonia tigna* (Table 4.1). Tannins have been detected in 13 of the 33 plant species namely: *Acacia karoo, Acacia robusta, Acanthospermum australe, Catharanthus roseus, Hypoxis hemerocallidea, Maytenus heterophylla, Melia azedarach, Psidium guajava, Schotia brachypetala, Sclerocarya birrea, Syzygium cordatum, Vernonia oligocephala* and *Vernonia tigna* (Table 4.1). From these findings, the dominant chemical components detected in the different plant material were saponins (20) flavonoids (19), tannins (18) and cardiac-glycosides (16) and the least detected components were alkaloids (15) and anthrax-quinones (14) (Table 4.1).

Antidiarrhoeal activity properties of medicinal plants could possibly be due to the presence of tannins, alkaloids, saponins, anthraquinones and flavonoids. These constituents may be responsible for the antidiarrhoeal activity as some of the findings corroborate with that of Havagiray *et al.*, 2004. For example, *Acacia karroo* was reported to contain tannins (Van Wyk and Van Wyk, 1997, Saphwan *et al.*, 2004), as this study also found (Table 4.1). None of the six chemical components have been detected in *Acacia robusta*. This finding corresponds with that of Pooley (2003), who reported the presence of only pheylanine and trypatamine in the plant. *Aloe arborescens* was reported to contain aloin, aloenin, as anthranoid and phenolic compounds (Gutterman

and Chauservolfson, 2000, Beppu *et al.*, 2006), whereas in the present study the plant tested positive for cardiac–glycosides and saponins (Table 4.1). Alkaloids were detected in *Azima tetracantha* as previously isolated by Hutchings and co-workers (1996). *Baccharoides adoensis* was found to contain alkaloids, anthra-quinones and flavonoids (Table 4.1) and these findings have not been previously reported. *Cantharanthus roseus* contains alkaloids and tannins (Table 4.1), these results correspond with the research of Wu and co-workers (Wu *et al.*, 2004).

Saponins and cardiac-glycosides have been detected in *Chenopodium ambrosioides* (Table 4.1) and this finding compares well with that of Ketzis and co-workers (2002) who report also the presence of ferulic acid, geraniol limonene and malic acid. *Chromolaena odarata* have been reported to be very poisonous (Browmilow, 2001). In this study the plant tested positive for cardiac-glycosides and saponins (Table 4.1). The presence of saponins, alkaloids and tannins have been indicated in the roots and leaves of *Dichrostachys cinerea* (Hutchings and Van Staden, 1994, Eisa *et al.*, 2000., Smith *et al.*, 2005., Mlambo *et al.*, 2008) and corresponds with results in Table 4.1. Literature showed that *Hypoxic hemerocallidea* contains tannins and cardiac–glycosides (Hutchings *et al.*, 1996, Steenkamp *et al.*, 2006., Nair *et al.*, 2007). None were detected during this study and it should be repeated again. It could be that the quantities in the extract were not enough to be observed during the test. Flavonoids were detected from *Lippia javanica* as previously investigated (Van Wyk and Wink, 2004). In this study *Maytenus heterophylla* (*Gymonosporia buxifolia*) tested positive for flavonoids and tannins and this corresponds to research done by Orabi (2001).

It has been reported that *Melia azedarach* contains the following chemical components: 24-methylenecyclartanone; cyclo-eucalenone; 4-stigmastene-3-one-2, 4-camestene-3-one; ß-sitosterol; ß-isosterol-D-glucoside, vanillic glucose; vanillic aldehyde; transcomanainic acid and vanillic acid (Coria *et al.*, 2008). *Melia azedarach* tested positive for saponins, tannins and alkaloids (Table 4.1). *Psidium guajava* was reported to contain tannins (Van Wyk and Wink, 2004, Gutieriez *et al.*, 2008) as the results showed in Table 4.1.

Sclerocarya birrea is commonly tanniferous and contains flavonoids (Hutchings *et al.*, 1996., Roodt, 1998., Ndhlala *et al.*, 2007) as were detected during the phytochemical screening. *Schotia brachypetala* contains alkaloids and tannins, which corresponds with the findings of McGaw *et al.* (2002) as well as Stafford and co-workers (2007). Flavonoids were detected in *Strychnos henningsii* as reported by Massiote *et al.* (1991), Tits (1991) and Hutchings (1996). *Syzygium cordatum* was reported to contain tannins (Hutchings *et al.*, 1996) as Table 4.1 also indicated. Alkaloids, triterpenoids friedelin and essential oils have been isolated from *Tetradenia riparia* by Hutchings and co-wokers (1996) and Omlo and co-wokers (2004). In the present study only alkaloids have been detected. All parts of the plant *Vernonia tigna* contain saponins, especially the roots (Watt and Brayer-Brandwijk, 1962, Pooley, 2003) the phytochemical screening corresponds with results in Table 4.1.

The antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydro electrolytic secretions which are known to be altered in diarrheic conditions (Venkatesan *et al.*, 2005). Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which assist resistant chemical alteration and reduce secretion (Havagiray *et al.*, 2004). Plants containing cardiac glycosides have previously been reported as used for their therapeutic effects (Kaplan, 2005) and could be the source of toxicity (Demiryurek and Demiryurek, 2005).

CHAPTER FIVE

Bio-autographic assay

5.1 Introduction

A thin layer chromatographic (TLC) bio-autographic method was developed for detection of antibiotic resistance agents. Bio-autographic assays identified antimicrobial substances in the extract. The method outlines the chemical profile of extract constituents and phases related to the antimicrobial activity against target micro-organisms. TLC is a simple, quick and inexpensive procedure that gives the researcher a quick answer as to how many chemical components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f (retention factor) of a compound is compared with the R_f of a known compound (Sherma and Fried, 1996).

A TLC plate is a sheet of glass, metal or plastic, which is coated with a thin layer of a solid adsorbent (usually silica or alumium). A small amount of a mixture to be analyzed is spotted near the bottom of the plate. The TLC plate is placed in the shallow pool of a solvent. This liquid or eluent is the mobile phase and slowly rises up the TLC plate by capillary action. As the solvent moves past the spot that was applied, equilibrium is established for the component of the mixture between the molecules of the component which are adsorbed on the solid and the molecules which are in solution. In principle, the component will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried further up the plate than others. This briefly outlines the different chemical components that are contained in the plant extract and that different chemical components have different retention factor values. The solvent used in extraction was dichloromethane/methane which is a polar and a non polar solvent. This solvent was used in order to be able to extract both polar and non polar components from different plant extracts.

5.2 Bio-autographic technique

Thin layer bio-autographic assays were carried out by placing 5 µl of the acetone plant extract (100 mg/ml) on silica gel thin layer chromatography (TLC) plates (Alugram r Sil G/UV_{254}, 0,2 mm). The plates were eluted with toluene 72%, dioxane 20% and acetic acid 8% (Merck). Two TLC plates were prepared under identical conditions, one to be incubated with the test organism *S. aureus* and Mueller-Hinton agar, and a reference plate without test organism and agar, kept at ambient temperature. A base layer (15 ml) of Tryptone Soya was poured into a sterile Petri dish. Thereafter the TLC plate was placed onto the agar surface with the silica side facing upwards. An overlay of Mueller-Hinton agar (15 ml) containing a bacterial culture *S. aureus* (approximate inoculum's size, 1×10^6 CFU/ml) was poured on top of the TLC plate. The plates were allowed to prediffuse at 4 °C for 1 hour prior to incubating at 37 °C for 24 hours. The resulting inhibition zones were visualized around active compounds by spraying with 0.04% (w/v) INT and compared to the reference TLC plate (Van Vuuren, 2007).

5.3 Results

The plant extracts used in this assay were selected based on their effectiveness to inhibit the growth of bacteria species causing diarrhoea, with minimum inhibition concentration values ranging from 1 mg/ml to 0.4 mg/ml. Plant extracts were spotted in duplication on one plate for verification of the results obtained. On the development and viewing of the TLC plate, the starting point and the solvent front (the level the solvent reached when the plate was removed from the developing chamber) were marked and all spots observed on the plate were circled in pencil. The locations of each spot on the plates were then represented numerically by calculating a Retention factor (R_f). This was accomplished by making the following measurements and calculations (Figure 5.1).

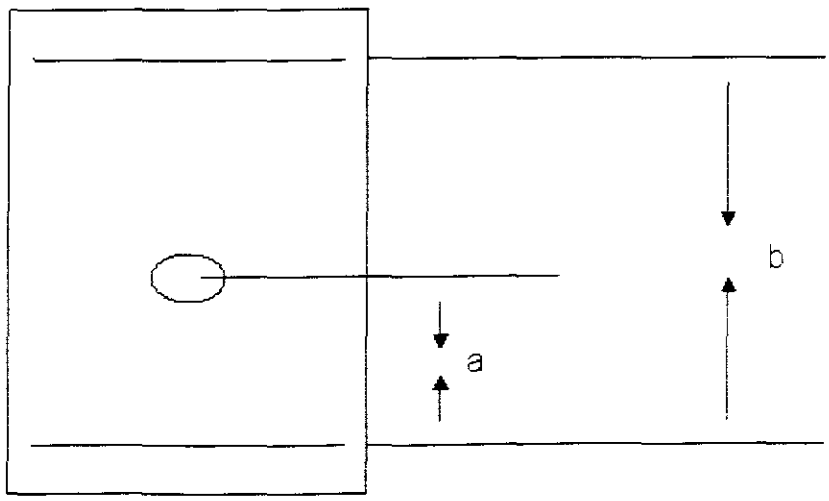


Figure 5.1 TLC plate showing how to calculate the R_f values.

Measured distance from the starting point to the center of the spot on the TLC plate (distance a). Measured distance from the starting point to the solvent front (distance b)

Calculation of Retention factor as $R_f = \frac{a}{b}$

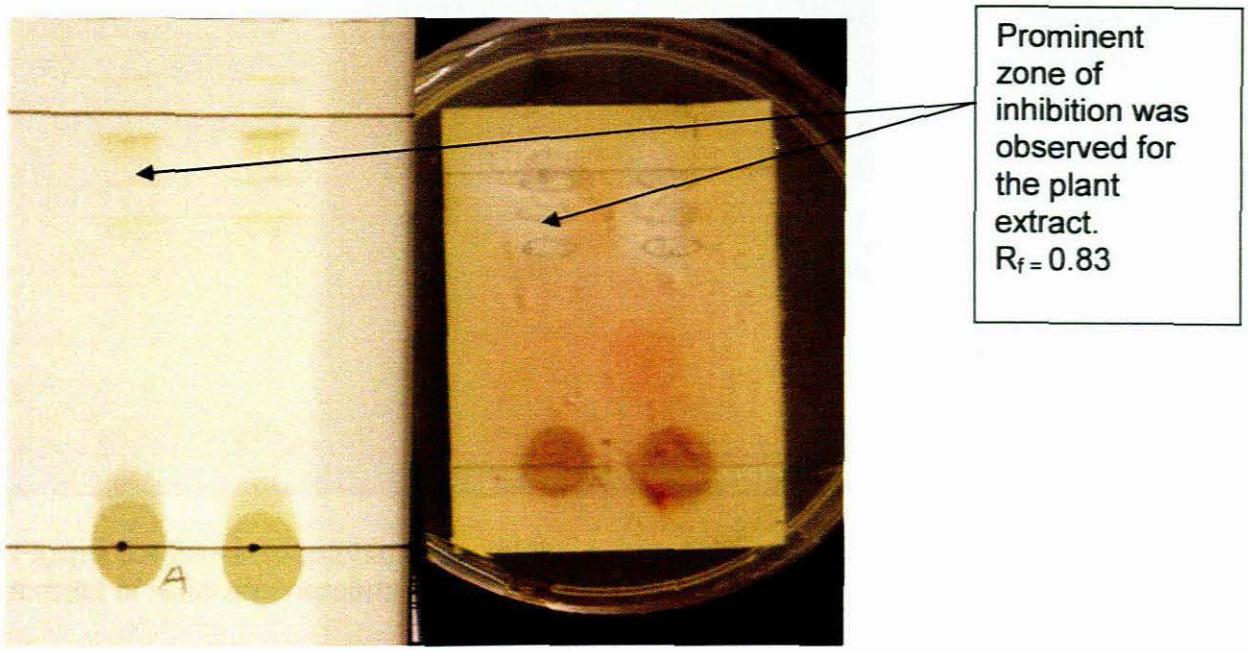


Figure 5.2 Bio-autographic assay of the crude dichloromethane\methanol extract of *Syzygium cordatum* against *S. aureus*.

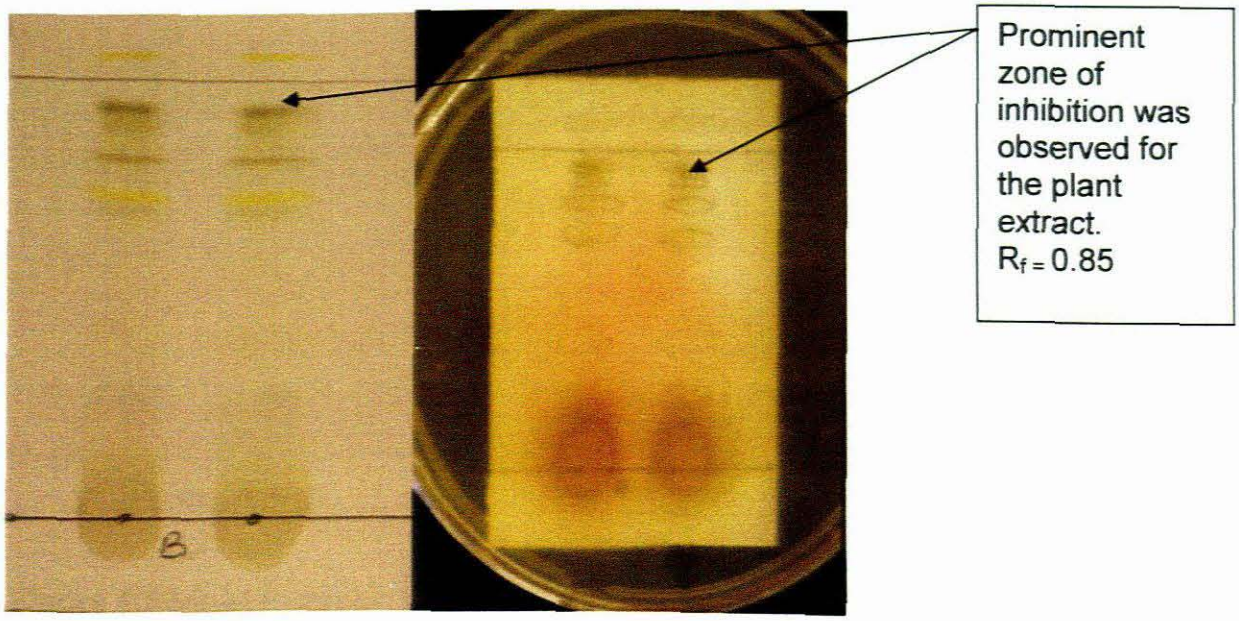


Figure 5.3 Bio-autographic assay of the crude dichloromethane\methanol extract of *Acacia robusta* against *S.aureus*.

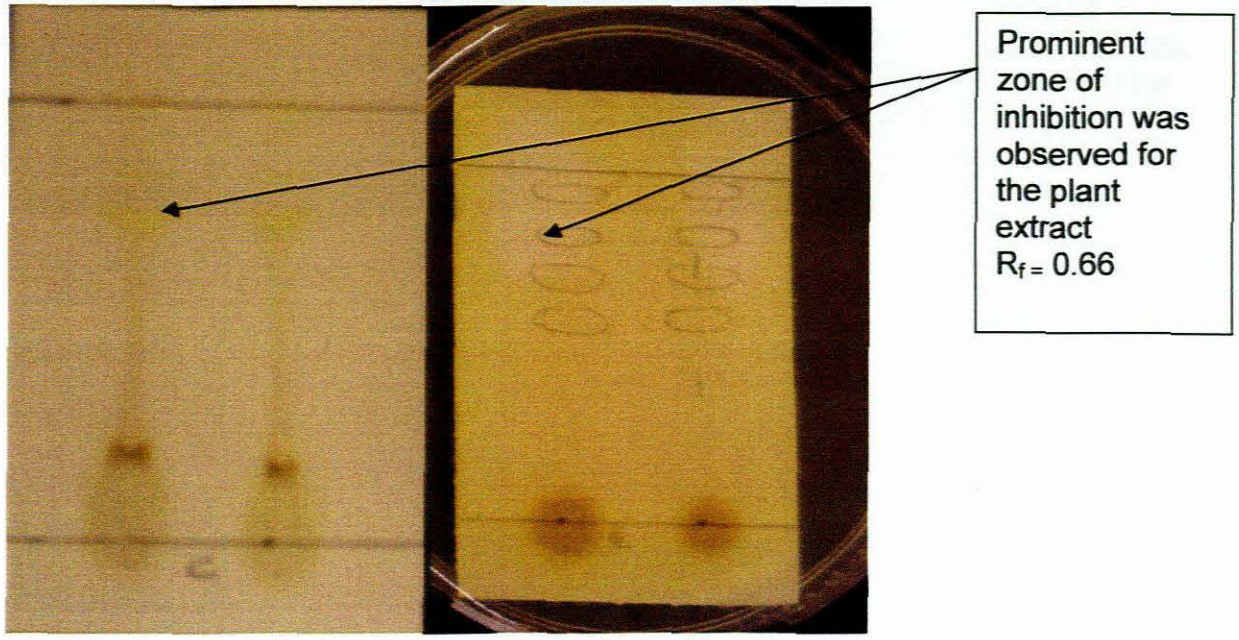


Figure 5.4 Bio-autographic assay of the crude dichloromethane\methanol extract of *Aloe arborescens* against *S.aureus*.

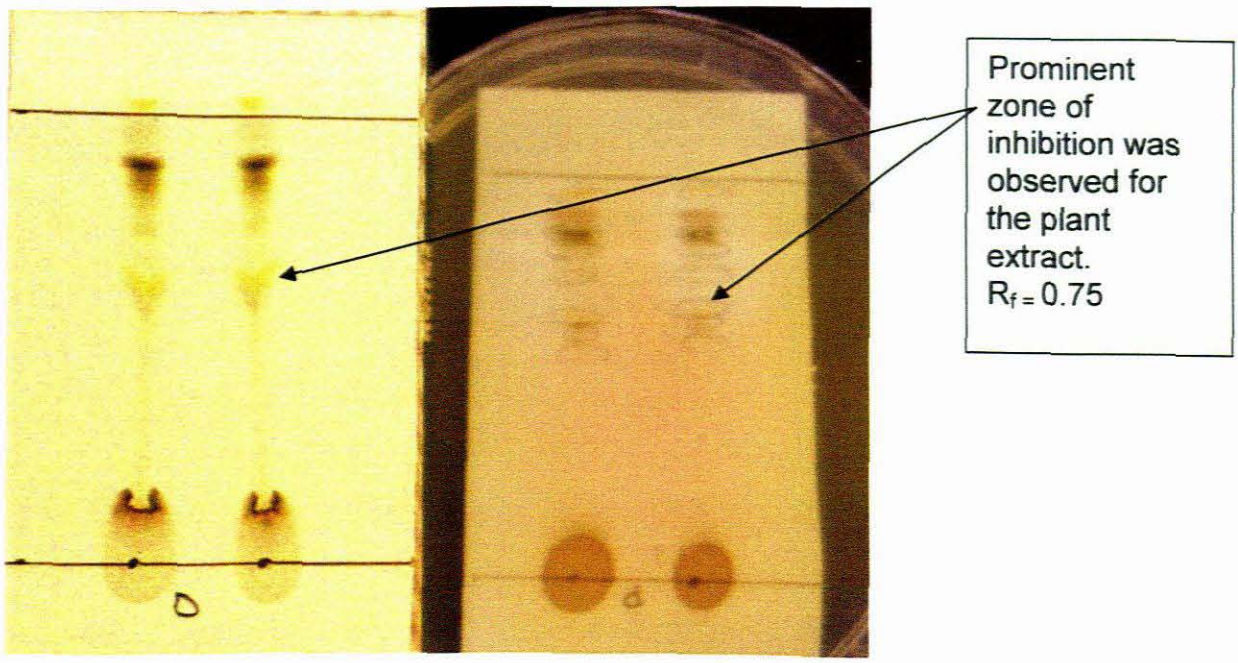


Figure 5.5 Bio-autographic assay of the crude dichloromethane\methanol extract of *Psidium guajava* against *S.aureus*.

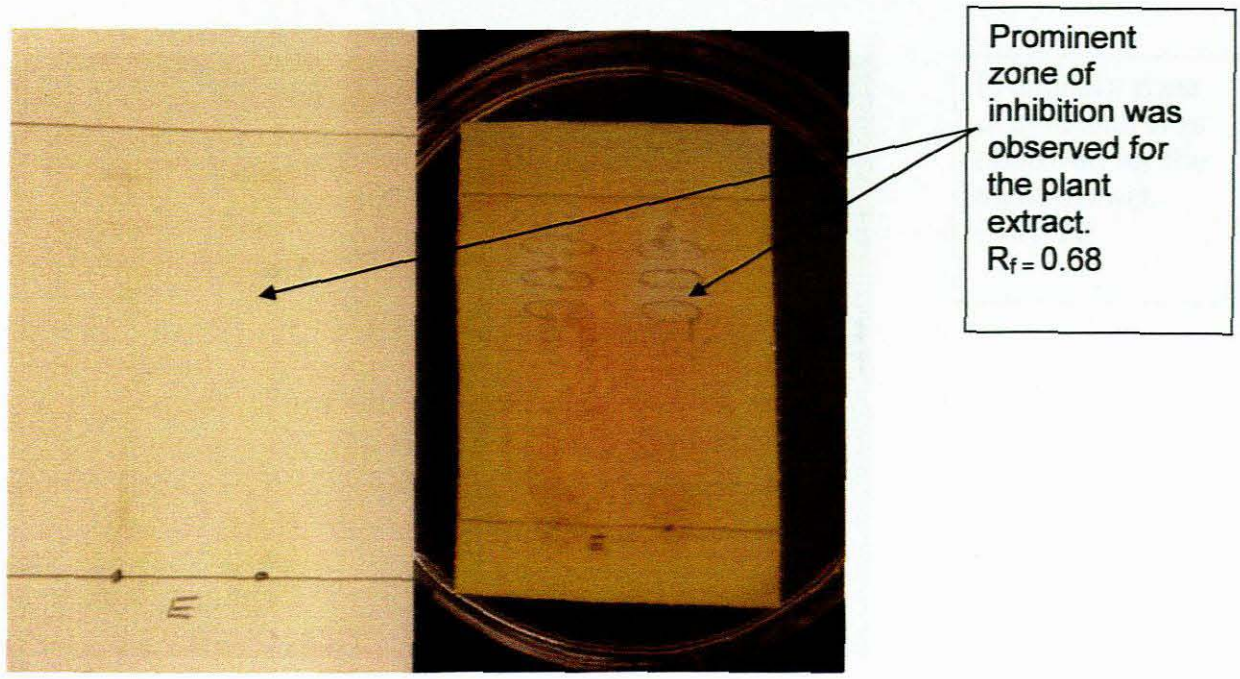


Figure 5.6 Bio-autographic assay of the crude dichloromethane\methanol extract of *Vernonia tigna against S.aureus*.

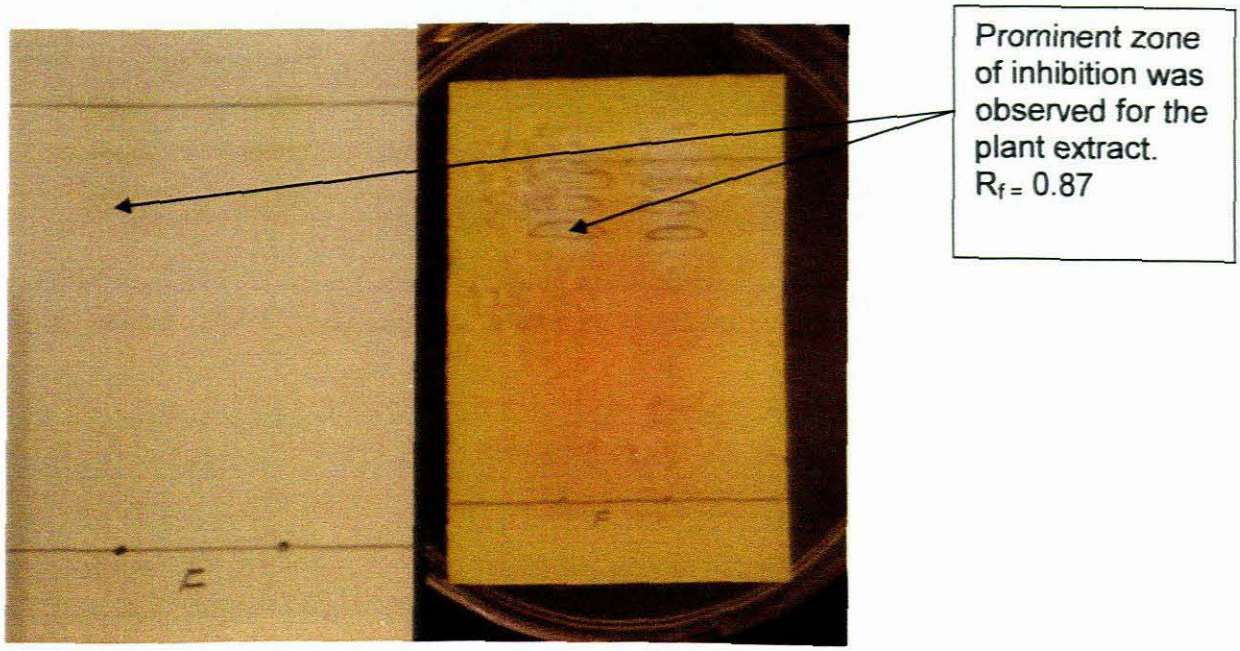


Figure 5.7 Bio-autographic assay of the crude dichloromethane\methanol extract of *Tetradenia riparia against S.aureus*.

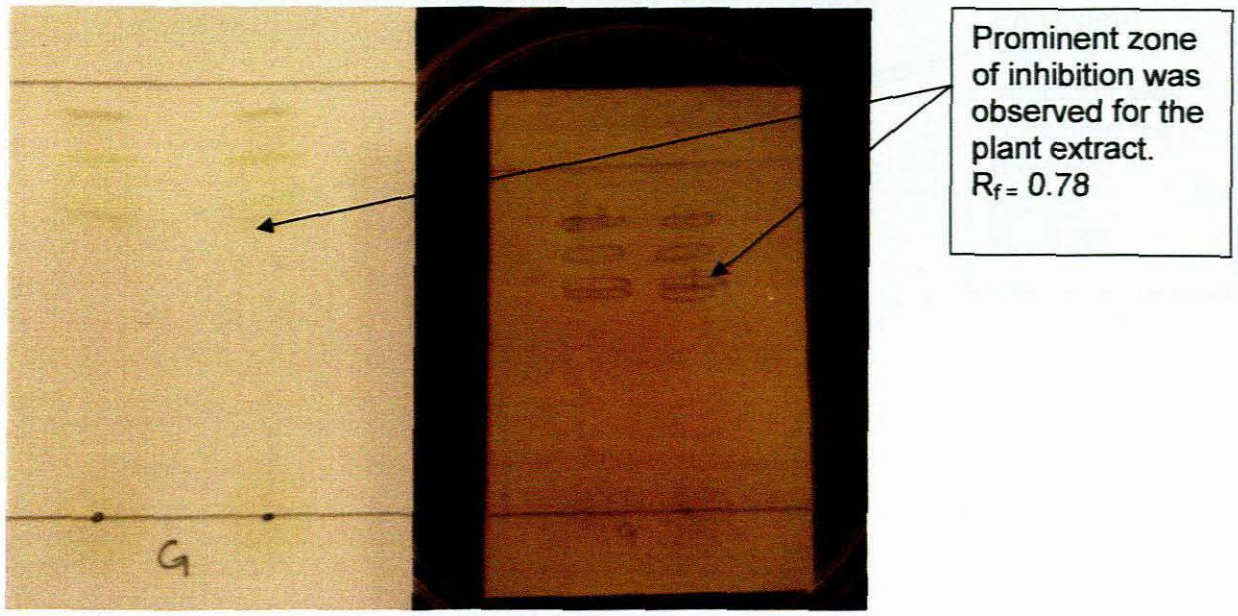


Figure 5.8 Bio-autographic assay of the crude dichloromethane\methanol extract of *Lippia javanica against S.aureus*.

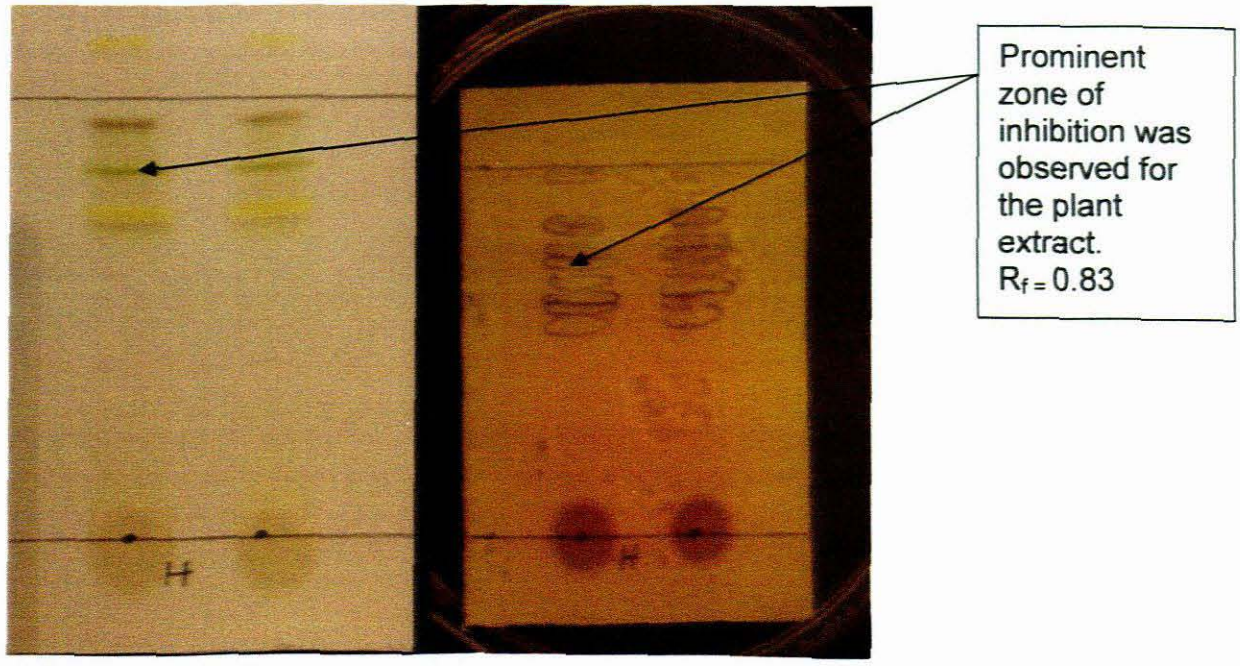


Figure 5.9 Bio-autographic assay of the crude dichloromethane\methanol extract of *Schotia brachypetala against S.aureus*.

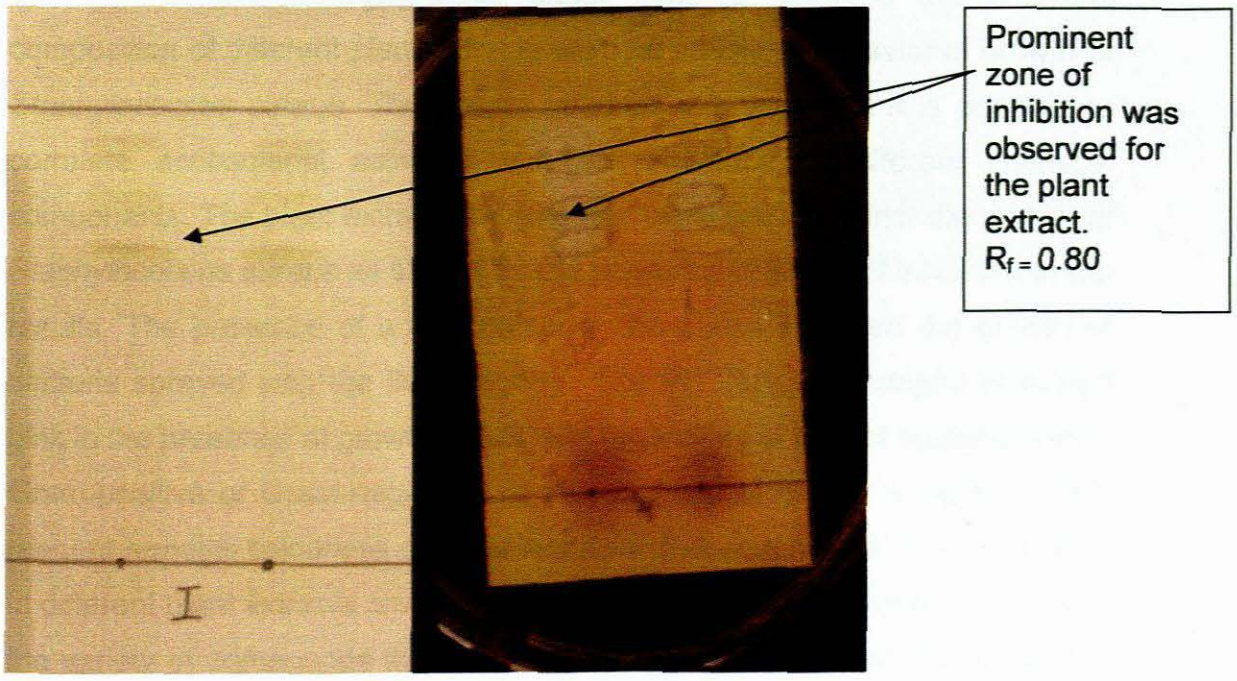


Figure 5.10 Bio-autographic assay of the crude dichloromethane\methanol extract of *Chromolaena odarata against S. aureus*.

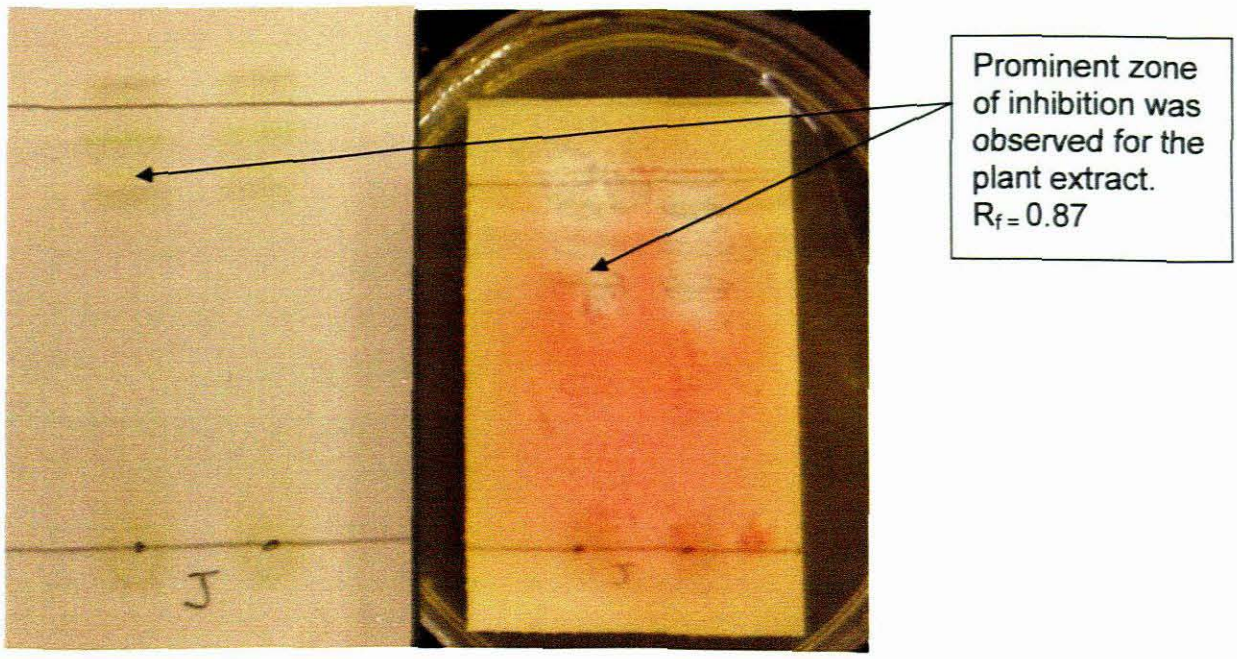


Figure 5.11 Bio-autographic assay of the crude dichloromethane\methanol extract of *Baccharoides adoensis* against *S.aureus*.

5.4 Discussion

The aim of the bio-autographic assay was to correlate the chemical composition of different plant extracts with the inhibitory behavior of growth of *Staphylococcus aureus*. The results obtained indicated that it is possible to correlate antibacterial activities with the presence of different chemical components. The plant extracts investigated were active against the growth of *Staphylococcus aureus* as shown by the presence of zones of inhibition in the results. The presence of a pink colour in the plates indicated the growth of bacteria sprayed with the INT reagent. The INT reagent changed or turned pink in the presence of growth of bacteria regardless of type of bacteria (either Gram-positive or Gram-negative). In the absence of growth of bacteria, INT reagent remains colourless or clear in colour. Different chemical compositions in different plant extracts are responsible for antibacterial activities. The larger the variety of compounds that are extracted by the extractant, the better the biologically active component inhibits the growth of bacteria. The crude extracts of *Psidium guajava* (Figure 5.5), *Chromolaena odarata* (Figure 5.10) and *Aloe arborescens* (Figure 5.5) consist of many chemical compounds, perhaps all compounds contribute.

CHAPTER SIX

Conclusions

The purpose of the ethnomedical survey and documentation were to catalogue the plants traditionally used against diarrhoea. The results of this study showed that 35 plants are traditionally used in the treatment of diarrhoea among the community around Ongoye forest namely: *Acacia karoo; Acacia robusta; Acanthospermum australe; Acridocarpus natalitius; Alepidea amatymbica; Aloe arborescens; Baccharoides adoensis; Callilepis laureola; Catharanthus roseus; Chenopodium ambrosioides; Chromolaena ambrosioides; Clerodendrum glabrum; Cyphostemma cirrhosum; Dichrostachys cinerca; Faurea macnaughton; Ficus sur; Helichrysum odoratissimum; Hewittia malambarica; Hypoxis hemerocallidea; Lippia javanica; Maytenus heterophylla; Melia azedarach; Physalis viscose; Portulacaria afra; Prunus persica; Schotia brachypetala; Schkuhria pinnata; Sclerocarya birrea; Senecio quinquelobus; Strychnos henningsii; Syzygium cordatum; Tetradenia riparia; Trichilia dregeana; Vernonia oligocephala and Vernonia tigna.*

The following plants were documented for the first time during this study to treat diarrhoea: *Acacia robusta, Acanthospermum australe. Acridocarpus natalius, Azima tetracantha, Cantharanthus roseus, Chromolaena odarata, Clerodendrum glabrum, Faurea macnaughton, Helichrysum odoratissimum, Hypoxis hemerocallidea, Lippia javanica, Physalis viscose, Portulacaria afra, Prunus persica, Schkuhria pinnata, Senecio quinquelobus* and *Tetradenia riparia.* This study revealed that women (61.4%) living in the homestead possessed more knowledge than the men (38.6%) who were interviewed at the homesteads regarding the medicinal uses of plants.

Research done on the antibacterial activities of South African medicinal plant species have typically been extracted with solvents of varying polarity and subsequently been subjected to antibacterial testing with little regard to how traditional healers prepare and use extracts (Lin *et al.*, 1999, Eloff, 1999.,

Pillay *et al.*, 2001). In this study remedies to treat diarrhoea were prepared according to traditional practice except those that were extracted using methanol and acetone and tested for antibacterial activity. The greatest advantages of acetone are the volatility, miscibility with polar and non-polar solvents and its relatively low toxicity to the test organism. Due to the ease of handling extract fractions at subsequent stages, acetone is probable preferable to methanol and more hydrophilic compounds were tested because acetone extracted highly polar components.

All the plants reported by people to have the potential to treat diarrhoea tested positive for antibacterial activity (those sourced at homesteads, as well as those bought at the muthi market). In this study, *Staphylococcus aureus* was mostly inhibited by all the different plant extracts. The micro-organisms that were the least inhibited were *Shigella sonnie*, *Shigella flexnerie* and *Salmonella typhii*. The results of the disk-diffusion were confirmed by the agar-well diffusion assay. The measurements of the zone of inhibition for both the methods ranged from 10 mm to about 30 mm in diameter. The lowest MIC value of the plant extracts was 1 mg/ml and 0.4 mg/ml. From the results that were obtained in this study it is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins and alkaloids are generally better in their antimicrobial activities and this corroborates with what has been investigated by Cowan (Cowan, 1999). This suggests that the strength of antimicrobial activity of a plant depends on the diversity and quantity of chemical component it contains.

The results lend some support to traditional knowledge and can serve as a basis for selecting the most active medicinal plants to use in traditional medicine practices in the future. These plants include: *Acacia robusta, Aloe arborescens, Baccharoides adoensis, Chromolaena odarata, Ihlaza, Lippia javanica, Psidium guajava, Schotia brachypetala, Syzygium cordatum, Tetradenia riparia* and *Vernonia tigna*. The inhibitory effects of most of the plant extracts from the above results can be considered as good antidiarrhoeal agents. Further work needs to be done in order to isolate and identify antimicrobial compounds from the plant extracts. This finding lends

some support to traditional knowledge and can 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 A

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:

Male	
Female	

Age:

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

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? _____

_____

_____

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

_____

_____

c) Are there any known side effects? _____

_____

_____

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

_____

_____

Age Group:

Infants	
Children	
Adults	

APPENDIX B

Agar-well diffusion assay

Table 1. Results of sensitivity of different bacteria against acetone plant extracts.

Sensitivity of different bacteria against acetone plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf Bs	Sal Kp
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	15 mm	-	27 mm	-	20 mm	15 mm	15 mm	-	-	-	-	-	-
Syzygium cordatum	11 mm	16 mm	11 mm	22 mm	26 mm	21 mm	13 mm	17 mm	10 mm	14 mm	-	21 mm	12 mm
Schotia brachypetala	17 mm	10 mm	24 mm	-	22 mm	18 mm	12 mm	-	14 mm	-	-	-	-
Sclerocarya birrea	-	15 mm	16 mm	-	16 mm	14 mm	16 mm	10 mm	16 mm	-	-	20 mm	-
Ungazini	15 mm	13 mm	18 mm	-	16 mm	14 mm	17 mm	-	10 mm	-	-	-	-
Flower													
Vernonia tigna	-	-	20 mm	-	20 mm	11 mm	15 mm	-	-	-	10 mm	-	-
Leaves													
Acacia karroo	11 mm	13 mm	16 mm	-	18 mm	19 mm	22 mm	-	13 mm	-	-	-	-
Acacia robusta	-	10 mm	10 mm	-	18 mm	10 mm	11 mm	-	-	-	-	-	-
Acanthospermum australe	-	12 mm	21 mm	-	16 mm	12 mm	14 mm	11 mm	-	-	-	-	-
Aloe arborescens	-	-	22 mm	-	25 mm	20 mm	16 mm	10 mm	11 mm	10 mm	9 mm	-	-
Catharanthus roseus	-	18 mm	12 mm	-	10 mm	10 mm	12 mm	11 mm	11 mm	-	-	-	-
Chromolaena odarata	15 mm	-	21 mm	-	20 mm	18 mm	16 mm	-	11 mm	-	-	-	-
Dichrostachys cinerea	14 mm	-	17 mm	-	22 mm	17 mm	18 mm	-	16 mm	-	-	22 mm	-
Faurea macnaughton	-	-	-	-	12 mm	16 mm	10 mm	-	-	11 mm	-	-	-
Hypoxis hemerocallidea	-	-	10 mm	-	-	16 mm	12 mm	10 mm	-	-	10 mm	-	-
Lippia javanica	-	11 mm	20 mm	-	15 mm	14 mm	18 mm	10 mm	-	-	-	16 mm	-
Maytenus heterophylla	17 mm	-	20 mm	-	19 mm	10 mm	14 mm	10 mm	-	-	11 mm	-	-
Melia azedarach	10 mm	15 mm	14 mm	-	10 mm	11 mm	14 mm	10 mm	-	-	-	-	-
Psidium guajava	-	-	27 mm	-	14 mm	12 mm	10 mm	11 mm	-	-	-	15 mm	-
Schotia brachypetala	20 mm	19 mm	20 mm	-	21 mm	12 mm	10 mm	14 mm	13 mm	10 mm	-	13 mm	-
Sclerocarya birrea	-	-	20 mm	-	18 mm	14 mm	18 mm	10 mm	-	-	-	-	-
Syzygium cordatum	15 mm	-	20 mm	12 mm	15 mm	18 mm	15 mm	18 mm	17 mm	15 mm	15 mm	20 mm	14 mm
Tetradenia riparia	18 mm	-	25 mm	-	14 mm	19 mm	12 mm	-	-	15 mm	10 mm	-	-
Trichilia dregeana	11 mm	17 mm	12 mm	-	16 mm	16 mm	15 mm	-	10 mm	-	-	-	-
Vernonia oligocephala	14 mm	-	16 mm	16 mm	15 mm	18 mm	14 mm	-	-	12 mm	-	-	-
Vernonia tigna	-	-	21 mm	-	24 mm	11 mm	21 mm	-	-	-	10 mm	-	-
Plants													
Baccharoides adoensis	-	11 mm	10 mm	-	10 mm	18 mm	15 mm	-	-	13 mm	-	-	-

Table 1. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Se	Sf	Sal
	Bs	Kp	Sa	Ec									
Plants													
Chenopodium ambrosioids	-	-	10 mm	-	15 mm	10 mm	13 mm	-	11 mm	-	-	-	-
Hewittia malambarica	-	-	-	-	12 mm	18 mm	15 mm	11 mm	11 mm	-	-	-	-
Stems													
Acacia robusta	11 mm	15 mm	12 mm	-	11 mm	20 mm	16 mm	-	-	-	-	-	-
Acanthospermum australe	14 mm	-	15 mm	-	16 mm	12 mm	14 mm	11 mm	-	-	-	-	-
Acridocarpus natalitius	15 mm	-	19 mm	15 mm	22 mm	20 mm	14 mm	-	10 mm	-	-	-	-
Catharanthus roseus	-	11 mm	-	10 mm	9 mm	14 mm	15 mm	-	-	10 mm	-	-	10 mm
Chromolaena odarata	20 mm	16 mm	19 mm	-	15 mm	20 mm	17 mm	11 mm	14 mm	15 mm	-	-	-
Dichrostachys cinerca	18 mm	12 mm	20 mm	18 mm	22 mm	19 mm	12 mm	10 mm	11 mm	-	-	-	-
Faurea macnaughton	-	-	-	-	14 mm	15 mm	10 mm	-	13 mm	-	-	-	-
Hypoxis hemerocallidea	-	-	18 mm	12 mm	18 mm	15 mm	20 mm	11 mm	13 mm	10 mm	-	12 mm	-
Lippia javanica	-	16 mm	35 mm	-	14 mm	16 mm	12 mm	-	-	14 mm	-	-	-
Maytenus heterophylla	10 mm	14 mm	21 mm	13 mm	20 mm	16 mm	20 mm	16 mm	-	11 mm	-	-	-
Melia azedarach	17 mm	-	12 mm	-	12 mm	14 mm	18 mm	16 mm	-	11 mm	-	-	-
Psidium guajava	11 mm	26 mm	-	-	20 mm	16 mm	10 mm	15 mm	-	-	-	-	-
Schotia brachypetala	-	11 mm	20 mm	15 mm	25 mm	15 mm	20 mm	12 mm	-	-	11 mm	-	-
Sclerocarya birrea	11 mm	12 mm	12 mm	-	21 mm	13 mm	16 mm	15 mm	13 mm	-	-	-	-
Tetradenia riparia	16 mm	11 mm	10 mm	-	17 mm	17 mm	11 mm	-	-	10 mm	-	-	-
Trichilia dregeana	16 mm	10 mm	12 mm	-	15 mm	18 mm	17 mm	10 mm	12 mm	-	-	-	-
Vernonia oligocephala	12 mm	-	16 mm	-	14 mm	13 mm	-	12 mm	-	10 mm	-	-	-
Vernonia tigna	10 mm	-	25 mm	-	21 mm	12 mm	15 mm	12 mm	-	-	-	-	-
Tuber													
Callilepis laureola	-	20 mm	17 mm	-	12 mm	15 mm	16 mm	15 mm	-	12 mm	-	-	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	14 mm	15 mm	30 mm	31 mm	16 mm	18 mm	21 mm	24 mm	19 mm	20 mm	25 mm	17 mm	22 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.DMSO- dimethly sulfoxide, – = no minimum inhibition concentration.

Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

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 2. Results of sensitivity of different bacteria against methanol plant extract.

Sensitivity of different bacteria against methanol plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	-	-	13 mm	12 mm	22 mm	21 mm	10 mm	-	-	-	-	-	-
Syzygium cordatum	12 mm	18 mm	20 mm	10 mm	20 mm	20 mm	14 mm	-	20 mm	-	15 mm	-	-
Schotia brachypetala	-	10 mm	10 mm	-	15 mm	16 mm	-	13 mm	-	-	-	12 mm	-
Sclerocarya birrea	-	-	11 mm	-	19 mm	11 mm	-	-	-	16 mm	-	-	-
Ungazini	-	-	15 mm	15 mm	17 mm	17 mm	10 mm	-	-	-	-	-	-
Flower													
Vernonia tigna	-	-	18 mm	12 mm	10 mm	-	-	12 mm	-	-	17 mm	-	-
Leaves													
Acacia karroo	-	12 mm	19 mm	-	16 mm	17 mm	18 mm	12 mm	-	-	13 mm	-	-
Acacia robusta	-	-	16 mm	11 mm	20 mm	14 mm	11 mm	-	-	-	-	-	-
Acanthospermum australe	10 mm	-	19 mm	-	11 mm	18 mm	16 mm	-	-	-	-	10 mm	-
Aloe arborescens	-	10 mm	11 mm	11 mm	16 mm	14 mm	12 mm	16 mm	14 mm	11 mm	-	10 mm	-
Catharanthus roseus	-	11 mm	-	15 mm	15 mm	14 mm	14 mm	-	-	-	-	-	-
Chromolaena odarata	-	-	10 mm	17 mm	12 mm	25 mm	16 mm	11 mm	-	-	-	-	-
Dichrostachys cinerca	-	-	13 mm	16 mm	16 mm	17 mm	12 mm	-	-	-	-	10 mm	-
Faurea macnaughton	-	-	19 mm	-	14 mm	10 mm	-	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	-	-	14 mm	10 mm	-	14 mm	-	-	-	-	-
Lippia javanica	-	-	17 mm	-	19 mm	16 mm	-	-	-	-	-	-	-
Maytenus heterophylla	-	15 mm	12 mm	17 mm	-	18 mm	-	14 mm	-	-	-	10 mm	-
Melia azedarach	-	-	15 mm	-	18 mm	-	-	21 mm	11 mm	-	-	11 mm	-
Psidium guajava	-	15 mm	15 mm	12 mm	19 mm	11 mm	-	-	-	-	-	12 mm	-
Schotia brachypetala	-	-	10 mm	13 mm	10 mm	-	11 mm	11 mm	-	-	-	-	-
Sclerocarya birrea	-	-	12 mm	-	12 mm	17 mm	12 mm	-	10 mm	-	-	-	-

Table 2. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Leaves													
Syzygium cordatum	16 mm	17 mm	13 mm	-	20 mm	23 mm	17 mm	18 mm	27 mm	-	13 mm	12 mm	14 mm
Tetradenia riparia	12 mm	13 mm	10 mm	10 mm	14 mm	14 mm	17 mm	10 mm	-	-	-	-	-
Trichilia dregeana	-	-	14 mm	-	14 mm	17 mm	14 mm	12 mm	-	-	-	-	-
Vernonia oligocephala	-	-	15 mm	-	12 mm	15 mm	11 mm	15 mm	-	-	-	-	-
Vernonia tigna	-	-	11 mm	10 mm	11 mm	20 mm	13 mm	-	-	-	-	17 mm	-
Plants													
Baccharoides adoensis	-	-	10 mm	-	15 mm	16 mm	12 mm	-	10 mm	-	-	-	10 mm
Chenopodium ambrosioides	-	-	10 mm	16 mm	18 mm	11 mm	10 mm	10 mm	-	-	-	-	-
Hewittia malambarica	-	-	11 mm	17 mm	15 mm	14 mm	13 mm	-	-	-	-	11 mm	-
Stems													
Acacia robusta	-	16 mm	11 mm	12 mm	11 mm	-	14 mm	-	-	-	-	10 mm	-
Acanthospermum australe	-	-	11 mm	-	14 mm	15 mm	12 mm	15 mm	10 mm	12 mm	10 mm	-	18 mm
Acridocarpus natalitius	-	-	-	-	19 mm	12 mm	-	-	-	-	-	-	-
Catharanthus roseus	-	10 mm	19 mm	16 mm	-	11 mm	-	-	-	-	-	-	-
Chromolaena odarata	-	-	16 mm	-	10 mm	16 mm	11 mm	-	14 mm	-	-	-	-
Dichrostachys cinerea	-	11 mm	17 mm	10 mm	17 mm	11 mm	21 mm	-	-	-	-	18 mm	-
Faurea macnaughton	-	-	10 mm	15 mm	10 mm	16 mm	18 mm	-	-	-	-	-	19 mm
Hypoxis hemerocallidea	-	-	17 mm	11 mm	18 mm	20 mm	18 mm	-	-	12 mm	-	13 mm	-
Lippia javanica	-	-	15 mm	-	14 mm	16 mm	10 mm	14 mm	-	-	-	-	-
Maytenus heterophylla	-	-	17 mm	-	-	10 mm	-	11 mm	12 mm	-	-	-	-
Melia azedarach	-	-	12 mm	17 mm	16 mm	19 mm	-	-	-	-	-	17 mm	-
Psidium guajava	-	11 mm	14 mm	15 mm	12 mm	18 mm	21 mm	-	17 mm	-	13 mm	-	-
Schotia brachypetala	-	-	17 mm	14 mm	18 mm	14 mm	19 mm	-	10 mm	12 mm	-	-	-
Sclerocarya birrea	-	-	15 mm	-	10 mm	12 mm	18 mm	10 mm	-	-	-	10 mm	-
Tetradenia riparia	-	-	15 mm	-	14 mm	-	10 mm	-	16 mm	-	-	-	-
Trichilia dregeana	11 mm	-	16 mm	11 mm	10 mm	17 mm	16 mm	10 mm	13 mm	-	-	12 mm	14 mm
Vernonia oligocephala	12 mm	14 mm	-	16 mm	-	16 mm	-	-	12 mm	-	-	-	-
Vernonia tigna	-	-	15 mm	-	13 mm	17 mm	11 mm	-	-	-	-	15 mm	-
Tuber													
Callilepis laureola	-	10 mm	17 mm	-	12 mm	16 mm	10 mm	-	-	-	10 mm	15 mm	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	17 mm	15 mm	30 mm	21 mm	18 mm	20 mm	18 mm	19 mm	13 mm	16 mm	18 mm	20 mm	20 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli,* Ss – *Shigella sonnei*, Sf – *Shigella flexneri,* Sal – *Salmonella typhii, Staphylococcus aureus* strains - P 5020, P4790 , T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403. DMSO- dimethly sulfoxide, – = no minimum inhibition concentration. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

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 3. Results of sensitivity of different bacteria against cold distilled plant extracts.

Sensitivity of different bacteria against cold distilled water plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	-	14 mm	20 mm	-	11 mm	16 mm	11 mm	-	-	11 mm	-	15 mm	-
Syzygium cordatum	-	10 mm	20 mm	14 mm	18 mm	20 mm	18 mm	17 mm	10 mm	-	16 mm	-	-
Schotia brachypetala	-	17 mm	18 mm	-	19 mm	16 mm	10 mm	-	-	-	-	17 mm	-
Sclerocarya birrea	16 mm	-	10 mm	-	11 mm	23 mm	17 mm	14 mm	-	-	-	11 mm	10 mm
Ungazini	-	15 mm	16 mm	-	17 mm	-	15 mm	-	-	-	-	-	-
Flower													
Vernonia tigna	-	-	16 mm	11 mm	-	10 mm	-	-	-	-	-	10 mm	-
Leaves													
Acacia karroo	-	14 mm	18 mm	-	18 mm	19 mm	16 mm	13 mm	-	11 mm	13 mm	-	-
Acacia robusta	25 mm	25 mm	16 mm	-	31 mm	20 mm	16 mm	-	-	-	-	-	-
Acanthospermum australe	-	-	-	14 mm	17 mm	11 mm	15 mm	-	-	-	16 mm	-	13 mm
Aloe arborescens	11 mm	-	20 mm	-	13 mm	19 mm	10 mm	-	-	10 mm	10 mm	13 mm	-
Catharanthus roseus	11 mm	10 mm	-	18 mm	16 mm	10 mm	18 mm	-	-	-	14 mm	10 mm	-
Chromolaena odarata	13 mm	13 mm	20 mm	16 mm	14 mm	11 mm	16 mm	-	-	-	-	-	-
Dichrostachys cinerea	17 mm	18 mm	15 mm	-	10 mm	-	18 mm	-	-	-	12 mm	11 mm	11 mm
Faurea macnaughton	-	-	17 mm	-	16 mm	15 mm	12 mm	-	-	-	-	-	-
Hypoxis hemerocallidea	-	10 mm	15 mm	11 mm	-	20 mm	10 mm	-	-	-	12 mm	-	9 mm
Lippia javanica	11 mm	13 mm	13 mm	-	-	20 mm	-	-	-	-	-	-	-
Maytenus heterophylla	-	-	21 mm	-	11 mm	17 mm	-	14 mm	11 mm	-	-	-	-
Melia azedarach	-	-	14 mm	-	10 mm	13 mm	14 mm	-	10 mm	-	-	11 mm	-
Psidium guajava	14 mm	15 mm	19 mm	-	13 mm	-	10 mm	-	-	-	-	13 mm	-
Schotia brachypetala	20 mm	11 mm	10 mm	15 mm	17 mm	-	16 mm	-	-	-	-	12 mm	-
Sclerocarya birrea	-	13 mm	17 mm	-	20 mm	15 mm	13 mm	-	12 mm	10 mm	10 mm	11 mm	-
Syzygium cordatum	16 mm	19 mm	15 mm	18 mm	23 mm	26 mm	19 mm	17 mm	19 mm	10 mm	-	10 mm	11 mm
Tetradenia riparia	20 mm	-	22 mm	-	25 mm	11 mm	10 mm	-	-	-	-	11 mm	-
Trichilia dregeana	-	-	13 mm	-	13 mm	16 mm	-	-	-	-	11 mm	-	-
Vernonia oligocephala	14 mm	13 mm	18 mm	10 mm	17 mm	-	13 mm	-	-	-	11 mm	15 mm	-
Vernonia tigna	-	-	20 mm	17 mm	10 mm	17 mm	11 mm	-	-	-	-	15 mm	-
Plants													
Baccharoides adoensis	27 mm	-	10 mm	-	-	11 mm	-	-	-		-	-	-
Chenopodium ambrosioides	-	14 mm	18 mm	-	11 mm	15 mm	17 mm	-	-	-	-	-	-
Hewittia malambarica	-	-	13 mm	-	15 mm	17 mm	-	-	-	-	-	-	-

Table 3. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Stems													
Acacia robusta	-	14 mm	19 mm	-	13 mm	10 mm	25 mm	-	-	-	-	11 mm	-
Acanthospermum australe	-	10 mm	15 mm	13 mm	17 mm	13 mm	19 mm	10 mm	-	11 mm	-	10 mm	-
Acridocarpus natalitius	-	11 mm	20 mm	-	16 mm	10 mm	13 mm	-	-	-	13 mm	-	-
Catharanthus roseus	17 mm	12 mm	15 mm	10 mm	10 mm	-	10 mm	-	-	-	-	15 mm	-
Chromolaena odarata	12 mm	10 mm	15 mm	11 mm	12 mm	10 mm	12 mm	11 mm	-	-	10 mm	-	-
Dichrostachys cinerea	15 mm	18 mm	19 mm	-	10 mm	17 mm	11 mm	-	-	-	-	16 mm	-
Faurea macnaughton	16 mm	11 mm	-	14 mm	-	20 mm	19 mm	-	-	-	12 mm	9 mm	-
Hypoxis hemerocallidea	13 mm	17 mm	15 mm	12 mm	17 mm	18 mm	19 mm	-	-	-	14 mm	-	-
Lippia javanica	16 mm	15 mm	17 mm	10 mm	23 mm	15 mm	10 mm	11 mm	-	-	-	11 mm	-
Maytenus heterophylla	-	17 mm	19 mm	-	13 mm	17 mm	19 mm	-	-	-	14 mm	12 mm	-
Melia azedarach	10 mm	17 mm	13 mm	17 mm	18 mm	-	-	-	11 mm	-	-	-	-
Psidium guajava	20 mm	18 mm	10 mm	11 mm	13 mm	15 mm	11 mm	-	-	-	-	-	-
Schotia brachypetala	23 mm	13 mm	17 mm	-	11 mm	-	-	-	-	-	-	-	-
Sclerocarya birrea	-	11 mm	16 mm	11 mm	18 mm	13 mm	15 mm	10 mm	-	-	13 mm	-	-
Tetradenia riparia	-	11 mm	15 mm	17 mm	19 mm	10 mm	13 mm	-	-	-	-	-	-
Trichilia dregeana	-	12 mm	18 mm	-	11 mm	19 mm	16 mm	-	10 mm	-	10 mm	19 mm	-
Vernonia oligocephala	-	14 mm	16 mm	-	17 mm	12 mm	15 mm	-	-	13 mm	-	-	-
Vernonia tigna	13 mm	15 mm	17 mm	-	16 mm	11 mm	12 mm	-	-	-	-	-	-
Tuber													
Callilepis laureola	-	-	12 mm	-	11 mm	13 mm	18 mm	-	-	-	-	17 mm	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	
Neomycin	38 mm	33 mm	13 mm	30 mm	21 mm	16 mm	15 mm	-	31 mm	28 mm	29 mm	26 mm	28 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulfoxide, – = no minimum inhibition concentration, Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark. 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. Results of sensitivity of different bacteria against of hot distilled water plant extracts.

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	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	-	-	10 mm	-	17 mm	15 mm	-	-	13 mm	-	17 mm	16 mm	-
Syzygium cordatum	21 mm	18 mm	17 mm	14 mm	23 mm	20 mm	18 mm	11 mm	10 mm	17 mm	16 mm	20 mm	-
Schotia brachypetala	11 mm	15 mm	15 mm	-	16 mm	19 mm	11 mm	-	-	10 mm	-	-	-
Sclerocarya birrea	-	14 mm	13 mm	-	20 mm	13 mm	16 mm	-	11 mm	-	11 mm	11 mm	-
Ungazini	12 mm	10 mm	-	-	12 mm	13 mm	-	-	-	-	-	15 mm	-
Flower													
Vernonia tigna	-	-	13 mm	-	-	11 mm	-	-	-	-	17 mm	15 mm	-
Leaves													
Acacia karroo	-	15 mm	20 mm	14 mm	18 mm	17 mm	18 mm	-	-	-	11 mm	-	-
Acacia robusta	-	17 mm	12 mm	12 mm	13 mm	12 mm	11 mm	-	11 mm	-	10 mm	-	11 mm
Acanthospermum australe	15 mm	12 mm	10 mm	-	13 mm	16 mm	15 mm	-	-	-	15 mm	10 mm	-
Aloe arborescens	17 mm	-	-	-	-	12 mm	14 mm	-	-	-	-	-	-
Catharanthus roseus	-	16 mm	13 mm	-	-	16 mm	17 mm	10 mm	14 mm	11 mm	-	15 mm	11 mm
Chromolaena odarata	-	11 mm	-	-	10 mm	-	15 mm	-	-	-	12 mm	-	14 mm
Dichrostachys cinerea	11 mm	14 mm	18 mm	-	14 mm	13 mm	11 mm	-	-	-	15 mm	17 mm	10 mm
Faurea macnaughton	15 mm	16 mm	11 mm	-	13 mm	18 mm	16 mm	-	-	10 mm	10 mm	10 mm	-
Hypoxis hemerocallidea	-	10 mm	14 mm	10 mm	15 mm	17 mm	18 mm	-	10 mm	-	16 mm	-	-
Lippia javanica	-	-	-	16 mm	-	13 mm	18 mm	-	-	-	12 mm	-	26 mm
Maytenus heterophylla	12 mm	-	-	10 mm	11 mm	10 mm	-	-	-	-	-	-	-
Melia azedarach	-	13 mm	-	15 mm	12 mm	14 mm	11 mm	-	-	-	10 mm	-	-
Psidium guajava	-	19 mm	15 mm	-	-	14 mm	16 mm	-	-	-	14 mm	-	-
Schotia brachypetala	-	20 mm	13 mm	-	10 mm	18 mm	13 mm	-	-	-	12 mm	14 mm	-
Sclerocarya birrea	10 mm	18 mm	20 mm	-	18 mm	13 mm	10 mm	-	-	11 mm	11 mm	-	-
Syzygium cordatum	16 mm	22 mm	18 mm	18 mm	20 mm	16 mm	19 mm	15 mm	11 mm	17 mm	20 mm	17 mm	15 mm
Tetradenia riparia	10 mm	15 mm	-	15 mm	-	11 mm	18 mm	-	-	-	18 mm	18 mm	-
Trichilia dregeana	10 mm	-	16 mm	-	-	-	16 mm	-	-	-	14 mm	-	14 mm
Vernonia oligocephala	-	-	12 mm	11 mm	12 mm	14 mm	19 mm	-	-	-	-	-	-
Vernonia tigna	-	-	-	10 mm	-	11 mm	13 mm	-	-	-	-	14 mm	-
Plants													
Baccharoides adoensis	15 mm	-	-	-	12 mm	-	-	-	-	-	-	-	-
Chenopodium ambrosioides	-	-	16 mm	17 mm	10 mm	11 mm	10 mm	-	-	-	18 mm	-	-
Hewittia malambarica	10 mm	13 mm	10 mm	12 mm	11 mm	12 mm	13 mm	10 mm	-	-	-	-	-

Table 4. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Stems													
Acacia robusta	10 mm	-	10 mm	14 mm	11 mm	15 mm	17 mm	-	19 mm	-	14 mm	-	-
Acanthospermum australe	11 mm	-	-	12 mm	16 mm	10 mm	13 mm	-	11 mm	-	11 mm	-	-
Acridocarpus natalitius	15 mm	14 mm	-	-	16 mm	-	14 mm	-	-	-	15 mm	11 mm	
Catharanthus roseus	-	17 mm	-	-	11 mm	-	10 mm	-	17 mm	-	13 mm	13 mm	-
Chromolaena odarata	10 mm	11 mm	12 mm	14 mm	-	16 mm	17 mm	-	-	-	10 mm	-	-
Dichrostachys cinerea	-	-	14 mm	18 mm	12 mm	11 mm	15 mm	-	-	-	-	-	-
Faurea macnaughton	-	19 mm	12 mm	17 mm	13 mm	14 mm	18 mm	-	-	-	12 mm	-	-
Hypoxis hemerocallidea	-	-	16 mm	13 mm	18 mm	15 mm	17 mm	-	-	11 mm	-	10 mm	-
Lippia javanica	-	13 mm	-	-	17 mm	14 mm	-	-	-	-	11 mm	-	-
Maytenus heterophylla	-	10 mm	15 mm	-	17 mm	14 mm	10 mm	-	-	-	-	-	-
Melia azedarach	-	10 mm	-	12 mm	13 mm	17 mm	13 mm	-	15 mm	-	10 mm	-	-
Psidium guajava	-	16 mm	-	-	14 mm	16 mm	-	-	-	-	15 mm	15 mm	-
Schotia brachypetala	18 mm	11 mm	12 mm	-	17 mm	13 mm	13 mm	-	-	-	14 mm	-	-
Sclerocarya birrea	16 mm	11 mm	15 mm	11 mm	17 mm	13 mm	11 mm	-	-	-	-	10 mm	-
Tetradenia riparia	16 mm	-	-	15 mm	-	11 mm	16 mm	-	-	10 mm	17 mm	15 mm	-
Trichilia dregeana	-	10 mm	13 mm	-	10 mm	13 mm	16 mm	-	-	-	-	-	-
Vernonia oligocephala	-	12 mm	16 mm	-	-	12 mm	12 mm	-	-	12 mm	-	-	-
Vernonia tigna	-	12 mm	17 mm	-	11 mm	10 mm	15 mm	-	-	-	-	-	-
Tuber													
Callilepis laureola	11 mm	16 mm	-	-	13 mm	13 mm	16 mm	-	-	-	20 mm	17 mm	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	20 mm	18 mm	16 mm	20 mm	25 mm	22 mm	20 mm	21 mm	20 mm	18 mm	26 mm	28 mm	18 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790 , T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulfoxide, – = no minimum inhibition concentration. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark. 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.

APPENDIX C

Disk-diffusion assay

Table 1. Results of sensitivity of different bacteria against of acetone plant extracts.

Sensitivity of different bacteria against acetone plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	15 mm	-	25 mm	-	16 mm	17 mm	14 mm	10 mm	-	-	-	-	-
Syzygium cordatum	12 mm	18 mm	11 mm	21 mm	20 mm	22 mm	17 mm	19 mm	10 mm	16 mm	-	24 mm	10 mm
Scholia brachypetala	18 mm	10 mm	22 mm	-	20 mm	17 mm	17 mm	-	18 mm	-	-	-	-
Sclerocarya birrea	-	15 mm	18 mm	-	19 mm	16 mm	18 mm	16 mm	18 mm	10 mm	-	16 mm	-
Ungazini	15 mm	14 mm	20 mm	-	19 mm	16 mm	19 mm	-	13 mm	11 mm	10 mm	-	-
Flower													
Vernonia tigna	-	-	20 mm	-	18 mm	14 mm	15 mm	-	-	-	12 mm	10 mm	-
Leaves													
Acacia karroo	-	14 mm	13 mm	-	16 mm	13 mm	12 mm	13 mm	-	-	-	-	-
Acacia robusta	-	15 mm	10 mm	-	19 mm	15 mm	16 mm	-	11 mm	10 mm	16 mm	-	-
Acanthospermum australe	-	12 mm	20 mm	-	13 mm	17 mm	17 mm	15 mm	9 mm	-	-	-	-
Aloe arborescens	-	-	24 mm	15 mm	27 mm	10 mm	16 mm	20 mm	11 mm	15 mm	11 mm	-	-
Catharanthus roseus	20 mm	16 mm	-	-	12 mm	10 mm	12 mm	11 mm	-	-	-	-	-
Chromolaena odarata	17 mm	-	21 mm	-	22 mm	20 mm	15 mm	-	17 mm	10 mm	-	-	-
Dichrostachys cinerea	16 mm	-	16 mm	-	20 mm	15 mm	18 mm	-	18 mm	10 mm	-	24 mm	-
Faurea macnaughton	-	-	-	-	15 mm	19 mm	10 mm	-	10 mm	13 mm	-	-	-
Hypoxis hemerocallidea	19 mm	-	20 mm	-	18 mm	10 mm	16 mm	11 mm	15 mm	-	-	-	11 mm
Lippia javanica	-	14 mm	22 mm	-	18 mm	19 mm	20 mm	10 mm	-	15 mm	-	16 mm	-
Maytenus heterophylla	19 mm	-	22 mm	10 mm	20 mm	13 mm	16 mm	12 mm	-	11 mm	15 mm	-	-
Melia azedarach	15 mm	10 mm	14 mm	-	16 mm	11 mm	15 mm	13 mm	20 mm	-	-	-	-
Psidium guajava	-	-	29 mm	-	17 mm	18 mm	10 mm	11 mm	-	11 mm	-	18 mm	10 mm
Scholia brachypetala	22 mm	22 mm	20 mm	-	20 mm	15 mm	11 mm	14 mm	10 mm	10 mm	-	16 mm	-
Sclerocarya birrea	-	-	24 mm	10 mm	16 mm	14 mm	15 mm	10 mm	-	11 mm	9 mm	-	-
Syzygium cordatum	15 mm	-	22 mm	14 mm	18 mm	18 mm	15 mm	16 mm	17 mm	18 mm	16 mm	22 mm	13 mm
Tetradenia riparia	20 mm	12 mm	20 mm	11 mm	16 mm	19 mm	15 mm	-	11 mm	15 mm	12 mm	10 mm	-
Trichilia dregeana	11 mm	20 mm	13 mm	-	20 mm	16 mm	18 mm	-	15 mm	-	-	-	-
Vernonia oligocephala	16 mm	-	18 mm	-	14 mm	10 mm	15 mm	-	-	10 mm	-	-	-
Vernonia tigna	-	-	25 mm	10 mm	20 mm	18 mm	16 mm	-	11 mm	-	15 mm	-	-

Table 1. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Plants													
Baccharoides adoensis	-	11 mm	15 mm	-	10 mm	15 mm	15 mm	10 mm	-	13 mm	-	-	-
Chenopodium ambrosioides	-	15 mm	19 mm	-	19 mm	13 mm	11 mm	12 mm	10 mm	-	-	-	-
Hewittia malambarica	-	-	12 mm	-	16 mm	15 mm	17 mm	13 mm	11 mm	10 mm	-	-	-
Stems													
Acacia robusta	15 mm	12 mm	16 mm	-	14 mm	20 mm	16 mm	11 mm	-	-	11 mm	-	-
Acanthospermum australe	15 mm	-	18 mm	-	16 mm	19 mm	20 mm	11 mm	-	11 mm	-	-	-
Acridocarpus natalitius	15 mm	-	19 mm	16 mm	20 mm	22 mm	15 mm	-	12 mm	-	-	-	-
Catharanthus roseus	10 mm	15 mm	-	11 mm	10 mm	16 mm	15 mm	-	-	10 mm	-	-	13 mm
Chromolaena odarata	20 mm	16 mm	-	-	12 mm	10 mm	12 mm	11 mm	-	-	-	-	-
Dichrostachys cinerca	16 mm	10 mm	18 mm	15 mm	20 mm	17 mm	15 mm	12 mm	12 mm	10 mm	-	10 mm	-
Faurea macnaughton	-	-	10 mm	-	16 mm	15 mm	13 mm	-	15 mm	10 mm	-	-	-
Hypoxis hemerocallidea	12 mm	14 mm	16 mm	-	13 mm	15 mm	17 mm	-	-	14 mm	-	-	-
Lippia javanica	10 mm	12 mm	30 mm	-	15 mm	16 mm	10 mm	10 mm	-	14 mm	10 mm	-	-
Maytenus heterophylla	12 mm	18 mm	24 mm	13 mm	22 mm	16 mm	22 mm	18 mm	-	12 mm	10 mm	15 mm	-
Melia azedarach	19 mm	10 mm	12 mm	-	18 mm	15 mm	18 mm	16 mm	-	13 mm	-	-	-
Psidium guajava	12 mm	26 mm	-	-	23 mm	18 mm	13 mm	15 mm	-	17 mm	-	-	-
Schotia brachypetala	-	12 mm	22 mm	18 mm	27 mm	15 mm	23 mm	12 mm	10 mm	-	15 mm	-	-
Sclerocarya birrea	11 mm	13 mm	17 mm	-	19 mm	13 mm	17 mm	15 mm	13 mm	10 mm	-	-	-
Tetradenia riparia	18 mm	11 mm	14 mm	10 mm	19 mm	17 mm	15 mm	-	10 mm	13 mm	11 mm	-	11 mm
Trichilia dregeana	18 mm	10 mm	15 mm	-	17 mm	18 mm	15 mm	10 mm	12 mm	-	-	-	-
Vernonia oligocephala	14 mm	12 mm	-	-	14 mm	16 mm	-	-	14 mm	-	-	15 mm	-
Vernonia tigna	13 mm	-	20 mm	-	20 mm	12 mm	16 mm	12 mm	13 mm	-	-	-	-
Tuber													
Callilepis laureola	-	18 mm	18 mm	-	15 mm	15 mm	16 mm	15 mm	-	12 mm	10 mm	-	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	15 mm	15 mm	30 mm	30 mm	20 mm	20 mm	25 mm	24 mm	20 mm	23 mm	28 mm	20 mm	22 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403, DMSO - dimethly sulfoxide, – = no minimum inhibition concentration.

Different plant parts: **l** – Leaves, **p** – Plant, **s** – Stem, **t** – Tuber, **f** – Flowers and **b** – Bar. 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.

Table 2. Results of sensitivity of different bacteria against of methanol plant extracts.

Sensitivity of different bacteria against methanol plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	-	-	18 mm	15 mm	20 mm	19 mm	16 mm	-	11 mm	15 mm	-	-	-
Syzygium cordatum	19 mm	10 mm	17 mm	19 mm	21 mm	20 mm	17 mm	-	-	11 mm	15 mm	-	-
Schotia brachypetala	11 mm	10 mm	17 mm	-	17 mm	20 mm	19 mm	-	-	-	-	-	-
Sclerocarya birrea	-	-	10 mm	15 mm	20 mm	16 mm	-	11 mm	-	-	-	15 mm	-
Ungazini	-	-	17 mm	17 mm	13 mm	19 mm	-	-	15 mm	-	-	11 mm	-
Flower													
Vernonia tigna	-	-	20 mm	-	10 mm	11 mm	12 mm	-	-	-	17 mm	-	-
Leaves													
Acacia karroo	-	12 mm	16 mm	-	15 mm	16 mm	16 mm	13 mm	-	-	13 mm	-	-
Acacia robusta	-	-	16 mm	20 mm	18 mm	22 mm	17 mm	-	-	-	12 mm	-	-
Acanthospermum australe	10 mm	-	17 mm	-	14 mm	16 mm	19 mm	-	13 mm	-	13 mm	10 mm	-
Aloe arborescens	-	15 mm	15 mm	20 mm	18 mm	13 mm	13 mm	20 mm	19 mm	15 mm	-	-	-
Catharanthus roseus	13 mm	10 mm	17 mm	19 mm	20 mm	10 mm	17 mm	-	11 mm	-	-	-	-
Chromolaena odarata	-	12 mm	17 mm	10 mm	17 mm	20 mm	24 mm	19 mm	-	-	11 mm	-	-
Dichrostachys cinerea	-	-	19 mm	-	17 mm	10 mm	18 mm	-	-	11 mm	-	-	-
Faurea macnaughton	-	-	23 mm	-	20 mm	19 mm	15 mm	-	17 mm	-	-	-	-
Hypoxis hemerocallidea	-	19 mm	-	15 mm	18 mm	20 mm	-	17 mm	-	14 mm	-	-	-
Lippia javanica	-	-	22 mm	-	20 mm	19 mm	15 mm	-	11 mm	-	-	11 mm	-
Maytenus heterophylla	-	18 mm	19 mm	-	16 mm	18 mm	15 mm	-	-	-	-	-	-
Melia azedarach	13 mm	-	19 mm	11 mm	16 mm	16 mm	-	11 mm	-	17 mm	-	-	-
Psidium guajava	-	11 mm	21 mm	12 mm	15 mm	17 mm	13 mm	-	17 mm	-	15 mm	10 mm	-
Schotia brachypetala	-	-	15 mm	13 mm	16 mm	16 mm	15 mm	-	-	12 mm	-	-	-
Sclerocarya birrea	-	-	16 mm	-	20 mm	19 mm	15 mm	-	10 mm	-	15 mm	-	-
Syzygium cordatum	14 mm	14 mm	18 mm	11 mm	20 mm	20 mm	13 mm	10 mm	17 mm	-	-	15 mm	13 mm
Tetradenia riparia	10 mm	-	17 mm	15 mm	22 mm	11 mm	17 mm	10 mm	-	-	11 mm	-	-

Table 2. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U18406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Leaves													
Trichilia dregeana	-	-	17 mm	16 mm	11 mm	20 mm	14 mm	17 mm	-	16 mm	-	15 mm	-
Vernonia oligocephala	-	-	20 mm	-	18 mm	19 mm	15 mm	18 mm	12 mm	-	14 mm	-	-
Vernonia tigna	14 mm	-	11 mm	-	15 mm	18 mm	19 mm	-	14 mm	-	-	-	-
Plants													
Baccharoides adoensis	11 mm	-	10 mm	-	17 mm	19 mm	10 mm	16 mm	10 mm	-	17 mm	-	-
Chenopodium ambrosioide s	-	-	14 mm	18 mm	19 mm	15 mm	10 mm	18 mm	12 mm	-	11 mm	-	-
Hewittia malambarica	-	-	17 mm	10 mm	19 mm	16 mm	12 mm	10 mm	-	11 mm	-	-	-
Stems													
Acacia robusta	-	-	16 mm	17 mm	19 mm	17 mm	20 mm	-	14 mm	-	-	-	-
Acanthospermum australe	-	-	20 mm	18 mm	19 mm	23 mm	20 mm	18 mm	18 mm	19 mm	15 mm	-	18 mm
Acridocarpus natalitius	-	-	15 mm	-	17 mm	18 mm	12 mm	11 mm	-	10 mm	-	-	-
Catharanthus roseus	11 mm	18 mm	20 mm	13 mm	13 mm	16 mm	11 mm	-	14 mm	-	-	-	-
Chromolaena odarata	-	-	18 mm	13 mm	15 mm	16 mm	10 mm	17 mm	14 mm	-	11 mm	-	-
Dichrostachys cinerea	-	-	13 mm	15 mm	12 mm	19 mm	15 mm	-	10 mm	-	-	14 mm	-
Faurea macnaughton	-	-	11 mm	18 mm	14 mm	13 mm	20 mm	-	-	-	-	-	-
Hypoxis hemerocallidea	-	-	16 mm	11 mm	15 mm	12 mm	19 mm	-	11 mm	-	12 mm	-	-
Lippia javanica	-	-	17 mm	-	16 mm	19 mm	14 mm	12 mm	11 mm	-	-	-	-
Maytenus heterophylla	-	-	15 mm	-	12 mm	13 mm	11 mm	-	15 mm	12 mm	-	-	-
Melia azedarach	-	-	18 mm	-	18 mm	17 mm	13 mm	-	13 mm	-	-	-	-
Psidium guajava	-	15 mm	18 mm	19 mm	15 mm	21 mm	15 mm	13 mm	-	11 mm	17 mm	-	-
Schotia brachypetala	-	18 mm	20 mm	15 mm	22 mm	19 mm	15 mm	-	-	18 mm	-	17 mm	-
Sclerocarya birrea	-	12 mm	19 mm	-	13 mm	18 mm	19 mm	14 mm	-	11 mm	-	-	-
Tetradenia riparia	-	-	19 mm	-	11 mm	16 mm	10 mm	11 mm	-	-	-	-	11 mm
Trichilia dregeana	11 mm	-	16 mm	11 mm	10 mm	17 mm	16 mm	10 mm	13 mm	-	-	12 mm	13 mm
Vernonia oligocephala	14 mm	-	12 mm	16 mm	14 mm	-	15 mm	-	-	-	-	-	-
Vernonia tigna	17 mm	-	19 mm	11 mm	20 mm	19 mm	15 mm	-	13 mm	11 mm	19 mm	18 mm	-
Tuber													
Callilepis laureola	10 mm	13 mm	15 mm	-	15 mm	13 mm	18 mm	-	13 mm	-	16 mm	-	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	30 mm	27 mm	28 mm	29 mm	26 mm	25 mm	27 mm	24 mm	22 mm	24 mm	22 mm	26 mm	24 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniea*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and

Escherichia coli strains - Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulfoxide, – = no minimum inhibition concentration. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

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 3. Results of sensitivity of different bacteria against of cold distilled water plant extracts.

Sensitivity of different bacteria against cold distilled water plant extracts (mm)													
PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Bark													
Ihlaza	-	16 mm	21 mm	-	15 mm	18 mm	15 mm	-	14 mm	10 mm	-	-	-
Syzygium cordatum	-	15 mm	17 mm	15 mm	17 mm	18 mm	20 mm	19 mm	14 mm	-	13 mm	10 mm	-
Schotia brachypetala	-	19 mm	20 mm	-	20 mm	18 mm	15 mm	-	15 mm	-	-	19 mm	-
Sclerocarya birrea	18 mm	-	14 mm	-	16 mm	21 mm	15 mm	12 mm	-	-	-	-	12 mm
Ungazini	-	18 mm	15 mm	-	19 mm	18 mm	16 mm	-	-	12 mm	-	11 mm	-
Flower													
Vernonia tigna	-	-	18 mm	15 mm	11 mm	17 mm	16 mm	10 mm	-	13 mm	-	13 mm	-
Leaves													
Acacia karroo	-	12 mm	19 mm	-	16 mm	17 mm	18 mm	12 mm	-	-	13 mm	-	-
Acacia robusta	23 mm	25 mm	19 mm	-	30 mm	21 mm	18 mm	-	15 mm	-	12 mm	-	-
Acanthospermum australe	-	-	18 mm	15 mm	19 mm	18 mm	17 mm	-	16 mm	-	17 mm	-	15 mm
Aloe arborescens	15 mm	-	23 mm	-	15 mm	20 mm	13 mm	-	-	14 mm	17 mm	15 mm	9 mm
Catharanthus roseus	11 mm	15 mm	18 mm	16 mm	13 mm	16 mm	16 mm	-	12 mm	-	16 mm	-	-
Chromolaena odarata	15 mm	18 mm	19 mm	15 mm	11 mm	16 mm	17 mm	-	10 mm	-	-	-	-
Dichrostachys cinerea	19 mm	20 mm	17 mm	11 mm	14 mm	11 mm	19 mm	-	12 mm	-	14 mm	-	11 mm
Faurea macnaughton	-	-	19 mm	-	15 mm	18 mm	14 mm	-	-	14 mm	-	12 mm	-
Hypoxis hemerocallidea	-	13 mm	17 mm	13 mm	12 mm	19 mm	11 mm	-	10 mm	-	-	-	10 mm
Lippia javanica	13 mm	17 mm	14 mm	11 mm	-	18 mm	-	-	-	11 mm	-	-	-
Maytenus heterophylla	-	-	19 mm	-	15 mm	19 mm	-	-	15 mm	-	-	13 mm	-
Melia azedarach	-	12 mm	17 mm	-	18 mm	15 mm	12 mm	11 mm	10 mm	11 mm	-	15 mm	-
Psidium guajava	18 mm	18 mm	17 mm	11 mm	15 mm	13 mm	16 mm	-	11 mm	-	15 mm	16 mm	-
Schotia brachypetala	19 mm	14 mm	-	14 mm	19 mm	-	18 mm	13 mm	-	10 mm	-	16 mm	-

Table 3. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sai
	Bs	Kp	Sa	Ec									
Leaves													
Sclerocarya birrea	10 mm	15 mm	17 mm	16 mm	14 mm	-	14 mm	12 mm	-	-	11 mm	-	-
Syzygium cordatum	19 mm	20 mm	17 mm	15 mm	20 mm	25 mm	17 mm	13 mm	20 mm	13 mm	-	13 mm	-
Tetradenia riparia	20 mm	-	22 mm	-	20 mm	11 mm	10 mm	-	-	-	-	11 mm	-
Trichilia dregeana	-	-	19 mm	-	16 mm	18 mm	-	-	-	12 mm	17 mm	-	-
Vernonia oligocephala	17 mm	19 mm	20 mm	17 mm	21 mm	-	14 mm	-	-	16 mm	17 mm	19 mm	-
Vernonia tigna	-	-	20 mm	17 mm	10 mm	15 mm	11 mm	-	-	-	-	-	-
Plants													
Baccharoides adoensis	26 mm	-	13 mm	-	13 mm	14 mm	-	-	15 mm	11 mm	-	-	-
Chenopodium ambrosioides	-	16 mm	19 mm	-	14 mm	17 mm	18 mm	-	-	-	-	-	-
Hewittia malambarica	-	-	18 mm	-	17 mm	19 mm	10 mm	-	9 mm	-	-	-	-
Stems													
Acacia robusta	-	13 mm	14 mm	-	18 mm	13 mm	20 mm	13 mm	-	11 mm	-	15 mm	-
Acanthospermum australe	-	10 mm	15 mm	13 mm	17 mm	13 mm	19 mm	10 mm	-	11 mm	-	10 mm	-
Acridocarpus natalitius	-	16 mm	22 mm	-	16 mm	10 mm	13 mm	-	-	-	13 mm	-	-
Catharanthus roseus	19 mm	14 mm	17 mm	13 mm	14 mm	12 mm	13 mm	15 mm	-	-	-	14 mm	-
Chromolaena odarata	15 mm	18 mm	17 mm	13 mm	17 mm	19 mm	22 mm	14 mm	11 mm	-	13 mm	-	-
Dichrostachys cinerea	14 mm	19 mm	18 mm	-	18 mm	15 mm	17 mm	11 mm	-	17 mm	-	-	-
Faurea macnaughton	17 mm	16 mm	11 mm	18 mm	16 mm	20 mm	18 mm	-	12 mm	-	11 mm	-	-
Hypoxis hemerocallidea	19 mm	11 mm	16 mm	20 mm	17 mm	19 mm	16 mm	-	-	11 mm	-	-	-
Lippia javanica	18 mm	14 mm	19 mm	13 mm	21 mm	17 mm	15 mm	11 mm	-	9 mm	-	15 mm	-
Maytenus heterophylla	-	18 mm	21 mm	-	15 mm	17 mm	20 mm	-	11 mm	-	15 mm	17 mm	-
Melia azedarach	15 mm	19 mm	14 mm	14 mm	20 mm	17 mm	-	13 mm	11 mm	-	12 mm	-	-
Psidium guajava	22 mm	19 mm	17 mm	15 mm	14 mm	17 mm	14 mm	-	-	-	-	-	-
Schotia brachypetala	24 mm	15 mm	19 mm	-	19 mm	-	-	-	12 mm	-	-	-	-
Sclerocarya birrea	-	13 mm	18 mm	15 mm	20 mm	19 mm	17 mm	15 mm	-	-	-	-	-
Tetradenia riparia	-	13 mm	17 mm	19 mm	20 mm	17 mm	18 mm	-	13 mm	-	16 mm	-	-
Trichilia dregeana	-	14 mm	17 mm	-	18 mm	20 mm	19 mm	18 mm	17 mm	-	13 mm	20 mm	-
Vernonia oligocephala	13 mm	15 mm	16 mm	12 mm	15 mm	13 mm	15 mm	-	-	12 mm	-	-	-
Vernonia tigna	13 mm	15 mm	17 mm	-	16 mm	11 mm	12 mm	-	-	-	-	-	-
Tuber													
Callilepis laureola	-	-	19 mm	-	-	15 mm	19 mm	-	14 mm	-	-	17 mm	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	38 mm	33 mm	13 mm	30 mm	21 mm	16 mm	15 mm	-	31 mm	28 mm	29 mm	26 mm	28 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, **Ec** – *Escherichia coli*.

Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulfoxide, – = no minimum inhibition concentration.

Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

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. *Results of sensitivity of different bacteria against of hot distilled water plant extracts.*

Sensitivity of different bacteria against hot distilled water plant extracts (mm)													
	ATTC CULTURES				Sa	Sa	Sa	Ec	Ec	Ec	Ss	Sf	Sal
PLANT NAMES	Bs	Kp	Sa	Ec	P5020	P4790	T1266	U1505S	U16406	U16403			
Bark													
Ihlaza	-	-	10 mm	-	18 mm	-	11 mm	-	-	-	19 mm	18 mm	13 mm
Syzygium cordatum	22 mm	19 mm	18 mm	16 mm	22 mm	24 mm	20 mm	10 mm	13 mm	18 mm	17 mm	10 mm	-
Schotia brachypetala	15 mm	17 mm	17 mm	11 mm	18 mm	17 mm	20 mm	-	-	-	-	11 mm	15 mm
Sclerocarya birrea	-	-	16 mm	-	13 mm	17 mm	19 mm	11 mm	-	-	15 mm	19 mm	-
Ungazini	10 mm	14 mm	-	-	16 mm	15 mm	-	-	16 mm	-	-	-	-
Flower													
Chenopod tigna	-	-	16 mm	-	17 mm	16 mm	12 mm	-	-	-	19 mm	17 mm	-
Leaves													
Acacia karroo	-	14 mm	13 mm	-	16 mm	13 mm	12 mm	13 mm	-	-	-	-	-
Acacia robusta	-	18 mm	16 mm	14 mm	18 mm	17 mm	19 mm	-	14 mm	11 mm	-	-	-
Acanthospermum australe	18 mm	16 mm	15 mm	15 mm	19 mm	20 mm	19 mm	-	15 mm	-	17 mm	15 mm	-
Aloe arborescens	19 mm	-	-	10 mm	-	15 mm	16 mm	-	-	13 mm	-	-	-
Catharanthus roseus	-	19 mm	10 mm	-	11 mm	19 mm	18 mm	14 mm	17 mm	-	12 mm	17 mm	15 mm
Chromolaena odarata	-	15 mm	-	-	14 mm	-	15 mm	-	-	-	16 mm	13 mm	11 mm
Dichrostachys cinerea	14 mm	17 mm	19 mm	-	16 mm	18 mm	14 mm	-	12 mm	-	16 mm	19 mm	-
Faurea macnaughton	18 mm	14 mm	-	-	19 mm	20 mm	14 mm	12 mm	-	-	14 mm	-	-
Hypoxis hemerocallidea	-	12 mm	16 mm	12 mm	16 mm	19 mm	19 mm	-	12 mm	16 mm	18 mm	-	11 mm
Lippia javanica	-	-	13 mm	18 mm	-	18 mm	19 mm	-	-	-	-	-	-
Maytenus heterophylla	14 mm	11 mm	15 mm	17 mm	15 mm	13 mm	-	-	-	-	-	-	-
Melia azedarach	-	16 mm	-	13 mm	15 mm	17 mm	15 mm	15 mm	-	-	-	-	-
Psidium guajava	-	19 mm	15 mm	-	-	14 mm	16 mm	-	-	-	14 mm	-	-
Schotia brachypetala	-	18 mm	16 mm	11 mm	-	20 mm	17 mm	-	-	14 mm	-	16 mm	-
Sclerocarya birrea	12 mm	19 mm	18 mm	-	20 mm	14 mm	16 mm	-	-	15 mm	10 mm	16 mm	-
Syzygium cordatum	13 mm	19 mm	22 mm	16 mm	19 mm	19 mm	17 mm	16 mm	14 mm	19 mm	18 mm	14 mm	-
Tetradenia riparia	-	16 mm	-	17 mm	18 mm	17 mm	16 mm	11 mm	-	-	16 mm	-	-
Trichilia dregeana	12 mm	-	17 mm	13 mm	19 mm	-	16 mm	-	11 mm	-	16 mm	-	-
Chenopod oligocephala	-	11 mm	-	15 mm	-	16 mm	20 mm	12 mm	-	-	-	13 mm	-
Chenopod tigna	-	-	11 mm	-	15 mm	-	17 mm	-	11 mm	-	13 mm	-	-
Plants													
Baccharoides adoensis	16 mm	-	-	16 mm	13 mm	-	16 mm	-	-	-	11 mm	-	-
Chenopod ambrosioides	11 mm	-	17 mm	19 mm	13 mm	11 mm	13 mm	11 mm	-	-	-	-	-
Hewittia malambarica	13 mm	17 mm	15 mm	17 mm	14 mm	19 mm	23 mm	-	13 mm	-	-	-	-

Table 4. (Continued)

PLANT NAMES	ATTC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Stems													
Acacia robusta	12 mm	-	-	12 mm	16 mm	18 mm	19 mm	-	18 mm	11 mm	18 mm	-	-
Acanthospermum australe	13 mm	-	-	-	17 mm	15 mm	15 mm	17 mm	19 mm	-	15 mm	-	-
Acridocarpus natalitius	17 mm	16 mm	-	-	18 mm	-	16 mm	11 mm	-	-	16 mm	13 mm	12 mm
Catharanthus roseus	-	19 mm	-	-	13 mm	-	13 mm	10 mm	18 mm	-	17 mm	15 mm	-
Chromolaena odarata	14 mm	13 mm	19 mm	13 mm	17 mm	20 mm	18 mm	15 mm	-	-	-	13 mm	-
Dichrostachys cinerea	12 mm	16 mm	-	16 mm	12 mm	14 mm	10 mm	-	11 mm	-	10 mm	-	-
Faurea macnaughton	-	18 mm	20 mm	19 mm	15 mm	18 mm	17 mm	-	-	-	-	11 mm	-
Hypoxis hemerocallidea	13 mm	15 mm	17 mm	16 mm	15 mm	18 mm	15 mm	-	-	12 mm	-	-	-
Lippia javanica	-	14 mm	12 mm	-	18 mm	16 mm	16 mm	-	-	-	-	-	-
Maytenus heterophylla	-	12 mm	16 mm	-	18 mm	15 mm	13 mm	10 mm	-	13 mm	-	-	-
Melia azedarach	-	12 mm	11 mm	13 mm	17 mm	18 mm	15 mm	11 mm	16 mm	-	-	-	-
Psidium guajava	-	19 mm	-	-	15 mm	18 mm	-	12 mm	-	13 mm	18 mm	12 mm	14 mm
Schotia brachypetala	19 mm	13 mm	15 mm	-	18 mm	18 mm	-	-	14 mm	-	-	-	-
Sclerocarya birrea	17 mm	13 mm	17 mm	12 mm	18 mm	16 mm	17 mm	-	13 mm	10 mm	-	-	-
Tetradenia riparia	17 mm	11 mm	10 mm	17 mm	-	14 mm	16 mm	-	-	14 mm	12 mm	17 mm	-
Trichilia dregeana	-	13 mm	16 mm	-	13 mm	14 mm	17 mm	-	-	12 mm	-	-	-
Vernonia oligocephala	16 mm	14 mm	-	10 mm	16 mm	14 mm	14 mm	-	-	12 mm	-	-	-
Vernonia tigna	10 mm	-	18 mm	-	13 mm	13 mm	17 mm	18 mm	-	11 mm	-	-	-
Tuber													
Callilepis laureola	12 mm	17 mm	18 mm	-	16 mm	14 mm	17 mm	-	14 mm	16 mm	21 mm	18 mm	-
Control													
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	22 mm	20 mm	21 mm	23 mm	25 mm	30 mm	27 mm	21 mm	20 mm	21 mm	26 mm	28 mm	19 mm

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790 , T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403. DMSO- dimethly sulfoxide, – = no minimum inhibition concentration. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

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.

APPENDIX D

Minimum inhibitory concentrations

Table 1. Minimum inhibitory concentrations from medicinal plants used for the treatment of diarrhoea in Ongoye forest.

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Acetone													
Ihlaza b	25	-	3.1	-	25	25	25	-	-	-	-	-	-
Syzygium cordatum b	50	25	50	0.01	3.1	3.1	25	12.5	50	25	-	0.01	-
Schotia brachypetala b	12.5	-	0.01	-	3.1	6.3	12.5	-	25	-	-	-	-
Sclerocarya birrea b	-	25	6.3	-	6.3	12.5	6.3	25	25	-	-	12.5	-
Ungazini b	50	50	25	-	25	25	25	-	50	50	50	-	-
Tinge tinge f	-	-	6.3	-	6.3	25	25	-	-	-	50	-	-
Acacia karroo l	50	25	6.3	25	50	6.3	6.3	50	25	6.3	50	25	50
Acacia robusta l	-	25	-	-	12.5	25	12.5	-	-	-	6.3	-	-
Acanthospermum australe l	-	-	6.3	-	-	50	50	50	-	-	-	-	-
Aloe arborescens l	-	-	3.1	12.5	1.0	-	25	6.3	-	25	-	-	-
Catharanthus roseus l	12.5	25	-	-	50	-	50	50	-	-	-	-	-
Chromolaena odarata l	25	-	3.1	-	3.1	0.01	3.1	-	25	-	-	-	-
Dichrostachys cinerea l	25	-	25	-	6.3	25	12.5	-	12.5	-	-	0.01	-
Faurea macnaughton l	-	-	-	-	12.5	6.3	-	-	-	50	-	-	-
Hypoxis hemerocallidea l	12.5	-	3.1	-	-	-	25	-	50	-	-	-	-
Lippia javanica l	-	25	3.1	-	25	25	3.1	-	-	25	-	12.5	-
Maytenus heterophylla l	3.1	-	0.01	-	6.3	50	25	50	-	50	25	-	-
Melia azedarach l	25	-	12.5	-	12.5	50	50	25	12.5	-	-	-	-
Psidium guajava l	-	-	0.01	-	6.3	25	-	-	-	50	-	25	-
Schotia brachypetala l	3.1	3.1	6.3	-	6.3	12.5	50	50	-	-	-	12.5	-
Sclerocarya birrea l	-	-	0.01	-	50	50	25	-	-	3.1	-	-	-
Syzygium cordatum l	25	-	0.01	25	12.5	12.5	25	12.5	12.5	12.5	12.5	0.01	50
Tetradenia riparia l	3.1	50	3.1	-	12.5	6.3	50	-	-	50	50	-	-
Trichilia dregeana l	-	3.1	50	-	3.1	6.3	12.5	-	25	-	-	-	-
Tinge oligocephala l	25	-	12.5	-	50	-	25	-	-	-	-	-	-
Tinge tinge l	-	-	0.01	-	3.1	12.5	25	-	-	-	25	-	-
Baccharoides adoensis p	-	-	12.5	-	-	6.3	25	-	-	-	-	-	-

Table 1. (Continued)

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Acetone													
Chenopodium ambrosioides p	-	50	3.1	-	6.3	-	-	3.1	-	-	-	-	-
Hewittia malambarica p	-	-	50	-	50	50	50	-	-	-	-	-	-
Acacia robusta s	25	12.5	12.5	-	12.5	3.1	6.3	12.5	-	-	50	-	-
Acanthospermum australe s	-	-	12.5	-	25	6.3	3.1	-	-	-	-	-	-
Acridocarpus natalitius s	25	-	6.3	25	6.3	3.1	25	-	50	-	-	-	-
Catharanthus roseus s	50	25	-	50	50	25	25	-	-	50	-	-	50
Chromolaena odarata s	6.3	12.5	6.3	-	6.3	6.3	12.5	50	50	25	-	-	-
Dichrostachys cinerca s	25	50	25	50	6.3	6.3	25	50	50	-	-	-	-
Faurea macnaughton s	-	-	50	-	12.5	25	25	-	25	25	-	-	-
Hypoxis hemerocallidea s	25	25	12.5	-	25	25	12.5	-	-	12.5	-	-	-
Lippia javanica s	-	50	0.01	-	50	25	50	-	-	50	-	-	-
Maytenus heterophylla s	25	12.5	0.01	25	0.01	12.5	0.01	12.5	-	50	50	25	-
Melia azedarach s	25	-	50	-	25	25	25	25	-	50	-	-	-
Psidium guajava s	50	6.3	-	-	3.1	6.3	50	50	-	25	-	-	-
Schotia brachypetala s	-	50	3.1	25	0.01	25	3.1	50	-	-	12.5	-	-
Sclerocarya birrea s	-	50	6.3	-	12.5	-	25	-	-	-	-	-	-
Tetradenia riparia s	25	50	25	-	6.3	6.3	25	-	50	50	50	-	-
Trichilia dregeana s	12.5	-	25	-	12.5	6.3	50	-	50	-	-	-	-
Vernonia oligocephala s	50	-	25	12.5	-	6.3	6.6	-	-	-	-	-	-
Vernonia tigna s	-	-	3.1	-	0.01	-	-	50	-	-	-	-	-
Callilepis laureola t	-	25	25	-	50	50	25	25	-	50	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	6.3	6.3	0.01	0.01	6.3	6.3	0.01	3.1	3.1	3.1	0.01	0.01	0.01

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, – = no minimum inhibition concentration. Bacteria collected at lancet laboratory: Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal – *Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulphoxide.

Different plant parts: **l** – Leaves, **p** – Plant, **s** – Stem, **t** – Tuber, **f** – Flowers and **b** – Bark.

Table 2. Minimum inhibitory concentrations from medicinal plants used for the treatment of diarrhoea in Ongoye forest.

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Methanol													
Ihlaza b	-	-	6.3	12.5	3.1	6.3	25	-	50	25	-	-	-
Syzygium cordatum b	6.3	-	6.3	3.1	3.1	3.1	6.3	-	-	50	25	-	-
Schotia brachypetala b	50	-	12.5	-	12.5	3.1	6.3	-	-	-	-	-	-
Sclerocarya birrea b	-	-	50	12.5	3.1	12.5	-	50	-	-	-	50	-
Ungazini b	-	-	6.3	6.3	25	6.3	-	-	12.5	-	-	50	-
Tinge tinge l	-	-	0.01	-	-	25	25	-	-	-	6.3	-	-
Acacia karroo l	50	6.3	50	25	6.3	6.3	6.3	25	50	25	-	-	-
Acacia robusta l	-	-	6.3	0.01	3.1	0.01	6.3	-	-	-	25	-	-
Acanthospermum australe l	-	-	6.3	-	25	12.5	6.3	-	50	-	25	50	-
Aloe arborescens l	-	6.3	6.3	0.01	3.1	25	25	1.6	0.01	12.5	-	-	-
Catharanthus roseus l	25	-	12.5	3.1	1.6	-	6.3	-	50	-	-	-	-
Chromolaena odarata l	-	25	6.3	50	12.5	3.1	0.01	3.1	-	-	50	-	-
Dichrostachys cinerea l	-	-	3.1	-	6.3	-	6.3	-	-	25	-	-	-
Faurea macnaughton l	-	-	0.01	0.01	3.1	6.3	25	-	12.5	-	-	-	-
Hypoxis hemerocallidea l	-	3.1	-	12.5	6.3	3.1	-	12.5	-	25	-	-	-
Lippia javanica l	-	-	0.01	-	0.01	6.3	25	-	50	-	-	50	-
Maytenus heterophylla l	-	6.3	3.1	-	12.5	6.3	12.5	-	-	-	-	-	-
Melia azedarach l	25	-	3.1	25	25	25	-	50	-	12.5	-	-	-
Psidium guajava l	-	50	0.01	25	6.3	6.3	25	-	25	-	25	50	-
Schotia brachypetala l	-	-	12.5	25	6.3	6.3	25	-	-	50	-	-	-
Sclerocarya birrea l	-	-	6.3	-	3.1	3.1	12.5	-	-	-	25	-	-
Syzygium cordatum l	25	25	6.3	-	0.01	0.01	25	-	6.3	-	-	12.5	12.5
Tetradenia riparia l	50	-	6.3	12.5	0.01	50	6.3	50	-	-	50	-	-
Trichilia dregeana l	-	-	6.3	6.3	50	1.6	12.5	6.3	-	6.3	-	12.5	-
Tinge oligocephala l	-	-	0.01	-	3.1	3.1	3.1	6.3	25	-	25	-	-
Tinge tinge l	25	-	50	-	25	25	3.1	-	25	-	-	-	-
Baccharoides adoensis p	50	-	-	-	6.3	3.1	-	12.5	-	-	6.3	-	-

Table 2. (Continued)

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Chenopodium ambrosioides p	-	-	25	12.5	12.5	25	-	25	50	-	50	-	-
Hewittia malambarica p	-	-	6.3	-	6.3	12.5	25	-	50	-	-	-	-
Acacia robusta s	-	-	12.5	12.5	3.1	0.01	0.01	-	-	25	-	-	50
Acanthospermum australe s	-	-	3.1	6.3	6.3	0.01	3.1	6.3	6.3	12.5	12.5	-	6.3
Acridocarpus natelitius s	-	-	50	-	50	25	12.5	-	-	-	-	-	-
Catharanthus roseus s	-	12.5	6.3	50	-	50	-	-	50	-	-	-	-
Chromolaena odarata s	-	-	6.3	50	12.5	6.3	-	6.3	12.5	-	25	-	-
Dichrostachys cinerea s	-	-	50	25	25	3.1	25	-	50	-	-	25	-
Faurea macnaughton s	-	-	50	3.1	12.5	25	0.01	-	-	-	-	-	-
Hypoxis hemerocallidea s	3.1	50	12.5	3.1	6.3	6.3	12.5	-	-	50	-	-	-
Lippia javanica s	-	-	6.3	-	12.5	3.1	12.5	50	50	-	-	-	-
Maytenus heterophylla s	-	-	25	-	50	50	-	-	50	50	-	-	-
Melia azedarach s	-	-	3.1	-	3.1	6.3	25	-	25	-	-	-	-
Psidium guajava s	-	12.5	6.3	6.3	3.1	0.01	12.5	12.5	-	50	6.3	-	-
Schotia brachypetala s	-	6.3	3.1	12.5	0.01	6.3	12.5	-	-	3.1	-	6.3	-
Sclerocarya birrea s	-	50	6.3	-	50	6.3	6.3	50	-	50	-	-	-
Tetradenia riparia s	-	-	6.3	3.1	3.1	6.3	-	50	-	-	-	-	50
Trichilia dregeana s	50	-	6.3	50	-	6.3	6.3	50	25	-	-	50	25
Vernonia oligocephala	-	50	25	6.3	12.5	12.5	6.3	-	-	3.1	6.3	-	-
Vernonia tigna s	6.3	-	3.1	25	3.1	6.3	25	-	25	50	3.1	6.3	-
Callilepis laureola t	-	-	50	-	25	-	6.3	-	25	-	12.5	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, – = no minimum inhibition concentration. Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains- P 5020, P4790 , T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403.

DMSO - dimethly sulphoxide. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

Table 3. Minimum inhibitory concentrations from medicinal plants used for the treatment of diarrhoea in Ongoye forest.

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Cold water													
Ihlaza b	-	12.5	0.01	-	25	6.3	25	-	25	50	-	-	-
Syzygium cordatum b	-	25	12.5	25	12.5	6.3	3.1	3.1	25	-	25	-	-
Schotia brachypetala b	6.3	-	25	-	25	3.1	25	-	25	-	-	3.1	-
Sclerocarya birrea b	6.3	-	25	-	6.3	0.01	25	50	-	-	-	-	50
Ungazini b	-	6.3	25	-	6.3	6.3	25	-	-	50	-	50	-
Vernonia tigna l	-	-	6.3	12.5	50	6.3	6.3	-	-	50	-	50	-
Acacia karroo l	6.3	25	3.1	25	3.1	12.5	25	50	-	-	-	12.5	-
Acacia robusta l	0.01	0.01	0.01	-	25	6.3	-	25	50	-	50	-	-
Acanthospermum australe l	-	-	6.3	25	6.3	6.3	6.3	-	6.3	-	6.3	-	12.5
Aloe arborescens l	25	-	0.01	-	6.3	3.1	50	-	-	50	12.5	25	-
Catharanthus roseus l	50	12.5	6.3	6.3	50	12.5	12.5	6.3	-	-	6.3	-	-
Chromolaena odarata l	6.3	3.1	3.1	6.3	50	6.3	6.3	-	-	-	-	-	-
Dichrostachys cinerea l	3.1	3.1	6.3	50	25	50	3.1	-	50	-	25	-	50
Faurea macnaughton l	-	-	3.1	-	25	6.3	25	-	-	25	-	50	-
Hypoxis hemerocallidea l	-	50	6.3	50	50	3.1	50	-	50	-	-	-	50
Lippia javanica l	50	6.3	25	50	-	3.1	-	-	-	50	-	-	-
Maytenus heterophylla l	-	-	6.3	-	3.1	50	0.01	50	-	50	-	25	
Melia azedarach l	-	25	6.3	-	3.1	25	50	-	-	50	-	25	-
Psidium guajava l	6.3	6.3	6.3	50	6.3	50	6.3	-	50	-	25	25	-
Schotia brachypetala l	3.1	25	-	12.5	3.1	-	6.3	50	-	-	-	12.5	-
Sclerocarya birrea l	-	25	12.5	-	12.5	6.3	6.3	-	25	50	50	25	-
Syzygium cordatum l	3.1	3.1	6.3	6.3	0.01	0.01	6.3	50	3.1	50	-	50	-
Tetradenia riparia l	3.1	-	0.01	-	3.1	50	-	-	-	-	-	50	-
Trichilia dregeana l	-	-	3.1	-	6.3	6.3	-	-	-	50	6.3	-	-
Vernonia oligocephala l	6.3	3.1	0.01	3.1	0.01	-	50	-	-	6.3	6.3	3.1	-
Vernonia tigna l	-	-	3.1	12.5	-	25	50	-	-	-	-	-	-
Baccharoides adoensis p	0.01	-	50	-	25	25	-	-	25	50	-	-	-
Chenopodium ambrosioides p	-	12.5	3.1	-	25	12.5	6.3	-	-	-	-	-	-
Hewittia malambarica p	-	-	6.3	-	6.3	3.1	-	-	-	-	-	-	
Acacia robusta s	-	50	25	-	6.3	25	3.1	25	-	-	-	25	-
Acanthospermum australe s	-	50	6.3	50	25	50	6.3	50	-	50	-	50	-
Acridocarpus natalitius s	-	6.3	3.1	-	6.3	50	25	-	-	-	50	-	-

Table 3. (Continued)

Plant species	Extracts and bacteria* tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Catharanthus roseus s	3.1	12.5	6.3	25	12.5	25	12.5	6.3	-	-	-	25	-
Chromolaena odarata s	12.5	6.3	6.3	50	6.3	3.1	0.01	25	-	-	50	-	-
Dichrostachys cinerca s	50	6.3	6.3	-	6.3	12.5	6.3	50	-	6.3	-	-	-
Faurea macnaughton s	6.3	6.3	50	6.3	6.3	3.1	6.3	-	50	-	50	-	-
Hypoxis hemerocallidea s	6.3	50	12.5	0.01	6.3	3.1	6.3	-	-	50	-	-	-
Lippia javanica s	6.3	25	3.1	25	3.1	12.5	25	50	-	-	-	12.5	-
Maytenus heterophylla s	-	6.3	0.01	-	6.3	6.3	0.01	-	50	-	12.5	6.3	-
Melia azedarach s	25	6.3	12.5	12.5	0.01	3.1	-	25	-	-	50	-	-
Psidium guajava s	0.01	6.3	12.5	25	25	12.5	25	-	-	-	-	-	-
Schotia brachypetala s	0.01	50	6.3	-	6.3	-	-	-	50	-	-	-	-
Sclerocarya birrea s	-	50	6.3	12.5	3.1	3.1	6.3	50	-	-	-	-	-
Tetradenia riparia s	-	50	6.3	3.1	0.01	6.3	6.3	-	50	-	25	-	-
Trichilia dregeana s	-	50	6.3	-	6.3	3.1	3.1	3.1	3.1	-	50	3.1	-
Vernonia oligocephala	50	-	6.3	6.3	12.5	6.3	12.5	-	-	6.3	-	-	-
Vernonia tigna s	25	25	6.3	-	6.3	50	50	-	-	-	-	-	-
Callilepis laureola t	-	-	3.1	-	-	6.3	3.1	-	25	-	-	6.3	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	0.01	0.01	0.01	0.01	3.1	6.3	-	0.01	3.1	3.1	3.1	6.3	3.1

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, – = no minimum inhibition concentration, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii, Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains- Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulphoxide. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.

Table 4. Minimum inhibitory concentrations from medicinal plants used for the treatment of diarrhoea in Ongoye forest.

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Hot water													
Ihlaza b	-	-	50	-	6.3	-	50	-	-	50	6.3	12.5	50
Syzygium cordatum b	0.01	3.1	3.1	6.3	0.01	0.01	3.1	50	50	3.1	3.1	50	-
Schotia brachypetala b	6.3	6.3	6.3	50	6.3	6.3	3.1	-	-	-	-	50	12.5
Sclerocarya birrea b	-	-	6.3	-	50	6.3	6.3	50	-	-	12.5	6.3	-
Ungazini b	50	6.3	-	-	12.5	12.5	-	-	12.5	-	-	-	-
Vernonia tigna f	-	-	6.3	-	6.3	6.3	50	-	-	-	3.1	3.1	-
Acacia karroo l	6.3	25	12.5	-	6.3	6.3	-	-	25	-	-	-	-
Acacia robusta l	-	6.3	12.5	25	6.3	6.3	6.3	-	25	50	-	-	-
Acanthospermum australe l	3.1	3.1	6.3	6.3	3.1	3.1	6.3	-	12.5	-	6.3	25	-
Aloe arborescens l	-	6.3	12.5	-	-	12.5	12.5	-	-	-	25	-	-
Catharanthus roseus l	50	25	6.3	50	50	12.5	12.5	-	50	-	25	-	-
Chromolaena odarata l	-	12.5	-	-	25	-	25	-	-	-	12.5	50	50
Dichrostachys cinerea l	25	50	3.1	50	6.3	3.1	6.3	12.5	-	-	-	50	-
Faurea macnaughton l	6.3	25	-	-	6.3	6.3	25	50	-	-	25	-	-
Hypoxis hemerocallidea l	-	50	6.3	50	12.5	6.3	6.3	-	50	12.5	6.3	-	50
Lippia javanica l	-	-	25	6.3	-	6.3	6.3	-	-	50	-	-	-
Maytenus heterophylla l	12.5	50	6.3	6.3	25	50	-	-	-	-	-	-	-
Melia azedarach l	-	12.5	-	50	12.5	12.5	50	25	-	-	-	-	-
Psidium guajava l	6.3	6.3	6.3	50	12.5	50	6.3	-	50	-	12.5	6.3	-
Schotia brachypetala l	6.3	25	-	50	6.3	-	6.3	50	-	-	-	25	-
Sclerocarya birrea l	50	6.3	6.3	-	3.1	12.5	6.3	-	-	6.3	50	12.5	-
Syzygium cordatum l	6.3	3.1	6.3	12.5	3.1	0.01	6.3	50	3.1	50	-	50	-
Tetradenia riparia l	3.1	-	3.1	-	3.1	3.1	50	-	-	-	-	50	-
Trichilia dregeana l	50	-	6.3	25	3.1	-	12.5	-	50	-	6.3	-	-
Vernonia oligocephala l	-	50	-	25	-	12.5	6.3	50	-	-	-	25	-
Vernonia tigna l	-	-	50	-	12.5	-	6.3	-	50	-	50	-	-
Baccharoides adoensis p	12.5	-	-	12.5	25	-	12.5	-	-	-	50	-	-
Chenopodium ambrosioides p	50	-	6.3	3.1	25	50	25	50	-	-	-	-	-
Hewittia malambarica p	50	6.3	12.5	6.3	25	3.1	1.6	-	50	-	-	-	-
Acacia robusta s	50	-	-	50	12.5	3.1	3.1	-	3.1	50	3.1	-	-
Acanthospermum australe s	25	-	-	-	6.3	12.5	12.5	6.3	6.3	-	12.5	-	-
Acridocarpus natalitius s	6.3	12.5	-	-	3.1	-	25	50	-	-	25	50	-

Table 4. (Continued)

Plant species	Extracts and bacteria[a] tested (MIC in mg/ml)												
	ATCC CULTURES				Sa P5020	Sa P4790	Sa T1266	Ec U1505S	Ec U16406	Ec U16403	Ss	Sf	Sal
	Bs	Kp	Sa	Ec									
Catharanthus roseus s	-	3.1	-	-	50	-	50	50	6.3	-	12.5	25	-
Chromolaena odarata s	25	50	3.1	50	6.3	3.1	6.3	12.5	-	-	-	50	-
Dichrostachys cinerea s	-	-	6.3	3.1	3.1	6.3	12.5	12.5	12.5	-	-	-	-
Faurea macnaughton s	-	6.3	3.1	3.1	12.5	6.3	6.3	-	-	-	-	50	-
Hypoxis hemerocallidea s	-	50	12.5	50	12.5	3.1	6.3	-	50	50	6.3	-	50
Lippia javanica s	-	25	50	-	3.1	6.3	6.3	-	-	-	-	-	-
Maytenus heterophylla s	-	50	12.5	-	3.1	12.5	50	-	-	50	-	-	-
Melia azedarach s	-	50	50	50	6.3	6.3	12.5	-	12.5	-	-	-	-
Psidium guajava s	-	6.3	-	-	12.5	6.3	-	50	-	25	3.1	50	25
Schotia brachypetala s	6.3	25	12.5	-	6.3	6.3	-	-	25	-	-	-	-
Sclerocarya birrea s	6.3	25	6.3	50	3.1	6.3	6.3	-	25	50	-	-	-
Tetradenia riparia s	6.3	50	50	6.3	-	12.5	6.3	-	-	50	50	6.3	-
Trichilia dregeana s	-	50	12.5	-	25	25	25	-	-	50	-	-	-
Vernonia oligocephala	50	25	12.5	6.3	-	25	6.3	-	6.3	-	6.3	-	-
Vernonia tigna s	50	-	3.1	-	50	25	6.3	6.3	-	50	-	-	-
Callilepis laureola t	50	6.3	6.3	-	12.5	25	12.5	-	50	25	0.01	3.1	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-
Neomycin	3.1	3.1	3.1	3.1	0.01	0.01	0.01	3.1	3.1	3.1	0.01	0.01	6.3

Bs – *Bacillus subtilis*, Kp – *Klebsiella pneumoniae*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, – = no minimum inhibition concentration, Ss – *Shigella sonnei*, Sf – *Shigella flexneri*, Sal –*Salmonella typhii*, *Staphylococcus aureus* strains - P 5020, P 4790, T 1266 and *Escherichia coli* strains - Ec U1505s, Ec U16406, Ec U16403. DMSO - dimethly sulphoxide. Different plant parts: l – Leaves, p – Plant, s – Stem, t – Tuber, f – Flowers and b – Bark.