An ethnopharmacological study of plants used for treating respiratory infections in rural Maputaland

by

Talita York

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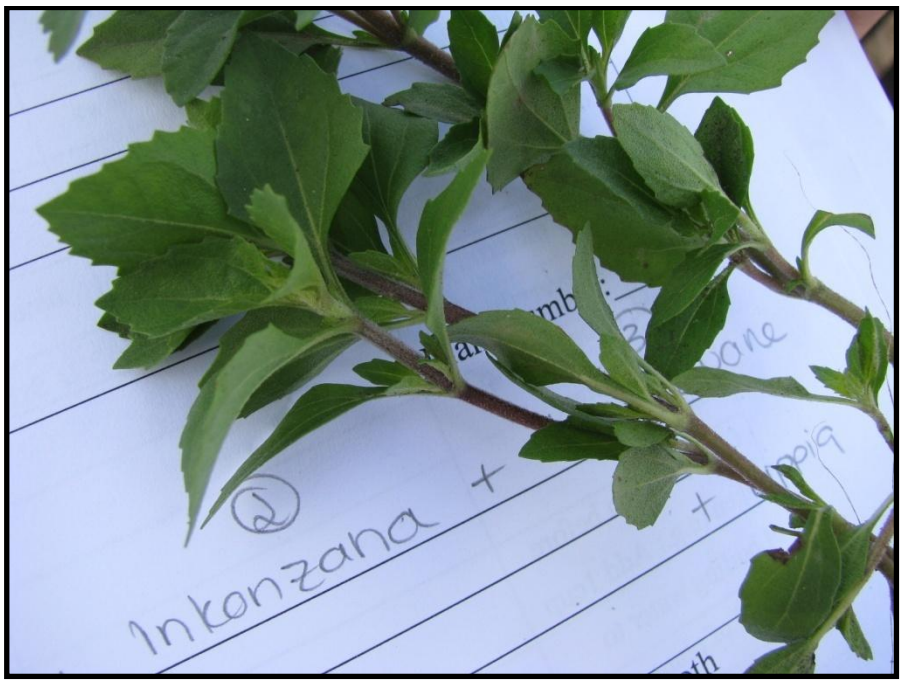

A dissertation submitted to the Faculty of Science and Agriculture, in fulfilment of the requirements for the degree of Masters in Science in Botany in the Department of Botany at the University of Zululand

Supervisors: Dr H. de Wet and Prof S.F. van Vuuren

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Declaration

I, Talita York declare that this dissertation is my own work. It is being submitted for the fulfilment for the degree of Masters of Science in Botany at the University of Zululand, KwaDlangezwa, KwaZulu-Natal. It has not been submitted before for any degree or examination at this or any other University.

Talita York: _____

Date: _____

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Abstract

This study aimed, firstly, to perform an ethnobotanical survey, focusing on lay people's knowledge on plants used for treating respiratory infections in northern Maputaland, KwaZulu-Natal, South Africa. Eighty homesteads were visited purposively and ethnobotanical information was gathered using structured questionnaires. The study documented 30 plant species, with *Acanthospermum glabratum, Aloe marlothii, Krauseola mosambicina, Ozoroa obovata, Parinari capensis* subsp. *incohata* and *Plectranthus neochilus* recorded for the first time, globally, as medicinal plants used for treating respiratory ailments and related symptoms. The two most frequently used plant species were the indigenous *Lippia javanica*, followed by the exotic *Eucalyptus grandis*. Both these plant species are highly aromatic. For eight of the 30 plant species used, new vernacular names, not recorded before, were given by the interviewees. Although six of the 80 interviewees were healers, the current study focused on lay knowledge, which makes the information recorded during the current survey invaluable.

The antimicrobial activity of all 30 plant species were undertaken using the minimum inhibitory concentration (MIC) assay against pathogens associated with respiratory infections. Organic and aqueous extracts, as well as essential oils of aromatic plant species, were tested. Selected plant parts were assessed for antimicrobial activity against a range of pathogens involved in respiratory infections i.e. *Cryptococcus neoformans, Klebsiella pneumoniae, Moraxella catarrhalis, Mycobacterium smegmatis* and *Staphylococcus aureus*. The most active aqueous extracts were that of *O. obovata* and *Sclerocarya birrea*, (MIC values of 0.10 mg/mL against *C. neoformans*), while the most active organic extracts were *P. capensis* subsp. *incohata* (0.03 mg/mL against *C. neoformans*), *S. birrea* (0.04 mg/mL against *C. neoformans*) and *Tetradenia riparia* (0.03 mg/mL against *S. aureus*). *Sclerocarya birrea* and *Syzygium cordatum* had the broadest antimicrobial activities, having noteworthy MIC values against four of the five test organisms. For the essential oils, *L. javanica* displayed the lowest MIC value (0.25 mg/mL against *C. neoformans*).

Fifty of the 80 homesteads used plants in different combinations, and the efficacy of these combinations was evaluated using the ∑FIC index. Twenty-three of the 30 plant species documented during the current survey were combined (in combinations of two-, three- or four

plant species) to produce 24 different plant combinations. Besides these 24 different plant combinations, there was also a combination in which the roots and the leaves of the same species (*L. javanica*) were mixed. The most synergistic interaction noted was from the combination of *L. javanica* essential oil with *Brachylaena* cf. *uniflora* organic extract (∑FIC value = 0.18 against *C. neoformans*). Other combinations displaying synergism, include *E. grandis* essential oil with *O. obovata* organic extract (∑FIC value = 0.28 against *K. pneumoniae*). Also, *S. cordatum* with *Terminalia sericea* organic extracts (∑FIC value = 0.20 against *C. neoformans* and 0.31 against *K. pneumoniae*) and *L. javanica* with *Trichilia emetica* organic extracts (∑FIC value = 0.47 against *M. smegmatis*).

For a selection of plant combinations the isobologram ratio method has been used to determine if synergistic interactions were optimal at varied ratios. When using the isobologram method, the 1:1 organic extract combination of *S. cordatum* with *T. sericea* displayed the strongest synergistic interaction (against *C. neoformans*).

Overall, results demonstrate that frequency of use do not necessarily always correlate with high antimicrobial efficacy. The most frequently used plant species (*L. javanica* and *E. grandis*) did not show the strongest antimicrobial activity. Some of the fewer mentioned plant remedies (e.g. *P. capensis* subsp. *incohata*, *O. obovata*, *S. birrea* and *S. cordatum*) revealed some of the best overall antimicrobial activities. Analyses of selected plant extracts validate the antimicrobial efficacy of most of these plants. In the case of the combined remedies, the predominantly additive interactions of the aqueous extracts against certain test organisms lent credibility to the traditional use of most of these plant combinations in the treatment of respiratory infections.

Publications

York, T., De Wet, H., Van Vuuren, S.F., 2011. Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology 135, 696-710.

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Table of contents

List of figures

List of tables

List of abbreviations

ADC - Bovine albumin fraction V 2.5, Dextrose and Catalase

AIDS - Acquired Immunodeficiency Syndrome

ATCC - American Type Culture Collection

CDC - Centres for Disease Control and Prevention

CFU - Colony Forming Units

CLSI - Clinical and Laboratory Standards Institute

COPD - Chronic Obstructive Pulmonary Disease

DMSO - Dimethyl Sulphoxide

FIC - Fractional Inhibitory Concentration

g - Gram

HIV - Human Immunodeficiency Virus

h - Hour(s)

INT - Iodonitrotetrazolium chloride

L - Litre

LD_{50} - Lethal Dose, 50%

MIC - Minimum Inhibitory Concentration

mg - Milligram

mL - Millilitres

mm - Millimetres

NNIS - National Nosocomial Infections Surveillance System

spp. - Species

STI - Sexually Transmitted Infection

subsp. - Subspecies

TB - Tuberculosis

TSB - Tryptone Soy Broth

WHO - World Health Organization

WSIALD - Wood-Smoke Inhalation-Associated Lung Disease

∑FIC - Sum of the Fractional Inhibitory Concentration Index

µL - Micro Litre

°C - Degrees Celsius

Chapter 1

Introduction

1.1 Background

This study is part of an on-going research project registered by Dr De Wet (S378/08) in northern Maputaland, KwaZulu-Natal. The project investigates lay people's knowledge on the use of plants to treat various infectious diseases. Besides the current study, which has been conducted in northern Maputaland, the other studies in this area include both antidiarrhoeal plants (De Wet et al., 2010), as well as plants used to treat sexually transmitted infections (De Wet et al., 2012). Infectious diseases are a major cause of mortality globally, and specifically in developing countries, with acute respiratory infections being the most significant. Sexually transmitted infections, diarrhoea, as well as most respiratory illnesses, are communicable. Small rural communities, as found in the study area of northern Maputaland, are at high risk of developing these infections and are of great concern to epidemiologists (Nester et al., 2001; Boutayeb, 2006).

1.2 Respiratory infections - globally and in South Africa

Millions of people worldwide suffer daily from chronic respiratory diseases (WHO, 2010a). Currently 210 million people have Chronic Obstructive Pulmonary Disease (COPD) globally, and according to the latest estimates COPD is predicted to become the third leading cause of death by the year 2030 (WHO, 2008a). Due to the high co-occurrence with human immunodeficiency virus (HIV) infections, acute bacterial pneumonia is responsible for a significant amount of deaths globally (Feikin et al., 2004). Pneumonia is also the leading cause of childhood deaths worldwide, especially in developing countries (Shann et al, 1999), killing an estimated 1.6 million children every year (WHO, 2010b). Otitis media is one of the most frequently occurring bacterial infections among children (Huebner et al., 2003). Even though it is often fatal in developing countries, pneumonia is typically curable in developed countries (Scott et al., 2008). In low-income countries of Africa pneumonia is still the main cause of childhood deaths (Scott et al., 2008), and in South Africa, in 2000, almost 6% of all childhood deaths were due to lower respiratory tract infections (Bradshaw et al., 2003). Some of the issues effecting children's health are the high levels of poverty and unemployment present in this country (Bradshaw et al., 2003). In South Africa some of the leading causes of death include influenza, pneumonia and TB (Statistics South Africa, 2005), with a significant degree of

coinfection with TB and HIV being evident (Taylor, 2003). Seven of the countries with the highest rates of TB, globally, are in southern Africa, with more than 300 000 cases recorded every year in South Africa (Naidoo, 2008). Globally 9.2 million new cases and 1.7 million deaths occurred in 2006 due to tuberculosis (TB). Of these deaths, 12% were in HIV-positive individuals (WHO, 2008b).

1.3 Respiratory pathogens and related symptoms

There are a large variety of respiratory illnesses, which can be divided into infections of the upper part of the respiratory system, mainly the head and neck, and infections of the lower respiratory system, the chest (Nester et al., 2001). Some of the known respiratory microorganisms are Gram-negative bacteria such as *Haemophilus influenzae* and *Moraxella catarrhalis* (Nester et al., 2001). *Streptococcus pneumoniae*, a Gram-positive bacterium, is the common cause of pneumonia - an acute illness in which the lung's alveolar air spaces become inflamed and filled with white blood cells and fluid. Even though pneumonia can be caused by a viral or parasitic infection, the most severe cases are caused by bacteria. Studies show that 50% of pneumonia cases in children in developing countries are caused by either *H. influenza* or *S. pneumoniae* (Scott et al., 2008). *Klebsiella pneumoniae* and other Gram-negative rod shaped bacteria, often cause pneumonia in immunocompromised people. Symptoms of *Klebsiella* pneumonia include chills, fever, mucoid sputum, cough, chest pain and bloody sputum (Nester et al., 2001). Another pathogen able to infect the respiratory system of people with a compromised immune system is *Cryptococcus neoformans*. Cryptococcosis is an invasive fungal infection, most commonly manifesting itself in the form of meningitis. In recent years the annual death rate due to fungal infections has risen considerably (Hemaiswarya et al., 2008), and the formerly rare incidence of pulmonary cryptococcosis has become more common due to its frequent occurrence in acquired immunodeficiency syndrome (AIDS) patients. Patients with AIDS or other types of immunocompromising diseases are most commonly susceptible to this disease. *Cryptococcus neoformans* is not part of the normal flora of the respiratory tract in humans or animals and is commonly found in pigeon droppings, but inhalation of cryptococcal particles can cause cryptococcosis (Kerkering et al., 1981; Levitz, 1991; Aberg et al., 1999; Colen et al., 2007). Pulmonary cryptococcosis leads to pleural effusions, causing symptoms like coughing and chest pain (Núñez et al., 2000; Lindell et al., 2005). *Moraxella catarrhalis* is part of the natural flora of the nasopharynx, but has recently emerged as a real pathogen causing upper respiratory tract infections in healthy elderly people and children (Nester et al., 2001; Verduin et al., 2002).

Moraxella catarrhalis is also an important cause of infections of the lower respiratory tract-specifically with adults suffering from COPD. Young children often fall victim to otitis media, a painful inflammation of the middle ear commonly caused by *M. catarrhalis* (Rovers et al., 2004). Otitis media frequently occur together with sinusitis, and these highly common upper respiratory infections are often caused by the same causative agent. Sinusitis is the inflammation of one or more of the sinuses, causing a painful sensation in the region of infection (Nester et al., 2001). During a survey conducted by Wald et al. (1984), it was found that *M. catarrhalis* accounted for approximately 20% of sinusitis cases in children. Chronic sinusitis can lead to chronic bronchitis, initially characterised by coughing and eventually leading to shortness of breath and respiratory insufficiency (Nester et al., 2001; Van Wyk and Wink, 2004). *Staphylococcus aureus*, an opportunistic pathogen, is carried in the noses of about 20% of healthy people, and can also cause otitis media (Nester et al., 2001). Lower respiratory tract infections such as pneumonia can also be caused by *S. aureus*, and according to the National Nosocomial Infections Surveillance System (NNIS) of the Centers for Disease Control and Prevention (CDC), *S. aureus* was the most common cause of hospital acquired pneumonia during the period of January 1990 to May 1999 (CDC NNIS System, 1999). Another disease that affects the lower respiratory tract is TB. Tuberculosis is usually contracted when airborne organisms, coming from a person already suffering from the disease, are inhaled. The disease starts in the lungs, thereafter it can spread to the rest of the body. Most cases of Tuberculosis are caused by *Mycobacterium tuberculosis*, and this disease has recently risen in incidence due to the HIV/AIDS epidemic. It is a chronic disease, and symptoms characteristic of this illness include night sweats, a chronic cough, weight loss, fever, and often also blood in the sputum (Nester et al, 2001).

1.4 Medicinal plants and traditional medicine

Medicinal plant seeds have been excavated in Neanderthal-settlements, indicating the use of plant remedies since the existence of mankind (Hedberg and Staugård, 1989). This early dependence on nature was led by instinct, taste and experience and passed on from one generation to the next (Kim, 2005). One concept on which the uses of medicinal plants have been based on is the Doctrine of Signatures. This perception is based on the belief that a plant's appearance gives clues to the ailment it should be used for (Court, 1985; Van Wyk and Wink, 2004). If a plant shape looks similar to a specific human organ it will then be seen as the correct remedy for healing that body part. This doctrine has developed independently in countries like

China, India, South America and Africa, but according to Pujol (1990) this form of medicine administration is more part of the African herbalist than any other type of healer worldwide. The traditional healers of the South African Zulu culture believe that all herbal remedies contain signs from God (Pujol, 1990). This dogma, where organoleptic properties of plants play an important role in their medicinal selection, can also be seen in the doctrine of the bodily humors. It is based on the selection of medicinal plants as remedies for ailments having opposite qualities (e.g. hot vs. cold). Based on this doctrine, the medicinal beliefs of Hippocrates (460-377BC) and Aristotle (384-322BC) is believed to have been the main influence on the European healing system (Van Wyk and Wink, 2004; Leonti, 2011). In turn their ideas, on which Greek and Roman medicine is based, were derived from the ancient Indian and Egyptian principles, and first defined by Galen ($_{AD}$ 131-199). The concept of this doctrine is founded on the belief that the world consists of four elements, each of which is linked to its corresponding humor. The four humors are the four vital fluids of the body (blood, phlegm, black bile and yellow bile), which are each associated with a sensory experience (cold, heat, dry, damp) that needed to be in balance in order to promote a healthy body. Herbs containing opposite qualities were then applied to restore this balance. One of the two concepts on which ancient Chinese medicine is based on is very similar to this system. Even though ancient Chinese herbalism is believed to be over 5000 years old, traditional Indian medicine (Ayurvedic medicine), which is also based on the doctrine of the bodily humors, is possibly even older (Van Wyk and Wink, 2004). As concluded from many studies, the Euasterids (e.g. Asteraceae and Lamiaceae) are generally the most popular plants for medicinal use. It has been noted that these plants are highly detectable, showing distinct noticeable characteristics. It is these exact characteristics that could link such plants to one of the medicinal doctrines. Although such characteristics are often present in plants to attract pollinators, they can also attract unwanted predators. Secondary metabolites are then present in such plants to deter these predators, and it is believed that it is these biological substances that give them medicinal value (Leonti, 2011).

Supernatural powers, such as religion or magic, have been associated with medicinal plant use worldwide, but since 200 years ago modern allopathic medicine has been slowly displacing these practices in Europe and North America (Leonti, 2011). This slow change to modern medicine probably originated when the importance of the correct dose for a medical treatment was first stressed (Van Wyk and Wink, 2004). In 1804 the first active substance in a medicinal plant had been identified when morphine was isolated from *Papaver somniferum* L.. During the rapid development of modern medicine the importance of medicinal plants decreased in comparison to

products from the pharmaceutical industry. During the 70's and the 80's, however, many countries have regained interest in the area of herbal medicine (Hedberg and Staugård, 1989). Increasingly more scientific evidence has started to emerge on the healing properties of herbs. The traditional healers and their wisdom in herbal healing, previously demoted by modern medicine and science, are now attaining recognition as a powerful source of medicinal knowledge (Voeks, 2007). While conventional medicines aim to cure a disease, herbal remedies can be used as a preventative rather than a cure (Farmer-Knowles, 2010). This current popularity of herbal remedies has, amongst others, been influenced by the spread of traditional Chinese medicine to the rest of the world. Herbal medicines are still used today but by means of supporting modern medicine. In many cultures herbal medicines are favoured for chronic ailments, while acute or severe diseases are treated by modern medicine (Van Wyk and Wink, 2004). The recent rise in microbial drug resistance has also been a great cause of concern, catalysing the search for novel antimicrobial agents from plants (Nascimento et al., 2000; Kamatou et al., 2006).

1.5 The history and future of African traditional medicine

There is a general belief that illiterate societies transmit cultural knowledge orally (Leonti, 2011). Even though the earliest written records found on the use of medicinal plants are over 5 000 years old, this form of healthcare is believed to be much older, and some of the oldest medicinal systems originated in Africa (Raskin et al., 2002). Unlike the enormous historical and modern written documentation available on European, Indian and Chinese medicine, African traditional medicine has unfortunately been poorly recorded (Pujol, 1990; Van Wyk and Wink, 2004; Leonti, 2011). The traditional African is highly aware of his natural surroundings, often believing that a plant's appearance indicates its medicinal application (Dugmore and Van Wyk, 2008). In South Africa, and specifically in the Zulu culture, the method of gathering and preparing plants as therapeutic agents is practiced by the *inyanga* or herbalist, who is in charge of the physical health of his people. The *sangoma*, or Zulu spiritual healer uses more powerful medicine as well as magic. The patient initially consults the *inyanga* when ill. The *inyanga* will then attempt to heal his patient if it is believed that his disease is physical. If bewitchment is suspected the patient is then sent to the *sangoma* for spiritual healing (Pujol, 1990). Traditionally, the Zulu culture believed that plants are the most powerful source of healing, and that Africans are children of Mother Nature (Pujol, 1990). The colonization of South Africa brought the coexistence of various different cultural groups, influencing the traditional medicinal

system. At first the traditional medicinal system was regarded as 'backward' by the European settlers (Cocks and Dold, 2000). These thoughts were based on the fact that the use of these traditional medicines is not based on scientific verification and is often seen as superstitious or magical (Hedberg and Staugård, 1989). However, in the late 1800s and early 1900s there was a re-emergence of interest in the southern African traditional medicine, when some of the first ethnobotanical literature was recorded. John Mitchell Watt and Maria Breyer-Brandwijk are some of the pioneers of ethnobotany in South Africa, and the first edition of their book "The Medicinal and Poisonous Plants of Southern and Eastern Africa" was published in 1932 (Hutchings et al., 1996).

The traditional knowledge systems in Africa are mostly oral, passed to the next generation by word of mouth and personal experience (Louw et al., 2002; Light et al., 2005). According to a survey done by Zobolo and Mkabela (2006) the indigenous knowledge of the Zulu tribe is not adequately passed on to the next generation anymore. The youth seem to disregard this form of health care. It is therefore of utmost importance to accurately document such knowledge before it is totally lost to the rest of the world (Hutchings et al., 1996, Van Wyk et al., 2009).

1.6 Combination therapy

During the 20th century efforts have focused on the one-target, one-drug concept, in which it was important to find a single chemical entity able to inhibit a distinct molecular target (Keith et al., 2005). It has lead to the isolation of some successful single-molecule compounds from various different plant species (e.g. artemisinin from *Artemisia annua* L. and quinine from *Cinchona* spp.) (Raskin et al., 2002). When fractionating plant extracts into individual chemical components, it has been found that many of these components didn't have the pharmacological activities that have been detected when testing the whole extracts (Keith et al., 2005). This occurrence might be due to synergistic interactions between compounds found within such plant extracts. There has been a slow movement away from this single drug paradigm (Wagner, 2011). Many illnesses comprise of a complex pathophysiology and a multi-causal aetiology (Wagner and Ulrich-Merzenich, 2009), which has led to the use of combination therapies. The value of multi-molecule therapy can't be ignored and, just like the ancient Chinese healers, modern physicians have discovered that combinations of different medicinal compounds could be much more efficient than a single agent (Keith et al., 2005; Li and Zhang, 2008). Many effective

pharmaceutical treatments are drug combinations, and even the highly specific molecules that target single proteins are hardly ever observed as single entities (Keith et al., 2005). Some of the most virulent diseases like TB and malaria are being treated through the application of multi-drug therapies. Tuberculosis would, for example, reach global epidemic proportions without the multidrug approach currently in use (ionazid, rifampicin, pyrazinamide and ethambutol) (Van Vuuren and Viljoen, 2011). Cancer therapy, hypertension- and HIV treatment are often dependent on multiple drug combinations (Williamson, 2001; Wagner and Ulrich-Merzenich, 2009). Many such drug combinations have been formulated from separate single-compound drugs. Examples of such drugs, currently on the pharmaceutical market, include Advair for asthma (GlaxoSmithKline), Advicor for hypercholesterolaemia (Kos Pharmaceuticals) and Combivir for HIV (GlaxoSmithKline).

In traditional medicine the mixing of plant extracts are claimed to cause improved efficacy through synergistic effects within the mixture (Wagner and Ulrich-Merzenich, 2009). As is the case with ancient Chinese and Ayurvedic medicine, African traditional healers often combine different plant parts or -species for enhanced efficacy, and single plant remedies are rarely used (Van Vuuren and Viljoen, 2011). In many ethnobotanical studies that have been conducted in Africa it has been found that certain plant species are used in combination with one or more other plant species to treat infectious diseases (Togola et al., 2005; Maregesi et al., 2007; Ssegawa and Kasenene, 2007; Dahlberg and Trygger, 2009; Moshi et al., 2009; De Wet et al., 2012). Even though plant combinations are often used to treat infectious diseases, the pharmacological validation of such remedies has been neglected (Van Vuuren and Viljoen, 2011). One main aim of synergy research in the field of phytomedicine is to scientifically explain the therapeutic success of certain herbal remedies (Wagner and Ulrich-Merzenich, 2009), and understanding the synergy involved in traditional medicine could validate their use in combination therapy. Understanding the synergistic mechanisms of such remedies could also be of great significance to drug discovery (Li and Zhang, 2008).

1.7 Primary health care and African traditional medicine

Many African communities are still using traditional healing in their primary health care (Louw et al., 2002), and this is especially true for people living in rural areas (Appidi, 2008). In South Africa, traditional healing is widely practiced by approximately 80% of the black population and forms the backbone of rural healthcare (Jäger et al., 1996; Light et al., 2005). It is crucial for

western health workers to accommodate people's traditional beliefs in order to be able to manage their health problems (Ngubane, 1981). Edginton et al. (2002) has found that the rural inhabitants of South Africa often go to a traditional healer first, before consulting a western healer. Even though traditional healers play an integral part in the primary health care of most African people (Edginton et al., 2002), lay people play an important, and often unnoticed, role in their own primary health care (Dahlberg and Trygger, 2009). Self-care, as it is defined by Levin (1981) is the dominant form of care for most of the world's rural people living in developing countries. Understanding the knowledge that lay people have about traditional remedies has been stressed in a recent survey done by De Wet et al. (2010). The various remedies recorded during this survey emphasized the role that a community's lay knowledge plays in their primary health care. Lay people have their own personal views, often associated with social meanings on a specific ailment. It is clear that lay people's understanding of disease symptoms is distinctly different from that of medical professionals, and it is important to know about, and understand these beliefs in the pursuit of improving the quality of health care (Haller et al., 2008; Prior et al., 2011). Dahlberg and Trygger (2009) proclaimed that most studies don't clearly state whether the information obtained came from a lay person or a traditional healer. Similar to Dahlberg and Trygger (2009), a Kenyan study suggested that field work focusing on professional knowledge only, misrepresented the use of traditional medicine in that area (Geissler et al., 2002). In most countries there are no government standards for the quality of plant remedies, and there is little scientific knowledge verifying their traditional use (Skalli et al., 2007). The current study aims to give preliminary scientific validation for the use of some of the plants reputed to have healing properties against respiratory ailments, and could warrant further studies to assess their potential as effective natural remedies (Hedberg and Staugård, 1989).

1.8 Aims and objectives of this study

The main objective of this study is to explore the knowledge that the lay people living in rural Maputaland have about plants used for respiratory ailments, how this knowledge is used in primary health care, and whether these plant remedies show any antimicrobial efficacy against respiratory pathogens.

In order to reach this objective, these subsequent aims have to be followed:

- To perform an ethnobotanical survey, focusing on lay people's knowledge on plants used for treating respiratory infections in northern Maputaland, KwaZulu-Natal, South Africa.

- To collect and identify the associated plants which are growing in and around the homesteads.
- To test for the antimicrobial activity against various organisms associated with respiratory infections using the minimum inhibition concentration (MIC) assay.
- To perform interactive antimicrobial plant combination studies to determine the efficacy of plants used in combination.

1.9 Significance of this study

Not many ethnobotanical surveys focus on plant use for a specific ailment, but focus instead on the different uses of medicinal plants in a specific study area (Van Wyk et al., 2008; Cakilcioglu and Turkoglu, 2010; Giday et al., 2010; Rokaya et al., 2010; Ayyanar and Ignacimuthu, 2011; Menković et al., 2011; Nadembega et al., 2011; Tangjang et al., 2011). Also, with many ethnopharmacological studies, a selection of medicinally used plant species have been screened against a set of test organisms not necessarily associated with any specific ailment(s). Only a handful of studies have focused on the antimicrobial screening of plant species specifically against organisms associated with the ailment that such plants are traditionally used for (Van Vuuren, 2008). The current study is the first to investigate plant use in the treatment of respiratory ailments in this specific region. It will focus on the antimicrobial screening of these plant species, specifically against organisms associated with these ailments.

In South Africa, many antimicrobial studies on plant use against respiratory ailments have been conducted on previously reported uses (Lall and Meyer, 1999; Seidel and Taylor, 2004; Mativandlela et al., 2006; Eldeen and Van Staden, 2007; Bamuamba et al., 2008; Pallant and Steenkamp, 2008; Buwa and Afolayan; 2009), and the few ethnobotanical surveys that have been conducted, aimed on gathering information from traditional healers only (Mativandlela et al., 2008; Green et al., 2010). Hardly any studies, like the current survey, have focused on finding information directly from the lay people. Novel information gathered from surveys like the present study is important in preserving indigenous knowledge. While most of the ethnobotanical studies on South African medicinal plants used to treat respiratory illnesses have focused on plant species with possible antimycobacterial properties (Lall and Meyer, 1999; Seidel and Taylor, 2004; Mativandlela et al., 2006; Eldeen and Van Staden, 2007; Bamuamba et al., 2008; Mativandlela et al., 2008; Green et al., 2010), few such studies have, as is the aim of the current study, specifically focused on antimicrobially analysing plants against other

organisms associated with respiratory diseases (Pallant and Steenkamp, 2008; Buwa and Afolayan, 2009; Suliman et al., 2010).

Chapter 2

Ethnobotany of plants used in northern Maputaland for the treatment of respiratory diseases

2.1 Introduction

One of the aspects involved in the field of ethnobotany, is the collection of information on the various medicinal uses of plants (Pande et al., 2008). "The Great Native Herbal" is one of the earliest records of ancient Chinese herbal medicine (2800BC), and one of the first ancient Indian herbal records (*The Veda*) was written around 2000BC (Van Wyk and Wink, 2004). Written documentation was available on Indian medicine and such cultural knowledge was often passed on to the next generation through songs and poems. According to general belief *De Materia Medica*, a book written in the first century AD, was the first documented information on European herbals. It served as a foundation for most medicinal recordings following thereafter. Even though these ancient written documentations exist, there is still a great deal of undocumented data available on the medicinal uses of plants – especially in African traditional medicine (Van Wyk and Wink, 2004).

Recently there has been a re-emerging connection between plants and medicine, drawing more attention to the field of ethnobotany (Raskin et al., 2002; Pande et al., 2008). A large proportion of modern medicines still contain phytochemicals, and worldwide about 25% of all drugs prescribed contain compounds derived from flowering plants (Rates, 2001; Raskin et al., 2002). There is a general belief that commonly used medicinal plants, not yet subjected to any phytochemical or pharmacological analysis, could hold valuable therapeutic qualities (Hedberg and Staugård, 1989; Leonti, 2011).

Even though the modern drugs of the pharmaceutical industry have had a tremendous positive impact in the developed world, approximately 75% of the world population don't have access to this form of healthcare. Most of the developing world still relies primarily on herbal medicines (Raskin et al., 2002). The use of these remedies may be dangerous to the health of these people, considering the lack of quality in production, trade and prescription of herbal products. Thus,

knowledge of basic ethnobotany is the first step in attaining knowledge on medicinal plants. Natural product use should be validated, as suggested by the WHO (Rates, 2001). Another motivation behind ethnobotanical and ethnopharmacological research is that indigenous plant derived drugs could be a cheap addition to the synthetic drugs available in developing countries. In the search for these novel, plant derived drugs two approaches can be followed. Plants can be randomly selected and screened for their pharmacological properties, or local practitioners could be approached to find information about the most effective and most frequently used plant remedies. Lastly, ethnobotany has shown to be the most effective link between field studies and pharmacological testing (Hedberg and Staugård, 1989; Heinrich et al., 2009).

Not many ethnobotanical surveys focus on plant use for a specific ailment, but focus instead on the different uses of medicinal plants in a specific study area. Such ailments would range from urinary complaints to skin problems and broken limbs, with respiratory diseases often part of this list (Cakilcioglu and Turkoglu, 2010; Giday et al., 2010; Rokaya et al., 2010; Ayyanar and Ignacimuthu, 2011; Menković et al., 2011; Nadembega et al., 2011; Tangjang et al., 2011). Even though globally not many studies have focused on the traditional treatments of respiratory problems, this subject has been the focus of some Indian surveys (Ballabh and Chaurasia, 2007; Gautam et al., 2007; Savithramma et al., 2007). In one such study the use of traditional remedies for cold, cough and fever, by the Buddhists of an Indian tribal community was investigated (Ballabh and Chaurasia, 2007). During this study it was found that the whole plant (40 %) and roots (25 %) were the most commonly used plant parts, which were mostly prepared into tablet form (Ballabh and Chaurasia, 2007). In another study the knowledge that Indian traditional healers had on plants used to treat asthma was recorded and they found that nearly 80 plants were used for treating this respiratory problem (Savithramma et al., 2007). In Mexico, respiratory diseases have been found to be one of the ailments most frequently treated with traditional medicine (Rojas et al., 2001). Rojas et al. (2001) investigated the traditional use of 6 plant species commonly used in the treatment of respiratory ailments, and found the aerial plant parts to be mostly used (50 %). In a Guatemalan study, ethnobotanical surveys and literature reviews were conducted on plants used to treat respiratory ailments and a total of 234 plant species were recorded. The traditional preparations from the 68 most frequently used plant species were found to be mainly from the leaves (76 %) (Caceres et al., 1991). In Kenya a study was done on remedies used against ear- , nose- and throat infections, and 67 species were found to be used against these respiratory afflictions (Njoroge and Bussmann, 2006). During this study, leaves were also found to be the most popular plant part used (49 %). Herbal preparations were

mostly administered by drinking the boiled plant material, while a cold was commonly treated with steam inhalation. In another Kenyan study, plants used in the treatment of lower respiratory tract infections were documented and antimicrobially analysed (Kariuki and Njoroge, 2011). This survey by Kariuki and Njoroge (2011) documented only the five most frequently used plant species. However, in this study area it was found that bark was by far the most popular plant part used (83 %), and plant parts were mainly boiled with water to produce extracts for medicinal use. Some studies have focused on plants specifically used in treating TB. During a survey conducted in Uganda, traditional healers were interviewed about the traditional treatments used to treat TB, and a total of 88 plant species were recorded. The most frequently used plant parts were the leaves, which were mostly taken orally as a decoction (Tabuti et al., 2010). In Malaysia a novel survey was conducted in which information on plants used for treating respiratory diseases with TB symptoms was gathered from herbalists. In addition to this, previously documented information was also used. A total of 70 plant species were screened for possible antituberculosis activity. This study also documented the leaves to be the most popular plant part used (Mohamad et al., 2011).

In South Africa many of the ethnobotanical surveys done on plants traditionally used in treating respiratory infections are based on previously recorded uses (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). Plants traditionally used to treat TB seem to be the main focus in most of these surveys (Lall and Meyer, 1999; Seidel and Taylor, 2004; Mativandlela et al., 2006; Eldeen and Van Staden, 2007; McGaw et al., 2008; Green et al., 2010). In one such study, plants were not selected based on previous literature but on the responses of two traditional healers. These healers were interviewed based on the plants they used for treating TB patients (Green et al., 2010). Pendota et al. (2008) did a survey in the Eastern Cape Province, South Africa, where they have documented 12 plant species that are used for the treatment of eye infections. Novel information gathered from surveys like this, and the present study, is very important in preserving indigenous knowledge.

2.2 Materials and methods

2.2.1 Ethnobotanical survey and collection of plant material

An ethnobotanical study was conducted in northern Maputaland (KwaZulu-Natal, South Africa) during February-March 2010 to acquire information on plants that are used to treat respiratory

infections. Ethics clearance was obtained from the University of Zululand Ethical Committee before the onset of the study (Reference number: S548/10). The survey was conducted in the four different localities (Mabibi, Mbazwana, Mseleni and Tshongwe), as indicated in Figure 2.1, and focused on the use of plants growing in and around the homesteads.

Homesteads are very spread out in all four areas, and a total of 80 homesteads were visited purposively (Tongco, 2007), where people were interviewed using a structured questionnaire (Appendix A). We began at the homestead nearest to the road that lead into the particular rural area (Mabibi, Mbazwana, Mseleni and Tshongwe) being visited and continued for approximately 10 km, until inhabitants from 20 homesteads had been interviewed. If the homestead inhabitants were available to interview we completed the questionnaire, if not we moved on to the next homestead. The objectives of the study were explained in IsiZulu to each of the interviewees and before conducting the interview, a consent form was signed (Appendix B). The questionnaire was designed to obtain the following information; Locality, sociodemographic data (age, gender and educational background), vernacular plant names, plant parts used, harvest amounts, preparation methods, dosage forms and exact dosage amounts, use of plants in combination as well as the symptoms it will normally be used for.

The plants mentioned in the interviews were collected from the homesteads during February and March 2010, and voucher specimens were prepared on site. Identity of plant samples was authenticated by Dr. Theo Mostert from the University of Zululand, as well as Mr Mkhiphene Ngwenya from the South African National Biodiversity Institute KwaZulu-Natal Herbarium.

2.3 Study area

Northern Maputaland is an area situated in the province of KwaZulu-Natal, South Africa and has a high concentration of endemism, making it floristically unique (Scott-Shaw, 1999; Van Wyk and Smith., 2001). The homesteads visited during the survey is situated between 32°22’ and 32°52’ latitudes and 27°15’ and 27°30’ longitudes (Fig. 2.1). Within this study area there are a few dominant vegetation types, namely Tembe Sandy Bushveld, Maputaland Coastal Belt, Northern Coastal Forest as well as the Maputaland Wooded Grass Land type (Mucina et al., 2005).

These regions are all situated in the Umkhanyakude District Municipality at Umhlabuyalingana Local Municipality. This study area have a population of 140 952 people who are mainly IsiZulu speaking. It is a very poor community with about 85% of the people having no formal income, and the average highest level of education obtained being grade four. Thirty-one percent of the people living in this area have obtained no education at all, while only about 6% have acquired a senior certificate. The majority of the local inhabitants are female (56%). Seventy-two percent of the people living in this area are under the age of 30, while only 8% are over the age of 60 (Municipal Demarcation Board South Africa, 2001). The South African Child Gauge 2009/2010 (Sanders and Bradshaw, 2010) identified exposure to environmental risk factors, such as poorly ventilated and overcrowded living spaces, as risk factor for infectious diseases. This type of lifestyle can be the cause of rapid spread of infections, especially considering that more than 10% of the study area's population shares overcrowded living spaces (Municipal Demarcation Board South Africa, 2001).

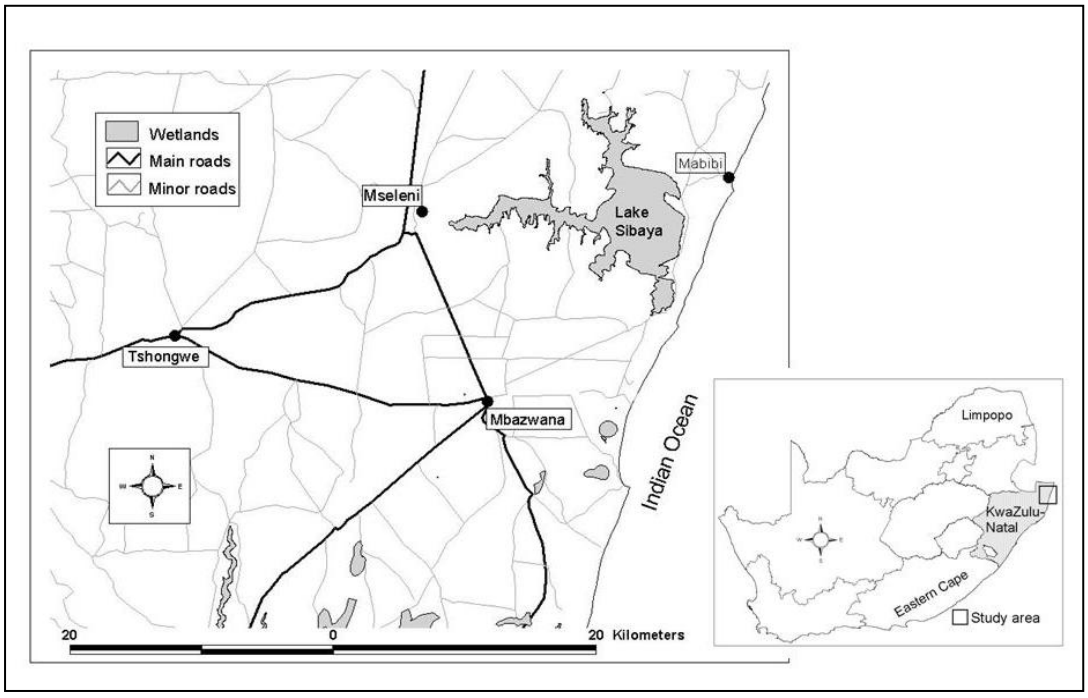


Fig.2.1 Study area: northern Maputaland, KwaZulu-Natal province, South Africa.

2.4 Results and discussion

2.4.1 Socio demographic information

The average age of the people being interviewed was 51, with 78% of them being female. Forty-six percent of the interviewees have received no education at all, while only 10% had a senior certificate. The average highest level of education obtained was grade four. Of the people being interviewed, 93% were lay people, while the rest were either a *sangoma* (diviner) or an *inyanga* (herbalist). Nearing the end of six interviews it was inadvertently discovered that those people in question were in fact some sort of traditional healer. Because the lay interviewees still formed the greater majority of our study group, we decided to keep this data as part of our survey, indicating which remedies were in fact suggested by them (Table 2.1 - 2.4). The majority of the interviewees claimed their grandparents (33%) or parents (33%) to be the source of their medicinal plant knowledge. Some of the other mentioned sources were their neighbours or friends (10%), the elderly (9%) or one of their other relatives (5%). Five percent of the interviewees claimed to be a healer (*inyanga* or *sangoma*). One of these healers shared with us that he received his information in his dreams. Not all of the healers received their knowledge through their healing profession. One of the six healers that were interviewed said that he got his knowledge from his father, even though he himself was an *inyanga*. One person said that, in addition to information received from her friends and neighbours, she also obtained some information from textbooks. When asked about the types of textbooks used, said interviewee showed us the Herbalist Handbook, written by Jean Pujol (Pujol, 1990). The other 5% of the interviewees did not share any knowledge with us, due to reasons discussed later in this chapter. It should also be mentioned that 16% of the interviewees said that their source of information, be it a friend or a family member, had been an *inyanga* or a *sangoma.* Irrespective of where their medicinal knowledge came from, 15% of the interviewees mentioned that they had a healer in the family.

Tables 2.1-2.4 give the lists of the interviewees' ages, as well as the GPS reading of each homestead and their homestead numbers. Also included in these tables is each interviewee's gender, highest level of education, whether he or she is a traditional healer, and, importantly, the medicinal plants mentioned. A detailed account of the plant species used is thereafter provided (Section 2.4.2).

Table 2.1 Interviewees and the plants mentioned to treat respiratory infections in the Mabibi area.

Homestead GPS reading	Homestead number of interviewee	Age	Gender	Highest level of education	Inyanga or sangoma?	Plants used to treat respiratory infections
S27°20'448" E32°43'044"	M48	68	M	Gr. 1	Yes	*Brachylaena discolor*
S27°20'255" E32°43'080"	M51	30	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*
S27°20'151" E32°43'184"	M42	±65	F	None	No	*Eucalyptus grandis*, *Ozoroa obovata*
S27°19'801" E32°43'413"	M61	44	F	Gr. 3	No	*Lippia javanica*, *Senecio serratuloides*
S27°19'724" E32°43'464"	M57	47	F	Gr. 2	No	*Lippia javanica*
S27°19'783" E32°43'574"	M56	±60	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°20'038" E32°43'132"	M41	55	F	Gr. 8	No	*Eucalyptus grandis*, *Lippia javanica*
S27°20'693" E32°43'136"	M45	90	F	None	No	*Sclerocarya birrea*, *Syzygium cordatum*
S27°29'520" E32°42'933"	M46	61	F	None	No	*Brachylaena* cf.*transvaalensis*, *Eucalyptus grandis*, *Lippia javanica*
S27°20'316" E32°43'055"	M50	31	F	Gr. 11	No	*Eucalyptus grandis*, *Lippia javanica*
S27°19'773" E32°43'230"	M52	58	M	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°19'795" E32°43'233"	M53	>70	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°19'699" E32°43'418"	M62	40	F	Gr. 8	No	*Eucalyptus grandis*, *Lippia javanica*, *Senecio serratuloides*
S27°19'880" E32°43'614"	M59	63	F	Gr. 7	No	*Acanthospermum glabratum*, *Eucalyptus grandis*, *Lippia javanica*
S27°19'934" E32°43'606"	M63	16	M	Gr. 9	No	*Senecio serratuloides*, *Lippia javanica*
S27°19'997" E32°43'602"	M60	58	F	Gr. 8	No	*Eucalyptus grandis*, *Senecio serratuloides*
S27°19'997" E32°43'602"	M64	>67	F	Gr. 7	No	*Lippia javanica*, *Trichilia emetica*
S27°19'921" E32°43'663"	M65	±66	F	None	No	*Eucalyptus grandis*, *Lippia javanica*, *Psidium guajava*, *Senecio serratuloides*
S27°19'583" E32°43'886"	M66	76	F	Gr. 1	Yes	*Acanthospermum glabratum*, *Senecio deltoideus*, *Senecio serratuloides*
S27°19'583" E32°43'886"	M67	55	M	None	No	No information

Table 2.2 Interviewees and the plants mentioned to treat respiratory infections in the Mbazwana area.

Homestead GPS reading	Homestead number of interviewee	Age	Gender	Highest level of education	Inyanga or sangoma?	Plants used to treat respiratory infections
S27°31'341" E32°32'297"	MB18	±60	F	None	No	*Lippia javanica*
S27°31'341" E32°32'294"	MB20	29	F	Gr. 7	No	*Eucalyptus grandis*, *Tetradenia riparia*
S27°30'714" E32°32'995"	MB10	±26	F	None	No	*Euphorbia tirucallii*, *Parinari capensis*
S27°30'633" E32°33'222"	MB11	49	F	Gr. 3	No	No information
S27°30'085" E32°33'691"	MB04	75	M	None	No	*Tetradenia riparia*
S27°30'036" E32°33'828"	MB06	37	F	Gr. 3	No	*Lippia javanica*
S27°30'026" E32°33'844"	MB07	56	M	Gr. 4	Yes	*Cyperus articulatus*
S27°29'835" E32°34'156"	MB12	25	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*
S27°29'843" E32°34'215"	MB14	55	F	Gr. 5	Yes	*Eucalyptus grandis*, *Lippia javanica*
S27°30'338" E32°33'390"	MB08	62	F	None	No	No information
S27°30'430" E32°33'334"	MB21	22	M	Gr. 11	No	*Eucalyptus grandis*, *Lippia javanica*
S27°30'449" E32°33'237"	MB22	47	F	Gr. 8	No	*Eucalyptus grandis*, *Lippia javanica*, *Tetradenia riparia*
S27°30'632" E32°33'126"	MB23	<60	F	None	No	*Acanthospermum glabratum*
S27°30'632" E32°33'126"	MB24	>60	F	None	No	*Helichrysum kraussii*, *Lippia javanica*, *Trichilia emetica*
S27°31'423" E32°32'179"	MB25	28	F	Gr. 9	No	*Cyperus articulatus*
S27°31'518" E32°32'089"	MB26	34	F	Gr. 4	No	*Eucalyptus grandis*, *Lippia javanica*, *Tetradenia riparia*
S27°31'541" E32°31'951"	MB27	32	F	Gr. 12	No	*Tetradenia riparia*
S27°31'669" E32°31'884"	MB28	65	M	None	No	*Lippia javanica*, *Trichilia emetica*
S27°31'701" E32°31'985"	MB29	>70	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°31'460" E32°32'240"	MB30	28	F	Gr. 9	No	*Clausena anisata*, *Eucalyptus grandis*, *Lippia javanica*

Table 2.3 Interviewees and the plants mentioned to treat respiratory infections in the Mseleni area.

Homestead GPS reading	Homestead number of interviewee	Age	Gender	Highest level of education	Inyanga or sangoma?	Plants used to treat respiratory infections
S27°22'126" E32°31'766"	MS21	40	F	Gr. 9	No	*Aloe marlothii*, *Erythrina caffra*, *Hypoxis* cf. *acuminata* *Scadoxis puniceus*
S27°22'089" E32°31'779"	MS22	50	M	Gr. 7	No	*Syzygium cordatum*, *Terminalia sericea*
S27°22'032" E32°31'827"	MS23	61	F	Gr. 8	No	*Clausena anisata*, *Eucalyptus grandis*, *Lippia javanica*, *Sansevieria hyacinthoides*
S27°21'684" E32°31'778"	MS07	59	F	Gr. 8	No	*Lippia javanica*, *Sclerocarya birrea*
S27°21'624" E32°31'891"	MS09	45	F	None	No	*Cyperus articulatus*, *Tetradenia riparia*
S27°21'487" E32°31'633"	MS32	47	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°21'298" E32°31'674"	MS13	80	F	None	No	*Lippia javanica*
S27°20'924" E32°31'486"	MS10	93	F	None	No	*Citrus limon*
S27°21'032" E32°31'379"	MS11	34	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*, *Psidium guajava*
S27°20'252" E32°31'942"	MS24	18	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*
S27°21'487" E32°31'633"	MS14	74	M	Gr. 10	No	*Brachylaena* cf. *uniflora*, *Lippia javanica*
S27°19'907" E32°33'614"	MS25	26	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*
S27°20'628" E32°31'775"	MS26	±60	F	Gr. 2	No	*Combretum molle*, *Eucalyptus grandis*, *Lippia javanica*
S27°20'785" E32°31'760"	MS27	38	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°21'082" E32°31'746"	MS12	>60	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°21'665" E32°31'944"	MS31	90	M	None	No	None for respiratory infections
S27°21'848" E32°31'205"	MS04	66	M	None	No	*Clematis brachiata*, *Lippia javanica*
S27°22'401" E32°32'407"	MS28	47	F	None	No	*Lippia javanica*
S27°22'593" E32°32'479"	MS29	44	F	Gr. 12	No	*Eucalyptus grandis*, *Lippia javanica*
S27°22'767" E32°32'527"	MS30	42	F	Gr. 8	No	*Acanthospermum glabratum*, *Ekebergia capensis*

Table 2.4 Interviewees and the plants mentioned to treat respiratory infections in the Tshongwe area.

Homestead GPS reading	Homestead number of interviewee	Age	Gender	Highest level of education	Inyanga or sangoma?	Plants used to treat respiratory infections
S27°25'823" E32°22'608"	T42	73	F	None	No	*Brachylaena* cf. *uniflora*, *Lippia javanica*
S27°25'370" E32°24'566"	T47	47	M	None	Yes	*Lippia javanica*
S27°24'756" E32°24'765"	T31	28	F	Gr. 12	No	*Krauseola mosambicina*
S27°24'916" E32°25'117"	T28	88	F	None	No	*Acanthospermum glabratum*, *Lippia javanica*
S27°25'046" E32°24'996"	T51	45	F	None	No	*Combretum molle*, *Lippia javanica*
S27°25'161" E32°24'739"	T46	63	F	None	Yes	*Acanthospermum glabratum*, *Lippia javanica*
S27°26'209" E32°21'627"	T38	58	F	None	No	*Hypoxis* cf. *acuminata*, *Lippia javanica*
S27°26'275" E32°21'633"	T41	56	F	None	No	*Eucalyptus grandis*, *Lippia javanica*, *Tetradenia riparia*
S27°26'186" E32°22'238"	T36	26	F	Gr. 11	No	*Combretum molle*, *Eucalyptus grandis*, *Lippia javanica*
S27°25'614" E32°22'570"	T43	60	F	None	No	*Eucalyptus grandis*, *Lippia javanica*
S27°26'392" E32°22'889"	T52	35	F	Gr. 7	No	*Lippia javanica*, *Plectranthus neochilus*
S27°27'508" E32°25'575"	T53	55	F	None	No	*Bridelia cathartica*, *Lippia javanica*
S27°27'506" E32°25'503"	T54	>60	M	None	No	*Lippia javanica*
S27°27'574" E32°25'493"	T55	31	F	Gr. 9	No	*Combretum molle*, *Lippia javanica*, *Terminalia sericea*
S27°27'475" E32°25'362"	T56	±63	F	None	No	*Krauseola mosambicina*, *Lippia javanica*
S27°27'517" E32°24'913"	T57	20	F	Gr. 10	No	*Lippia javanica*
S27°27'319" E32°24'836"	T58	32	M	Gr. 9	No	*Clematis brachiata*, *Lippia javanica*, *Tetradenia riparia*
S27°27'351" E32°24'925"	T59	40	F	None	No	*Acanthospermum glabratum*, *Lippia javanica*, *Tetradenia riparia*
S27°27'376" E32°24'786"	T60	45	M	Gr. 6	No	*Clematis brachiata*, *Lippia javanica*
S27°27'094" E32°24'199"	T61	34	M	Gr. 10	No	*Lippia javanica*

2.4.2 Plants used to treat respiratory infections

Voucher specimens of plants used to treat respiratory infections are deposited in the herbarium of the Botany Department of the University of Zululand, South Africa (Table 2.5). A complete botanical description of each of these plant species are given in Sections 2.4.2.1 – 2.1.2.30. Included with each plant description are the recorded English vernacular plant names (Eldridge et al., 1993; Brooker and Kleinig, 1996; Hutchings et al., 1996; Von Koenen, 1996; Van Wyk and Van Wyk, 1997; Pooley, 1998; Pooley, 2003; Smith and Crouch, 2009; Van Wyk et al., 2009). Unless otherwise stated all plants are not threatened or endangered with respect to their conservation status.

The average mass of a handful of whole plant (28 g), -leaves (40 g), -leaves with stems (39 g), and -stems (48 g) has been calculated. One cup is the equivalent of 250 mL, while one teaspoon has the volume of approximately 5 mL and one tablespoon is 15 mL.

Table 2.5 Medicinal plants used for the treatment of respiratory infections in rural Maputaland and their voucher numbers.

Plant scientific name	Voucher number(s)
Acanthospermum glabratum (DC.) Wild	TYORK 19
Aloe marlothii A. Berger	TYORK 42
Brachylaena discolor DC.	TYORK 32
Brachylaena cf. *uniflora* Harv.	TYORK 28
Brachylaena cf. *transvaalensis* E. Phillips & Schweick.	TYORK 39
Bridelia cathartica Bertol.f. subsp. *cathartica*	TYORK 27
Citrus limon (L.) Burm.f.	TYORK 36
Clausena anisata (Willd.) Hook. F. ex Benth.	TYORK 7
Clematis brachiata Thunb.	TYORK 26
Combretum molle R. Br. ex G. Don	TYORK 23
Cyperus articulatus L.	TYORK 4

Plant scientific name	Voucher number(s)
Ekebergia capensis Sparrm.	TYORK 20
Erythrina caffra Thunb.	TYORK 38
Eucalyptus grandis W. Hill ex Maiden	TYORK 33
Euphorbia tirucalli L.	TYORK 37
Helichrysum kraussii Sch. Bip.	TYORK 12
Hypoxis cf. *acuminata* Baker	TYORK 30
Krauseola mosambicina (Moss) Pax & K.Hoffm.	TYORK 21
Lippia javanica (Burm.f.) Spreng.	TYORK 1
Ozoroa obovata (Oliv) R.Fern. & A.Fern.	TYORK 34
Parinari capensis Harv. subsp. *incohata* F. White	TYORK 3
Plectranthus neochilus Schltr.	TYORK 24
Psidium guajava L.	TYORK 16
Sansevieria hyacinthoides (L.) Druce	TYORK 8
Scadoxis puniceus (L.) Friis & Nordal	TYORK 5
Sclerocarya birrea (A. Rich.) Hochst.	TYORK 9
Senecio deltoideus Less.	TYORK 41
Senecio serratuloides DC.	TYORK 35
Syzygium cordatum Hochst. ex C. Krauss.	TYORK 29
Terminalia sericea Burch. ex DC.	TYORK 6
Tetradenia riparia (Hochst.) Codd	TYORK 14
Trichilia emetica Vahl subsp. *emetica*	TYORK 13

2.4.2.1 *Acanthospermum glabratum* (Asteraceae)

Five seeded starbur

2.4.2.1.1 Botanical description

Acanthospermum glabratum is a prostrate annual herb (Figure 2.2). The leaves vary in shape and can be elliptic to broadly ovate and almost round, with the leaf base being wedge shaped or truncate. The leaves have toothed to subentire margins, while the leaf apex can be rounded or acute. The densely glandular leaves are initially hairy, but soon become hairless. The capitula contain yellow to greenish cream florets, with disc florets being glandular (Beentje et al., 2005).

2.4.2.1.2 Geographical distribution

Acanthospermum glabratum is native to tropical America, but currently occurs as a weed in tropical regions worldwide (Beentje et al., 2005).

2.4.2.1.3 Recorded medicinal uses

The whole plant is crushed, mixed with warm water and taken to treat diarrhoea (De Wet et al., 2010).

Figure 2.2 *Acanthospermum glabratum* leaves and flowers (photo: T. York).

2.4.2.1.4 Ethnobotanical information obtained from interviewees

(a) Crush a handful of the whole plant and mix with a half to one cup of warm water. Sieve and take the whole decoction as an enema once a day, or take one tablespoon three times a day orally to treat chills, fever, runny nose, chest pain, sore throat and/or headaches. Children take only a quarter of a cup of the decoction once a day as an enema or take one teaspoon of the decoction orally three times a day. One interviewee mentioned that children are not allowed to take the decoction as an enema.

(b) Boil a handful of the whole plant with a handful of leaves each of *T. riparia* and *L. javanica* in 1.5 L of water. Steam once a day and take one tablespoon three times a day to treat a cough and tiredness. Children under the age of three are not allowed to steam, while children over the age of three steam with parental supervision only.

(c) Crush a handful of the whole plant and add 1 L of warm water. Take a size 6-10 syringeful (180-300 mL) daily as an enema, while children only use a syringe size one (± 30 mL). This decoction is used to treat coughs, fever, chills and a runny nose. One interviewee mentioned that you only take this decoction once, and that you should not take it again the following day.

2.4.2.2 *Aloe marlothii* (Asphodelaceae)

Mountain aloe

2.4.2.2.1 Botanical description

Aloe marlothii is a single-stemmed leaf succulent (Figure 2.3). The unbranched stem is thickly covered with old, dried up leaves. Both surfaces of the dull grey-green leaves are usually covered in numerous sharp, hard spines. The leaf margin contains many rust-coloured spikes. The orange flowers have purple stamens, and are carried on relatively horizontal flower spikes. The fruit is a capsule (Van Wyk and Van Wyk, 1997).

2.4.2.2.2 Geographical distribution

Aloe marlothii occurs, specifically in rocky slopes, from Zululand northwards to Swaziland, Mpumalanga, Limpopo, Botswana, Zimbabwe and Mozambique (Pooley, 2003).

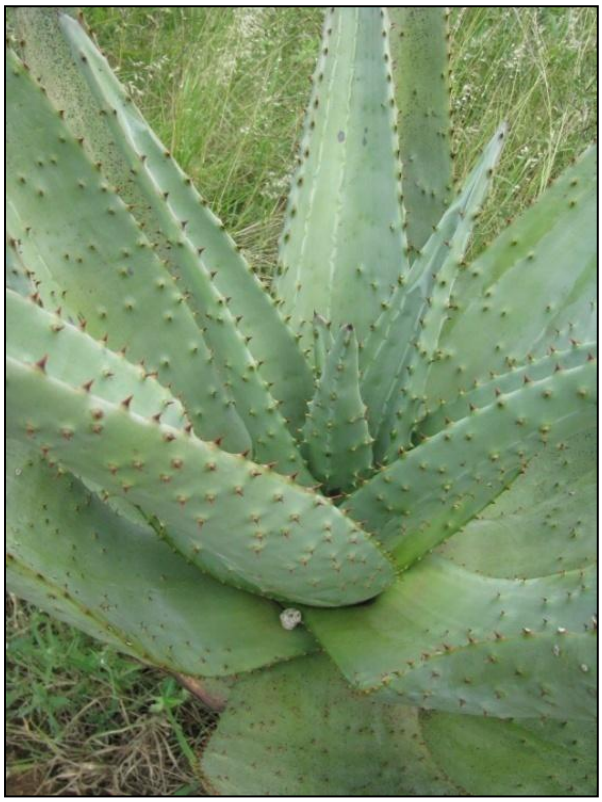


Figure 2.3 *Aloe marlothii* plant (photo: T. York).

2.4.2.2.3 Recorded medicinal uses

Decoctions made from the leaves and roots are taken orally or as enemas to treat roundworm (Watt and Breyer-Brandwijk, 1962). Leaf decoctions are used in treating horse sickness. Sap from the leaves is applied to the mother's breast to hasten weaning (Hutchings et al., 1996). Decoctions are made from the shoots and taken for stomach complaints (Watt and Breyer-Brandwijk, 1962).

2.4.2.2.4 Ethnobotanical information obtained from interviewees

Chop one leaf and mix it with the chopped bulb of a *Hypoxis* sp. (identified as *Hypoxis* cf. *acuminata*) as well as the chopped underground parts of *S. puniceus* and a handful of crushed *E. caffra* root. Add 1 L of boiling water and drink half a cup of the decoction once a day to treat chest pain, fever and a blocked nose. Children take one tablespoon of the decoction once daily.

2.4.2.3 *Brachylaena discolor* (Asteraceae)

Coast silver oak

2.4.2.3.1 Botanical description

This is a shrub or small tree with sexes on different plants. The slightly leathery leaves are dark green above with white felted undersides (Figure 2.4). The leaves are lanceolate to obovate, with an entire or unevenly toothed margin. Flowers develop in cream coloured flowerheads, arranged together in terminal panicles. The fruit is a small nutlet with rough hairs at the tip (Van Wyk and Van Wyk, 1997).

Figure 2.4 *Brachylaena discolor leaves* (photo: T. York).

2.4.2.3.2 Geographical distribution

Brachylaena discolor occurs from the Eastern Cape to southern Mozambique, including the coastal dunes of KwaZulu-Natal (Pooley, 2003).

2.4.2.3.3 Recorded medicinal uses

Leaf infusions are taken as a purgative to treat intestinal parasites (Bryant, 1966). Leaf infusions are also taken, by both Africans and Europeans, to treat diabetes and renal conditions. It is also taken as a tonic (Watt and Breyer-Brandwijk, 1962). The leaves are taken as an enema by children to treat coughs and fever. The bark and leaves are used by the Zulus to treat high fever and painful joints. A decoction is made from the bark and taken to act as a decongestant and for lessening constrictive breathing. The bark is also used for treating a bleeding stomach (Pujol, 1990).

2.4.2.3.4 Ethnobotanical information obtained from interviewees

Crush a handful of leaves and add one cup of warm water. Take a size 1 syringeful (30 mL) once a day as an enema and take one teaspoon three times a day to treat cough and a runny or blocked nose. Children take the same amount when an enema is used, but only drink one teaspoon twice daily.

2.4.2.3.5 Ethnobotanical information of other *Brachylaena* species

(a) Mix a handful of leaves from both *E. grandis* and *L. javanica* with the *Brachylaena* sp. identified as *Brachylaena* cf. *transvaalensis.* Bring to boil with 2 L of water and drink one cup of this decoction three times a day to treat cough, chest pain, runny nose and fever. Children take one teaspoon of the decoction three times a day.

(b) Mix three to four leaves of a *Brachylaena* sp. (identified as *Brachylaena* cf. *uniflora*) with two cups of water and bring the mixture to boil. Take one tablespoon daily to treat flu symptoms. Children only take one teaspoon daily.

(c) Mix two handfuls of leaves from the species identified as *Brachylaena* cf. *uniflora* with two handfuls of *L. javanica* leaves and bring to boil with 5 L water. Steam twice a day for 10 minutes and drink half a cup twice a day or use half a cup as an enema twice a day to treat chills, coughs and blocked nose. Children take it as an enema in the same way as the parents, but must steam with parental supervision. Children only drink half a cup once a day.

2.4.2.4 *Bridelia cathartica* (Euphorbiaceae)

Blue sweetberry

2.4.2.4.1 Botanical description

This is a small multistemmed tree or scrambling shrub with elliptic to broadly oblanceolate leaves (Figure 2.5). The leaves are dark green and glossy above with a grey-green to blue-green underside. The greenish to yellowish flowers are very small and formed in axillary clusters. The fruit is a berry and ripens from a red to a black colour (Van Wyk and Van Wyk, 1997).

2.4.2.4.2 Geographical distribution

Bridelia cathartica grows in the sandy soils of Maputaland and the Zululand coastline. It also occurs in Mpumalanga, Limpopo, Swaziland and Mozambique, extending into tropical Africa (Pooley, 2003).

2.4.2.4.3 Recorded medicinal uses

A decoction of the root- or stembark is taken to treat female infertility or menorrhagia, while the roots are used to treat sterility in men (Gelfand et al., 1985; Chhabra et al., 1990). It is drunk to treat gonorrhoea (Chhabra et al., 1990). Root- or rootbark decoctions are taken to treat headaches (Kokwaro, 1976; Gelfand et al., 1985), fever, asthma (Chhabra et al., 1990) and influenza (Kokwaro, 1976). Other ailments for which it is taken, include abdominal pains, constipation, diarrhoea, vomiting, anaemia and anorexia (Chhabra et al., 1990). Malaria (Kokwaro, 1976) or bilharzia (Chhabra et al., 1990) is treated with *B. cathartica* decoctions. According to Chhabra et al. (1990) roots can be used to treat cardiac pains (Chhabra et al., 1990). When milk is mixed with the root decoction, it is taken to treat liver pains (Kokwaro, 1976).

Figure 2.5 Collecting leaves from *B. cathartica* (photo: V. Nzama).

2.4.2.4.4 Ethnobotanical information obtained from interviewees

Mix a handful of leaves with a handful of *L. javanica* leaves and 2 L water, bringing to the boil. Steam twice a day, until the decoction cools down, to treat chills, headache, cough and a runny nose. Children under the age of four can steam only with adult supervision.

2.4.2.5 *Citrus limon* (Rutaceae)

Lemon

2.4.2.5.1 Botanical description

This is a small evergreen tree containing highly aromatic oil in the leaves (Figure 2.6), flowers and skin of the mature fruit. Leaves are adapted to form phylodiums (Van Wyk and Wink, 2004; Page, 2008). The leaves are dotted with translucent oil glands and aromatic when crushed (Dharani, 2002). The small flowers are purplish-white, with five sepals and petals. The sepals

have pointed tips and are fused into a cup-shaped calyx. The flower is followed by a unique type of fruit called a hesperidium. Each of the many ovary carpels develop into a fruit segment filled with juice sacs (Page, 2008). The yellow fruit has a relatively thick fruit rind and a very pronounced point or nipple (Van Wyk, 2005).

Figure 2.6 *Citrus limon* leaves and thorns (photo: T. York).

2.4.2.5.2 Geographical distribution

Originally from southern and south-eastern Asia, but cultivated worldwide in warm climates (Van Wyk and Wink, 2004).

2.4.2.5.3 Recorded medicinal uses

Juice from the lemon fruit is taken to treat coughs. The whole fruit can be eaten fresh to treat the same symptom (Watt and Breyer-Brandwijk, 1962; Tabuti et al., 2003; Ajibesin et al., 2008; Focho et al., 2009; Mesfin et al., 2009). A leaf decoction can also be drunk to treat coughs (Chinemana et al., 1985). The roots or a decoction made from the leaves or shoots is taken to treat TB (Frei et al., 1998; Kisangau et al., 2007). The fruit or the leaves are taken to treat colds, while symptoms such as headaches and fever are treated by applying a poultice made from the fruit (Watt and Breyer-Brandwijk, 1962; Camejo-Rodrigues et al., 2003; El-Hilaly et al., 2003; Scarpa, 2004). The diluted juice is used as an eye wash (Watt and Breyer-Brandwijk, 1962).

Other ailments for which *C. limon* is also used include goitre, impotence and appetite loss (Focho et al., 2009). *Citrus limon* is used in combination with various other plant species (including *Malus domestica* Borkh., *Olea europea* L. and *Ruta graveolens* L.) to treat diseases ranging from the common cold, to heart ailments or renal stones (Šarić-Kundalić et al., 2010).

2.4.2.5.4 Ethnobotanical information obtained from interviewees

Boil a handful of leaves for 10 minutes with 1 L of water. Drink half a cup of this decoction three times a day to treat a blocked nose and fatigue. Children take one tablespoon of the decoction three times a day.

2.4.2.6 *Clausena anisata* (Rutaceae)

Horsewood

2.4.2.6.1 Botanical description

It is a shrub or a small, slender tree with dark green leaves (Figure 2.7). The leaflets are gland-dotted and have an unpleasant aroma when crushed. The small, white to yellowish flowers occur in branched axillary sprays. The fleshy fruit are globose and ripen to a red, black or purple-black colour (Van Wyk and Van Wyk, 1997).

2.4.2.6.2 Geographical distribution

Clausena anisata commonly occurs in KwaZulu-Natal, while it is also found in the Eastern Cape, Mpumalanga, Limpopo, Swaziland, Mozambique and northwards into tropical Africa (Pooley, 2003).

Figure 2.7 *Clausena anisata* leaves (photo: T. York).

2.4.2.6.3 Recorded medicinal uses

Clausena anisata is used to treat respiratory illnesses such as headaches, influenza (Kokwaro, 1976), coughs and eye problems. Europeans have been reported to believe the plant acts as a diaphoretic, and use the leaves to treat ailments that cause a fever (Watt and Breyer-Brandwijk, 1962). Leaf juice is applied to the ear to treat ear infection (Teklehaymanot and Giday, 2007). Smoke inhalation is a common way of applying this plant medicinally. The leaves are placed on hot coals or ashes and inhaled by children for them to be protected from evil spirits. Newborn babies are exposed to the harsh smelling smoke to strengthen their lungs or heart, to cleanse the body internally, or cure fevers and rheumatism. Adults use this method to control bad body odour (Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Palgrave, 2002). This smoke has been reported to repel mosquitoes (Watt and Breyer-Brandwijk, 1962). The leaves or roots are used to expel parasitic worms, specifically treating hookworm infestations. The plant is also used as a laxative, an emetic, to treat gastro-enteritis (Watt and Breyer-Brandwijk, 1962) and indigestion (Kokwaro, 1976). A leaf decoction is taken as a blood purifier, while a root decoction is taken to treat heart ailments and vascular diseases (Pujol, 1990). It is used to treat swollen gums and toothache, while toothbrushes are made from the twigs (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976). Various other ailments are treated by *C. anisata*, including migraines, swelling,

furunculosis (a deep infection of the hair follicle), impotence, sterility (Watt and Breyer-Brandwijk, 1962), syphilis, malaria (Kokwaro, 1976), and lyprosy (Chhabra et al., 1991). Mental illnesses such as schizophrenia can be treated using this plant (Watt and Breyer-Brandwijk, 1962; Pujol, 1990).

2.4.2.6.4 Ethnobotanical information obtained from interviewees

(a) Mix a handful of leaves with a handful of leaves from both *L. javanica* and *E. grandis* and bring to boil with 4 L of water. Boil for 40 minutes before taking one tablespoon of the decoction twice a day to treat a cough, runny and blocked nose. Children take one teaspoonful of this decoction three times a day.

(b) Mix a handful of leaves with a handful of leaves from both *L. javanica* and *E. grandis* and boil for 10 minutes with 5 L of water. Drink half a cup of this decoction three times a day and steam once a day to treat cough and fever. For children, only a few leaves of each plant are boiled with 2 L of water. Children take one tablespoon of this decoction three times a day. Instead of steaming, two cups of this decoction may be splashed over a bathing child.

2.4.2.7 *Clematis brachiata* (Ranunculaceae)

Traveller's joy, Old man's beard

2.4.2.7.1 Botanical description

Clematis brachiata is a perennial climber with pinnate leaves (Figure 2.8) (Roberts, 1990; Von Koenen, 1996). The widely spaced pinnae are dentate and tapering to narrow tips, and are mostly five in number (Von Koenen, 1996; Pooley, 1998). The young stems are ridged and have soft hairs (Pooley, 1998). The small white flowers are arranged in panicles and the fruits are flattened nutlets, distributed through the wind by their long feathery styles (Von Koenen, 1996).

Figure 2.8 *Clematis brachiata* stems and leaves (photo: T. York).

2.4.2.7.2 Geographical distribution

Clematis brachiata is widespread in South Africa and Tropical Africa.

2.4.2.7.3 Recorded medicinal uses

A leaf- or stem decoction is often prepared from *C. brachiata* for steam inhalation (where after the decoction is often also drunk) to treat colds, or malaria (Watt and Breyer-Brandwijk, 1962). This decoction can be taken to treat headaches, coughs and chest ailments Snuff made from the leaves relieves headaches (Roberts, 1990), while the vapours from a crushed stem can be inhaled to clear your head when suffering from sinusitis or a cold (Watt and Breyer-Brandwijk, 1962; Njoroge and Bussmann, 2006). Juice is extracted from *C. brachiata*, and a few drops are then placed inside the nostrils to treat head colds (Watt and Breyer-Brandwijk, 1962). To relieve pain in the facial area, including the eyes, the roots are crushed and the sap rubbed into the temples. Another method used is to expose your face to a hot leaf decoction (Von Koenen, 1996). *Clematis brachiata* leaves are crushed and squeezed into infected eyes (Pendota et al., 2008). The plant is taken to treat abdominal problems (Roberts, 1990; Chhabra, 1991; Pooley, 1998),

and intestinal worms (Pooley, 1998). A decoction made from the leaves is taken orally or as an enema to treat stomach disorders (Roberts, 1990). In Kenya a root decoction is mixed with *Piper capense* L.f. and taken for depression (Koch et al., 2005). Other ailments for which this plant is used include venereal diseases and snakebite (Roberts, 1990; Pooley, 1998).

2.4.2.7.4 Ethnobotanical information obtained from interviewees

(a) Rub a small amount of stem and leaves between hands and inhale to treat headaches, coughs and a runny nose.

(b) Mix a handful of leaves and stem with a handful of *L. javanica* leaves and bring to boil with 4 L of water. Steam once a day for four minutes. The interviewee stated that the plant is very strong, and one shouldn't steam for longer than four minutes, while children are not allowed to steam. The decoction is used to treat coughs, chest pain and a runny or blocked nose.

(c) Bring a handful of leaves and stem to boil with 2 L of water. Steam once a day for five minutes to treat headaches, coughs and a runny nose. The interviewee also mentioned that steaming for longer than five minutes is "bad for you" and children are not allowed to steam.

(d) Mix a handful of stems and leaves with a handful of *L. javanica* leaves and bring to boil with 5 L of water. Steam twice a day, until the decoction cools down, or take one tablespoon three times a day to treat chest pain, cough, headaches and a runny nose. Children only take one teaspoon of the decoction three times a day, and are not allowed to steam.

2.4.2.8 *Combretum molle* (Combretaceae)

Velvet bushwillow

2.4.2.8.1 Botanical description

This is a small to medium-sized tree with exfoliation of bark from its branchlets. It is deciduous to semi-deciduous with autumn leaves having a red to purplish colour. The leaves are elliptic to almost circular and contain dense velvety hairs (Figure 2.9). The greenish yellow flowers develop in axillary spikes, each developing into a four-winged fruit. The young fruits are

yellowish green and flushed with red, drying to a coppery colour (Van Wyk and Van Wyk, 1997).

2.4.2.8.2 Geographical distribution

This species is found in KwaZulu-Natal and northwards to the Northern Province, Swaziland, Zimbabwe and into tropical Africa. It also occurs in Mozambique (Pooley, 2003).

Figure 2.9 Young leaves and branches of *C. molle* (photo: T. York).

2.4.2.8.3 Recorded medicinal uses

Leaf or root decoctions are used to treat fever (Kokwaro, 1976; Mabogo, 1990; Van Wyk, 1996; Pooley, 2003), headaches or chest complaints (Kokwaro, 1976; Grønhaug et al., 2008). Leaves of *L. javanica* are mixed with *C. molle* bark and a decoction is made to be taken for asthma (Amusan et al., 2002). Leaf decoctions are used for various respiratory diseases such as influenza (Fyhrquist et al., 2002; Koné et al., 2004). Decoctions are taken to treat stomach aches or abdominal pains (Kokwaro, 1976; Hedberg and Hedberg, 1982; Mabogo, 1990; Hutchings et

al., 1996; Van Wyk, 1996; Pooley, 2003; Grønhaug et al., 2008) and constipation (Hutchings et al., 1996; Van Wyk, 1996). Root decoctions are taken to treat diarrhoea or dysentery (Kokwaro, 1976). Leaf decoctions are taken to treat malaria (Grønhaug et al., 2008). Unknown plant parts are taken for schistostomiasis (McGaw et al., 2001) and root or leaf decoctions are taken as anthelmintics against hookworm (Kokwaro, 1976; Mabogo, 1990). Other uses include the treatment of wounds and skin diseases (Van Wyk, 1996; Fyhrquist et al., 2002; Koné et al., 2004) and leprosy (Kokwaro, 1976; Mabogo, 1990). Extracts of leaves and roots are used for the treatment of snakebite (Kokwaro, 1976; Mabogo, 1990; Hutchings et al., 1996; Van Wyk, 1996). Plant parts are also used in the treatment of gonorrhoea and syphilis (Fyhrquist et al., 2002), impotence (Grønhaug et al., 2008), or even convulsions (Mabogo, 1990) and madness (Grønhaug et al., 2008).

2.4.2.8.4 Ethnobotanical information obtained from interviewees

(a) Mix a handful of leaves with a handful of leaves from both *L. javanica* and *E. grandis* and boil with 1.5 L of water until 0.5 L has evaporated. Drink half a cup three times a day to treat cough and fever. Children take one tablespoon of the decoction three times a day.

(b) Mix a handful of leaves with a handful of *L. javanica* leaves and bring to boil with 2 L of water. Steam twice a day, until the decoction cools down, to treat chills, cough and a blocked nose. Children only steam with parental supervision.

(c) Take two handfuls of leaves and boil it with 2 L of water until the water colours. Steam once a day until the decoction cools down to treat chills, coughs and a blocked nose. Children are not allowed to steam.

(d) Mix a handful of leaves with a handful of leaves each of *L. javanica* and *T. sericea* and bring to boil with 1 L of water. Take one tablespoon daily to treat a chronic cough. If the cough is really severe, drink the decoction more often. Children take one teaspoon of the decoction per day.

2.4.2.9 *Cyperus articulatus* (Cyperaceae)

Jointed flat sedge

2.4.2.9.1 Botanical description

Cyperus articulatus has sturdy, leafless culms with solid piths and septate nodes (Figure 2.10). The brown flowers form in a terminal inflorescence, having short, sharp bracts. The erect culms emerge from a stoloniferous rhizome (Gordon-Gray et al., 2006). Pseudo-bulbs develop at the rhizome nodes (Watt and Breyer-Brandwijk, 1962; Gordon-Gray et al., 2006).

2.4.2.9.2 Geographical distribution

This species is found in the Eastern Cape, Limpopo, Mpumalanga, KwaZulu-Natal, Swaziland, Botswana and Namibia (including Caprivi) (Gordon-Gray et al., 2006).

Figure 2.10 Collection of *C. articulatus* pseudo-bulbs (photo: T. York).

2.4.2.9.3 Recorded medicinal uses

The root is used to treat abdominal ailments, vomiting with yellow fever, nausea, colic, toothache, parasitic worms, snake bite, and as a sedative (Watt and Breyer-Brandwijk, 1962). *Cyperus articulatus* is also taken for headaches and coughs (Abbiw, 1990).

2.4.2.9.4 Ethnobotanical information obtained from interviewees

(a) Crush four pseudo-bulbs and add half a cup of cold water and soak. Take one sip of this cold water infusion three times a day to treat chest pain, chills, cough and a blocked nose. For children the mixture is made a little bit weaker (dilution factor not specified by the interviewee).

(b) Crush two pseudo-bulbs and add two tablespoons of cold water. Take one tablespoon of this infusion twice a day to treat a blocked nose and shortness of breath. In this household this infusion is only used for children.

(c) Remove the skin from one pseudo-bulb, chew and swallow once a day to treat a cough, blocked nose and shortness of breath. To treat a small child, an adult will chew the corm and blow it into the child's nostrils once a day.

2.4.2.10 *Ekebergia capensis* (Meliaceae)

Cape ash

2.4.2.10.1 Botanical description

This is a medium-sized to large tree and is semi-deciduous (Figure 2.11). The leaves are compound with 9-13 glossy green leaflets per leaf. The bark is light to very dark grey and smooth, while the young branchlets are dotted with white lenticels (Van Wyk and Van Wyk, 1997; Palgrave, 2002). The small white or pinkish flowers form in axillary panicles and are followed by fleshy pink to bright red fruits (Van Wyk and Van Wyk, 1997).

2.4.2.10.2 Geographical distribution

Ekebergia capensis is found from the southern- and eastern Cape, to KwaZulu-Natal, Mpumalanga and Limpopo. It also occurs in Swaziland and Mozambique, and northwards through Botswana, Zimbabwe to Sudan and Ethiopia (Pooley, 2003).

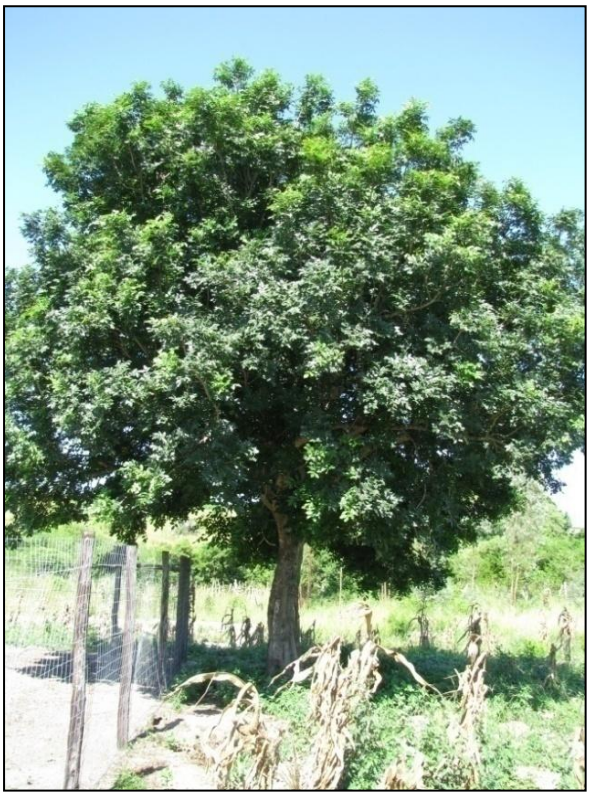


Figure 2.11 *Ekebergia capensis* tree (photo: T. York).

2.4.2.10.3 Recorded medicinal uses

Bark- leaf- or root decoctions are taken as a cough remedy (Bryant, 1966; Mabogo, 1990; Pujol, 1990; Palgrave, 2002; Pooley, 2003), and are also used to relieve headaches (Mabogo, 1990; Palgrave, 2002). *Ekebergia capensis* roots or bark is used for dysentery (Mabogo, 1990; Pooley, 2003), gastritis, hyperacidity, heartburn and stomach bleeds, while a bark decoction is taken to induce vomiting (Pujol, 1990). The leaves are taken to expel intestinal worms (Palgrave, 2002). Various skin ailments such as acne, abscesses or boils are treated by the application of leaf or bark decoctions (Mabogo, 1990; Pujol, 1990). A cold water infusion, prepared from the leaves, is applied to the nostrils to treat insanity (Pujol, 1990).

2.4.2.10.4 Ethnobotanical information obtained from interviewees

Crush a handful of leaves, add boiling water and sieve. Take one tablespoon of the decoction, or take a size eight syringeful (± 240 mL) as an enema once a day to treat a cough, chest pain and a runny nose. This preparation is not to be used by children.

2.4.2.11 *Erythrina caffra* (Fabaceae)

Coast coral tree

2.4.2.11.1 Botanical description

This is a deciduous tree and is medium to large in size with a rounded crown (Figure 2.12). The leaves are compound with broadly ovate to elliptic leaflets with a tapering apex. The two lateral leaflets are slightly smaller than the terminal one, and the branchlets contain prickles. The flowers are orange-scarlet and develop in stalked axillary or terminal racemes. The flowers curve backwards revealing their stamens. The hairless fruits are dehiscent cylindrical pods containing red seeds -each having a black spot at the point of attachment (Van Wyk and Van Wyk, 1997).

Figure 2.12 Collecting roots from *E. caffra* (photo: T. York).

2.4.2.11.2 Geographical distribution

Erythrina caffra is found from Humansdorp (Easter Cape) to Port Shepstone (KwaZulu-Natal), and further north to Lake St. Lucia, Lake Sibaya's swamp forest and Maputaland (Pooley, 2003).

2.4.2.11.3 Recorded medicinal uses

A leaf decoction is applied as ear drops to a painful ear (Bryant, 1966; Van Rensburg, 1982). *Erythrina caffra* leaf decoctions are also taken to treat urinary complaints as well as venereal diseases such as syphilis (Bryant, 1966). The bark is used as a bandage to treat a sprained limb, while crushed leaves are made into a paste and applied to sores to draw out pus (Van Rensburg, 1982).

2.4.2.11.4 Ethnobotanical information obtained from interviewees

It is used in combination with *A. marlothii, S. puniceus and Hypoxis* cf. *acuminate* as described previously (2.4.2.2.4) to treat chest pain, fever and a blocked nose.

2.4.2.12 *Eucalyptus grandis* (Myrtaceae)

Flooded gum, rose gum

2.4.2.12.1 Botanical description

Eucalyptus grandis is a very fast growing *Eucalyptus* species, reaching unusual heights (up to 80 m). The straight growing trunk has smooth white to grey-white bark with a short, rough basal stocking (Eldridge et al., 1993; Brooker and Kleinig, 1996). The stalked discolorous leaves are lanceolate to broadly lanceolate in shape, with a glossy dark green colour above. The flowers are white (Figure 2.13) (Brooker and Kleinig, 1996).

2.4.2.12.2 Geographical distribution

Eucalyptus grandis is native to eastern Australia, and propagated worldwide for industrial wood production. Most of these industrial plantations occur in Brazil and South Africa, while there are also large plantations in Argentina, Australia, India, Uruguay, Zambia and Zimbabwe. The southern African plantations probably originated from northern New South Wales or southern Queensland (Eldridge et al., 1993).

Figure 2.13 *Eucalyptus grandis* leaves and flowers (photo: T. York).

2.4.2.12.3 Recorded medicinal uses

In the Democratic Republic of Congo, *Eucalyptus grandis* is taken to treat constipation (Chifundera, 2001), while in Uganda, a bark decoction is taken for coughs (Ssegawa and Kasenene, 2007).

2.4.2.12.4 Ethnobotanical information obtained from interviewees

(a) Boil a handful of leaves with a handful of *L. javanica* leaves in 2 L to 5 L of water. The decoction is mostly made by adding 2 L of water. Take one tablespoon to half a cup of this decoction three times a day, while using what's left of it once or twice a day for steaming. Steam until the water cools down. When the decoction is made with more than 2 L of water it can be used to bath in, by method of splashing, once a day. Above methods are used to treat chills, coughs, a runny nose, headache, chest pain, tonsillitis, sore throat, fatigue, fever, or even ear ache. Some interviewees only drink half a cup of the decoction once a day. Steaming should not be used to treat children. Children can drink the decoction, but at half the dosages used for adults.

(b) Take a branch tip with young leaves (approximately a handful) and bring to boil with one cup of water. Boil for 20 minutes and take one teaspoon of this decoction four times a day to

treat chest pain and cough. When making a large amount of this decoction (about 5 L) it can also be used for steaming. Steam for 30 minutes. Young children take one teaspoon twice a day.

(c) Mix two handfuls of leaves with one handful of *L. javanica* leaves and bring to boil with 2 L of water. Drink a quarter cup three times a day to treat chest pain, cough, headaches and a runny nose. Children take only one tablespoon of the decoction three times a day.

(d) Mix three handfuls of leaves with one handful of *L. javanica* leaves and bring to boil with 4 L of water. Steam until the water cools down, twice a day, to treat chest pain, cough, headaches and a runny nose.

(e) It is used in combination with *C. anisata* and *L. javanica,* as described previously (2.4.2.6.4), to treat cough, fever and a runny or blocked nose.

(f) It is used in combination with *C. molle* and *L. javanica,* as previously described (2.4.2.8.4), to treat cough and fever.

(g) It is used in combination with *Brachylaena* cf. *transvaalensis* and *L. javanica,* as previously described (2.4.2.3.4), to treat a cough, chest pain, a runny nose and fever.

(h) Mix a handful of leaves with a handful of leaves from *O. obovata* and bring to boil with 2 L of water. Steam until the decoction cools down, once a day, to treat chest pain, cough, fever and a blocked or runny nose. Children can steam only for five minutes per day with parental supervision.

(i) Mix a handful of leaves with a handful of leaves each from *L. javanica*, *P. guajava* and *S. serratuloides* and bring to boil with 6 L of water. Use this decoction to steam twice a day to treat a cough, fever and runny nose. Children don't steam but they get bathed in this decoction.

(j) Mix a handful of leaves with a handful of leaves each from *S. serratuloides* and *L. javanica* with 5 L of water and bring to boil. Drink one cup daily to treat a cough and blocked nose. Children take two tablespoons three times a day.

(k) Mix a handful of leaves with a handful of leaves each from *L. javanica* and *S. serratuloides* and bring to boil with 1.5 L of water. Drink half a cup three times a day to treat chest pain, cough, headache and a runny nose. Children only take one tablespoon of this decoction three times a day.

(l) Mix a handful of leaves with a handful of leaves from *L. javanica* and *T. riparia* and bring to boil with 2 L of water. Boil until 1 L of the decoction is left and drink half a cup three times a day to treat chills, coughs, sleepless nights, fever and headaches.

2.4.2.13 *Euphorbia tirucalli* (Euphorbiaceae)

Rubber euphorbia

2.4.2.13.1 Botanical description

It is a spineless, succulent shrub or small tree with smooth cylindrical branches (Figure 2.14). The growth form is irregular and often develops into a dense crown. The leaves are small and slender and are rarely seen due to the fact that they fall off very early. The greenish yellow flowers form at the branch tips, followed by small green to pink capsule fruits (Neuwinger, 1996).

2.4.2.13.2 Geographical distribution

Euphorbia tirucalli is widespread and naturalised throughout Africa, while cultivated in Europe, America and India (Watt and Breyer-Brandwijk, 1962; Neuwinger, 1996).

2.4.2.13.3 Recorded medicinal uses

A root decoction is taken as an emetic in the occurrence of snakebite, and is also used to cure sterility (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976; Neuwinger, 1996), while the plant juice is used as a remedy for sexual impotence (Watt and Breyer-Brandwijk, 1962). Root extract is taken to cure gonorrhoea, while the latex is taken, or applied externally, to treat syphilis (Watt and Breyer-Brandwijk, 1962; Neuwinger, 1996). The latex is taken for various other ailments, including constipation (Watt and Breyer-Brandwijk, 1962; Chhabra et al., 1990; Neuwinger, 1996), stomach problems, swellings, epilepsy and as an antidote for poisoning. In India the latex has been applied externally to treat rheumatism, neuralgia, toothache, headache, earache, colds, tumours and warts (Neuwinger, 1996). The young branches of *E. tirucalli* are roasted and chewed to treat sore throats and stomach ailments (Kokwaro, 1976; Dharani, 2002). A root

extract is taken to treat bilharzia, while juice obtained from the branches are taken to treat intestinal worms. The pounded twigs are prepared into a plaster, which is applied to the swollen parts of a leg. Leaf juice is applied to the affected ear in the case of otitis (Neuwinger, 1996). Root- or stem bark extracts are taken internally to treat asthma (Neuwinger, 1996), while whole plant ashes are also taken for the same ailment (Savithramma et al., 2007).

Figure 2.14 The modified stems and branches of *E. tirucalli* (photo: T. York).

2.4.2.13.4 Ethnobotanical information obtained from interviewees

Boil a handful of modified stem in two cups of water until one cupful is left. Apply two drops of this decoction to an affected ear once at night to treat earache. Children only take one drop of the decoction to an affected ear.

2.4.2.14 *Helichrysum kraussii* (Asteraceae)

Straw everlasting

2.4.2.14.1 Botanical description

This is an aromatic shrublet growing up to 1 m in height. The small leaves have sharp tips and their margins are rolled under. The leaves have white felted undersides. The flowers form in cylindrical heads with yellow bracts. The inflorescences are dense and branched (Figure 2.15). Sometimes fluffy white galls develop at the branch tips (Pooley, 1998).

Figure 2.15 *Helichrysum kraussii* leaves and flowers (photo: T. York).

2.4.2.14.2 Geographical distribution

Helichrysum kraussii occurs in the coastal grassland or open woodland areas of the Eastern Cape Province and KwaZulu-Natal, to Angola, Zimbabwe and Mozambique (Pooley, 1998).

2.4.2.14.3 Recorded medicinal uses

Dried flowers and seeds are smoked to treat coughs and pulmonary tuberculosis (Watt and Breyer-Brandwijk, 1962), while whole plant's ash is taken orally for coughs. Leaf decoctions are used to wash keloid scars (a type of scar composed mainly of type I or type III collagen). (Lourens et al., 2008).

2.4.2.14.4 Ethnobotanical information obtained from interviewees

Mix a handful of leaf filled branches with a handful of *L. javanica* leaves and three leaflets from *T. emetica* and boil for 10 minutes in 1 L of water. Sieve the decoction and take one teaspoonful each day to treat chest pain, cough and a blocked nose.

2.4.2.15 *Hypoxis spp.* (Hypoxidaceae)

Star flower

2.4.2.15.1 Botanical description

Species from the genus *Hypoxis* are perennials with tuberous corms and strap-shaped leaves that are hairy (Figure 2.16) (Hutchings et al., 1996, Pooley, 1998; Van Wyk et al., 2009). The bright yellow flowers are star-shaped (Van Wyk et al., 2009).

2.4.2.15.2 Geographical distribution

Hypoxis spp. grows in grassland areas of sub-saharan Africa. Some species, like *Hypoxis acuminata* Baker, also occur in other countries such as Mauritius and the Seychelles (Pooley, 1998; Owira and Ojewole, 2009).

2.4.2.15.3 Recorded medicinal uses

Different *Hypoxis* species have been documented for their use in a range of diseases, including urinary tract infections, heart ailments, nervous disorders, cancers as well as diseases affecting the immune system (Owira and Ojewole, 2009). Corm infusions of *Hypoxis rigidula* Baker are used for treating wounds and rashes, while a decoction is taken to treat asthma and arthritis (Shale et al., 1999). Unidentified *Hypoxis* spp. have been recorded for their various medicinal uses. Corm infusions are taken as anthelmintics (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Hutchings et al., 1996), while other uses include the treatment of ulcers and skin infections (Hutchings et al., 1996). Another *Hypoxis* sp. is used to treat vomiting, appetite loss, abdominal pains, fever and even delirium (Watt and Breyer-Brandwijk, 1962).

Figure 2.16 A plant of the *Hypoxis* species (photo: T. York).

2.4.2.15.4 Ethnobotanical information obtained from interviewees

(a) It is used in combination with *A. marlothii*, *S. puniceus* and *E. caffra*, as described previously (2.4.2.2.4), to treat chest pain, fever and a blocked nose.

(b) Chop the corms and mix one handful of this with a handful of *L. javanica* leaves and bring to boil with one cup of water. Sieve the decoction and take one cupful a day as an enema to treat flu symptoms such as chills and a runny nose. Children only take a quarter cup of the decoction per day.

2.4.2.15.5 Conservation status

Most *Hypoxis* spp. are not threatened or endangered, except for *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. This species is threatened by extensive commercial use, causing a decline in the population. *Hypoxis patula* Nel. and *Hypoxis uniflorata* Markötter are both at high risk of extinction in the wild (Raimondo et al., 2009).

2.4.2.16 *Krauseola mosambicina* (Caryophyllaceae)

No English vernacular name could be found for *K. mosambicina*.

2.4.2.16.1 Botanical description

Krauseola mosambicina is an annual or perennial herb (Figure 2.17). Growing in a straggling manner, it grows up to 40 cm tall, branching near the base of the plant. The leaves and stems are sparsely covered in soft hairs. The leaves are oblanceolate to narrowly obovate, with 4-6 leaves arranged in a false whorl. The leaf apex is obtuse to mucronulate, while the leaf base is cuneate. The deltoid stipules are scarious. The greenish flowers have membranous petals and form in axillary or terminal cymes. The membranous stamens are broad with broadly oblong anthers, while the pistil has twisted stigmatic arms. The seeds develop in a capsule and are reddish brown in colour (Wild, 1961).

Figure 2.17 *Krauseola mosambicina* leaves and flowers (photo: T. York).

2.4.2.16.2 Geographical distribution

Krauseola mosambicina only occurs in northern KwaZulu-Natal, and extends into Mozambique (Jordaan, 2000).

2.4.2.16.3 Recorded medicinal uses

A cold water infusion, prepared from the whole plant, is taken orally or as an enema to treat diarrhoea (De Wet et al., 2010).

2.4.2.16.4 Ethnobotanical information obtained from interviewees

Mix a handful of stems and leaves with a handful of *L. javanica* leaves, crush and bring to the boil with 0.5 L of water. Take half a cup of the decoction as an enema once a day to treat cough and a runny or blocked nose. Children take only a quarter cupful of the decoction as an enema.

2.4.2.17 *Lippia javanica* (Verbenaceae)

Fever tea

2.4.2.17.1 Botanical description

It is an erect, woody shrub and can grow up to two metres high. The highly aromatic leaves are hairy with prominent veins (Figure 2.18). The leaves have a strong lemon aroma. The white and yellow flowers are small and produced in dense heads (Van Wyk et al., 2009).

Figure 2.18 *Lippia javanica* flowers and leaves (photo: T. York).

2.4.2.17.2 Geographical distribution

Lippia javanica occurs from South Africa, where it is distributed over large parts of the country, including the Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo, and up into tropical Africa (Van Wyk et al., 2009).

2.4.2.17.3 Recorded medicinal uses

Leaf decoctions are commonly taken for various respiratory complaints such as colds, coughs, fever, influenza and asthma (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985; Mabogo, 1990; Roberts, 1990). Smoke from the burning leaves and stems are inhaled to treat respiratory conditions such as cough and asthma, while the crushed leaves are stuffed into the nostrils to relieve cold symptoms (Watt and Breyer-Brandwijk, 1962; Roberts, 1990). Leaf decoctions are taken for malaria and measles (Watt and Breyer-Brandwijk, 1962; Mabogo, 1990; Roberts, 1990). Leaf- or root decoctions are taken to relieve headaches, backache (Gelfand et al., 1985; Mabogo. 1990) and sore muscles (Pooley, 1998). *Lippia javanica* is also used against various other ailments, including eye problems (Gelfand et al., 1985; Hedberg and Staugård, 1989), skin problems such as rashes (Mabogo, 1990; Pooley, 1998), diarrhoea or dysentery (Watt and Breyer-Brandwijk, 1962; Mabogo, 1990; De Wet et al., 2010), parasitic worms (Mabogo, 1990), convulsions or weak joints (Gelfand et al., 1985).

2.4.2.17.4 Ethnobotanical information obtained from interviewees

(a) Rub a few leaves between your hands and inhale the vapours to relieve cold and flu symptoms.

(b) Add a handful of leaves to 0.5 L water and bring to boil. Take one to two tablespoons three times a day, or one cupful once a day (the decoction must be warm) to treat cough, aching muscles, a sore throat and fever. Children only take one teaspoon of the decoction twice a day.

(c) Bring to boil a handful of leaves in 10 L of water. Steam until the decoction cools down and drink a quarter of a cup twice a day to treat a cough and blocked nose. Children take one teaspoonful three times a day.

(d) Bring to boil two handfuls of leaves in 2 L of water. Steam, until the water cools down, once or twice a day to treat coughs, chest pain, headaches, fever, chills, a sore throat or a blocked

nose. This decoction can also be taken orally by drinking half a cup daily, but it is mostly used for steaming. Young children are not allowed to steam, and only take one teaspoon of the decoction daily. One interviewee mentioned that young children steam for a maximum of three minutes each day.

(e) Bring to boil a handful of leaves with 2 L of water. Steam, until the water cools down, once or twice a day or take one tablespoon of the decoction once or twice a day to treat cough, headaches, fever, sore muscles, a runny nose or a blocked nose. The decoction can also be splashed over the body while in bathing. Children are generally not allowed to steam. When steaming takes place it is only for a few minutes, and with parental guidance. Children take one teaspoon of the decoction once a day.

(f) Bring to boil two handfuls of leaves in 5 L of water. Steam twice a day for 10 minutes to treat flu symptoms. Children are not allowed to steam.

(g) Crush a handful of leaves and mix it with one cup of warm water. Soak the decoction until the water turns green and sieve. Take one tablespoon of the decoction four times a day to treat flu symptoms. Children only take one teaspoon of this four times a day.

(h) Chop the root and mix a handful of this with a handful of leaves and one cup of hot water. Take one tablespoon of the decoction three times a day or steam with it three times a day to treat tonsillitis, or a cough and runny nose.

(i) Crush a handful of leaves and mix it with two cups of warm water. Take a size eight syringeful (± 240 mL) of this decoction once a day as an enema, to treat tonsillitis, coughs and a runny nose. Children take half the dosage.

(j) Mix one to two handfuls of leaves with an equal amount of *T. emetica* leaves and bring to boil with 4-5 L of water. Steam twice a day, until the decoction cools, to treat headache, fever, cough and a runny nose. This preparation is not to be used by children.

(k). Mix one handful of leaves with one handful of *T. riparia* leaves and add a cup of warm water. Take two tablespoons of this whenever feeling very ill. It is mostly used to treat coughs, sore throat and fever. Children take only one tablespoon three times a day.

(l) It is used in combination with *H. kraussii* and *T. emetica,* as described previously (2.4.2.14.4), to treat chest pain, cough and a blocked nose.

(m) It is used in combination with *C. anisata* and *E. grandis*, as described previously (2.4.2.6.4), to treat cough, fever, a runny and a blocked nose.

(n) Used in combination with *C. molle* and *E. grandis*, as described previously (2.4.2.8.4), to treat coughs and fever.

(o) It is used in combination with *C. molle*, as described previously (2.4.2.8.4), to treat chills, cough and a blocked nose.

(p) Used in combination with *C. molle* and *T. sericea*, as described previously (2.4.2.8.4), to treat a chronic cough.

(q) It is used in combination with *Hypoxis* sp., described previously (2.4.2.15.4), to treat chills and a runny nose.

(r) *Lippia javanica* is used in combination with *B. cathartica*, as previously described (2.4.2.4.4), to treat chills, headaches, coughs and a runny nose.

(s) It is used in combination with *C. brachiata*, as previously described (2.4.2.7.4), to treat headache, coughs, chest pain and a runny or blocked nose.

(t) Used in combination with *K. mosambicina*, as previously described (2.4.2.16.4), to treat coughs and a runny or blocked nose.

(u) It is used in combination with *A. glabratum* and *T. riparia*, as previously described (2.4.2.1.4), to treat cough and tiredness.

(v) Used in combination with *E. grandis* and *S. serratuloides*, as previously described (2.4.2.12.4), to treat coughs and a blocked nose.

(w) It is used in combination with *E. grandis, S. serratuloides* and *P. guajava*, as previously described (2.4.2.12.4), to treat coughs, fever and a runny nose.

(x) Used in combination with *Brachylaena* cf. *transvaalensis* and *E. grandis,* as previously described (2.4.2.3.4), to treat chest pain, fever and a runny nose.

(y) It is used in combination with *E. grandis*, as previously described (2.4.2.12.4) to treat chest pain, cough, headaches, a runny nose, chills, fatigue, fever, a sore throat or ear ache.

(z) Used in combination with *E. grandis* and *T. riparia*, as previously described (2.4.2.12.4), to treat chills, coughs, sleepless nights, headaches and fever.

(aa) Mix a handful of leaves with a handful of leaves from *P. neochilus* and bring to boil with 2 L of water. Add sugar to taste and take one tablespoon of the decoction three times a day to treat chills, cough and a runny or blocked nose. Children only take one teaspoon three times a day.

(ab) It is used in combination with *E. grandis* and *S. serratuloides*, as described previously (2.4.2.12.4), to treat chest pain, cough, headache and a runny nose.

(ac) Used in combination with *Brachylaena* cf. *uniflora*, as previously described (2.4.2.3.4), to treat chills, cough and a blocked nose.

2.4.2.18 *Ozoroa obovata* (Anacardiaceae)

Broad-leaved resin tree

2.4.2.18.1 Botanical description

It is a heavily branched shrub or small semi-deciduous tree. The sexes occur on different plants. The leaves, which are usually arranged in whorls of three, are obovate or elliptic (Figure 2.19). The presence of densely appressed hairs on the underside of the leaf makes it appear silvery in colour while the opposite side is dark green. The leaf's main side veins are parallel, and the leaf apex is rounded or broadly tapering. The small, creamy white flowers develop in terminal sprays and produce a sweet fragrance. The fleshy fruit is a drupe, and is bean-shaped. The initially green coloured fruit ripens to become black and wrinkled (Van Wyk and Van Wyk, 1997).

2.4.2.18.2 Geographical distribution

Ozoroa obovata occurs along the coast from Maputaland in KwaZulu-Natal, to Mozambique and northwards (Pooley, 2003).

2.4.2.18.3 Recorded medicinal uses

Root- and bark decoctions are taken for dysentery (Kokwaro, 1976; Verzár and Petri, 1987). An ointment made from the crushed leaves is applied to lesions, while a cold water extract is used

for treating a swollen body. A root extract is taken to treat rheumatism or indigestion (Verzár and Petri, 1987).

Figure 2.19 *Ozoroa obovata* leaves and flower buds (photo: T. York).

2.4.2.18.4 Ethnobotanical information obtained from interviewees

Mix a handful of leaves with a handful of *E. grandis* leaves and bring to boil with 2 L of water. Steam once or twice daily (depending on the severity) to treat chest pain, cough, fever and a runny- or blocked nose. Children are only allowed to steam for five minutes with parental supervision.

2.4.2.19 *Parinari capensis* subsp. *incohata* (Chrysobalanaceae)

The English vernacular name for *Parinari capensis* Harv. subsp. *capensis* is dwarf mobola. The English vernacular name for *P. capensis* subsp. *incohata* could not be found in the literature.

2.4.2.19.1 Botanical description

Parinari capensis subsp. *incohata* is a dwarf shrublet and a geoxylic suffrutex (Figure 2.20), with its branches arising from a subterranean rhizome. It is very similar to *Parinari curatellifolia* Planch. ex Benth., a medium- to large evergreen tree (Van Wyk and Van Wyk, 1997; Van Wyk and Smith, 2001). *Parinari capensis* subsp. *capensis* has smooth leaves that are lanceolate to elliptic, and flowers occuring in axillary panicles. The fruit is a drupe, and is edible (Von Koenen, 1996).

2.4.2.19.2 Geographical distribution

It is endemic to the coastal grasslands of Maputaland (Van Wyk and Van Wyk, 1997).

2.4.2.19.3 Recorded medicinal uses

Parinari capensis (subspecies not specified) is mixed with another unknown medicine to facilitate conception in women (Watt and Breyer-Brandwijk, 1962). A leaf decoction of *P. capensis* is taken to treat abdominal pain (Chinemana et al., 1985). No medicinal information could be found in the literature specifically for *P. capensis* subsp. *incohata*.

Figure 2.20 *Parinari capensis* subsp. *incohata* shrublet with fruits (photo: T. York).

2.4.2.19.4 Ethnobotanical information

Take a handful of roots, scrape off the outer layer, and chop the remainder before bringing to the boil with 2 L of water. Boil until 0.5 L evaporates and drink half a cup of the decoction a day to treat TB. Children are not allowed to use this medicine until they are spiritually mature.

2.4.2.20 *Plectranthus neochilus* (Lamiaceae)

Lobster flower, blue coleus, poor man's lavender

2.4.2.20.1 Botanical description

Plectranthus neochilus is an aromatic succulent herb with highly branched stems. The stems are hairy and the grey-green leaves are glandular and downy beneath. The leaf margins are slightly toothed and the leaves blunt tipped, folding along the midrib. The flowers develop in terminal inflorescences and have keel-shaped greenish-white bracts that drop off early. Each bract tip is four-angled. The blue to purple flowers have a short upper lip and a big, boat-shaped lower lip (Figure 2.21) (Pooley, 1998).

2.4.2.20.2 Geographical distribution

Plectranthus neochilus occurs in dry thicket and open, rocky woodland of the Eastern Cape, up to KwaZulu-Natal, Zimbabwe, Zambia and Namibia (Pooley, 1998).

2.4.2.20.3 Recorded medicinal uses

In southern Brazil *P. neochilus* leaves are taken for the relief of stomach ache and indigestion (Merétika et al., 2010).

Figure 2.21 *Plectranthus neochilus* flowers and leaves (photo: T. York).

2.4.2.20.4 Ethnobotanical information obtained from interviewees

Mix one handful of leaves with a handful of *L. javanica* leaves and bring to boil with 2 L of water. Sugar can be added to taste. Take one tablespoon three times a day to treat chills, cough and a runny or blocked nose. Children take one teaspoon of the decoction three times a day.

2.4.2.21 *Psidium guajava* (Myrtaceae)

Guava

2.4.2.21.1 Botanical description

This is an evergreen shrub or small tree with hairy, four-angled branchlets (Figure 2.22). The smooth bark is mottled with grey, auburn and whitish patches. The 40-80 mm long leaves are thick and rigid and hairy below. The leaves are ovate to oblong, with impressed venation above and raised venation below. White flowers develop in axillary clusters. The odorous fruit is an oval berry of up to 100 mm long. The green fruit ripens to a yellow colour, containing many seeds and white, yellow or pink flesh. The calyx is still present on ripe fruit (Van Wyk and Van Wyk, 1997).

2.4.2.21.2 Geographical distribution

Psidium guajava is native to tropical America but is cultivated worldwide for its edible fruit. In South Africa, it is an invasive species and found in the warm subtropical areas of KwaZulu-Natal, Mpumalanga and the Northern Province. (Van Wyk et al, 2009).

Figure 2.22 Some interviewees using a *P. guajava* tree for shade (photo: T. York).

2.4.2.21.3 Recorded medicinal uses

Leaf decoctions are commonly taken to treat diarrhoea in South Africa and many other countries, including Latin America, Mozambique, China, Philippines, Fiji and Senegal (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Girón et al., 1991; Gutiérrez et al., 2008; De Wet et al., 2010). Leaf decoctions are also taken as a cough remedy in many countries, including Hong Kong, Egypt, Mexico, Peru, Fiji and Guatemala (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985; Girón et al., 1991; Gutiérrez et al., 2008; Pradhan and Badola, 2008). In Brazil, India and the Philippines leaves and shoots are taken to treat mouth ulcers and throat afflictions (Pradhan and Badola., 2008). A bark decoction is taken in India for blood present in stools (Pradhan and Badola, 2008), and the leaf juice is given to children when they have allergies or headaches (Harsha et al., 2002). Root decoctions are taken to treat venereal diseases (Mabogo, 1990), while leaf and root decoctions are taken for infertility (Gelfand et al., 1985).

Leaf extracts are also taken for diabetes (Girón et al., 1991; Gutiérrez et al., 2008), gastrointestinal problems (Cano and Volpato, 2004) or convulsions (Gutiérrez et al., 2008).

2.4.2.21.4 Ethnobotanical information obtained from interviewees

(a) Take two handfuls of leaves and boil for 30 minutes after adding 2 L of water. Drink half a cup of this decoction three times a day to treat cough, fever, chills, sore throat and a blocked nose. Children only take one tablespoon of this decoction three times daily.

(b) It is used in combination with *E. grandis, S. serratuloides* and *L. javanica*, as described previously (2.4.2.12.4), to treat cough, fever and a runny nose.

2.4.2.22 *Sansevieria hyacinthoides* (Dracaenaceae)

Mother-in-law's-tongue, Piles root

2.4.2.22.1 Botanical description

Sansevieria hyacinthoides is a perennial herb with long and mottled strap-shaped leaves (Figure 2.23). These fibrous, succulent leaves arise from a fleshy rootstock. Small white flowers develop on a long flower cluster, and develop into tiny yellow berry-like fruits (Van Wyk et al., 2009).

2.4.2.22.2 Geographical distribution

Sansevieria hyacinthoides occurs in thicket and woodland from the Eastern Cape into KwaZulu-Natal and tropical Africa (Pooley, 1998).

2.4.2.22.3 Recorded medicinal uses

A very common way for Africans to use *S. hyacinthoides* is to heat the leaf and drip the juice in the affected ear to treat earache (Watt and Breyer-Brandwijk, Bryant, 1966; Roberts, 1990; Pooley, 1998). Toothache can also be treated in this way, while root decoctions are used as a remedy for haemorrhoids and intestinal worms (Watt and Breyer-Brandwijk, 1962; Roberts,

1990; Pooley, 1998). Roots are eaten to ease the pain during child birth (Roberts, 1990), while root infusions are taken as a remedy for miscarriages.

Figure 2.23 *Sansevieria hyacinthoides* plant (photo: T. York).

2.4.2.22.4 Ethnobotanical information obtained from interviewees

Take one whole leaf, and heat on the stove until extremely hot. Let it cool down before squeezing out the juice and applying one drop per affected ear at least three times a day to treat ear ache that is usually accompanied by a discharge. After applying the decoction, the ear must be sealed with a piece of cotton wool.

2.4.2.23 *Scadoxis puniceus* (Amaryllidaceae)

Red paintbrush

2.4.2.23.1 Botanical description

This plant has long and erect strap-shaped leaves arising from a bulb. The leaves have curvy margins with purple speckled leaf stalks. The flowers are orange-red and develop in dense clusters on a long, thick stalk (Figure 2.24) (Van Wyk et al., 2009). The fruit is a berry and is red in colour (Pooley, 1998).

Figure 2.24 *Scadoxis puniceus* plants with flowers (photo: T. York).

2.4.2.23.2 Geographical distribution

Scadoxis puniceus is distributed over all the summer-rainfall areas of South Africa (Van Wyk et al., 2009).

2.4.2.23.3 Recorded medicinal uses

Even though *S. puniceus* is poisonous (Watt and Breyer-Brandwijk, 1962), it is used as a remedy for various ailments, including coughs and chest complaints (Bryant, 1966). Warm water emetics are made from the underground parts and given to people believed to have been poisoned (Hutchings et al., 1996).

2.4.2.23.4 Ethnobotanical information obtained from interviewees

It is used in combination with *A. marlothii, E. caffra* and *Hypoxis* cf. *acuminata*, as described previously (2.4.2.2.4), to treat chest pain, fever and a blocked nose.

2.4.2.24 *Sclerocarya birrea* (Anacardiaceae)

Marula

2.4.2.24.1 Botanical description

It is a Medium sized to large deciduous tree with a spreading, rounded crown (Figure 2.25) and sexes on different plants. The erect trunk has rough, flaky bark with grey and light brown patches (Van Wyk and Van Wyk, 1997; Van Wyk et al., 2009). Leaves are compound with three to seven pairs of leaflets and a terminal one. The leaves are light blue-green below and dark green above with a smooth margin. Young leaves often have pinkish petioles while the margins are typically toothed (Van Wyk and Van Wyk, 1997). The small flowers have yellow petals and red sepals and are borne in unbranched sprays (Van Wyk and Van Wyk, 1997; Van Wyk et al., 2009). The rounded, fleshy fruit ripen to a yellow colour after falling to the ground, containing a hard stone with two to three lids (Van Wyk and Van Wyk, 1997).

2.4.2.24.2 Geographical distribution

Sclerocarya birrea occurs from the south coast of KwaZulu-Natal up to Mpumalanga, Limpopo, Mozambique, Swaziland and northwards to tropical Africa (Pooley, 2003).

2.4.2.24.3 Recorded medicinal uses

Decoctions are made from *S. birrea* bark and taken to treat diarrhoea, dysentery or Malaria (Watt and Breyer-Brandwijk, 1962; Roberts, 1990; Pujol, 1990; Palgrave, 2002; Ojewole et al., 2010). In Cameroon and South Africa stem-bark is taken for diabetes mellitus, while in Ghana various plant parts are used in the treatment of itchy skin, sore throat, goiter, splenomegaly (an enlargement of the spleen) and snake bite. Tanzanians use leaf or root decoctions to treat fungal infections, and in Zimbabwe the roots are used to treat sore eyes (Ojewole et al., 2010). *Sclerocarya birrea* is used as a remedy for various intestinal ailments such as proctitis (inflammation of the anus), gangrenous rectitis and abdominal problems (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Ojewole et al., 2010). Skin diseases such as ulcers and skin eruptions are treated with an application of the boiled bark (Roberts, 1990). It is also used as a heart tonic (Pujol, 1990); while fever, stomach ailments and ulcers are a few other ailments for which it is used (Mabogo, 1990; Ojewole, 2010).

Figure 2.25 *Sclerocarya birrea* tree (photo: T. York).

2.4.2.24.4 Ethnobotanical information obtained from interviewees

(a) Half a handful of bark from the young branches is soaked in half a cup of cold water. Take one tablespoon of this infusion three times a day to treat a dry cough. Children take only one teaspoon of this three times a day.

(b) Chop a piece of mature bark, as well as the bark of *S. cordatum* and mix a handful of each with 2 L of water before bringing to boil. Take two tablespoons of this decoction twice a day to treat cough, fever and a runny nose. Children only take one teaspoon of this twice a day.

2.4.2.25 *Senecio deltoideus* (Asteraceae)

Canary creeper

2.4.2.25.1 Botanical description

This is a slender, multi-stemmed herbaceous climber (Figure 2.26). The stems grow in a zigzag formation, and contain fine hairs. The thin, soft leaves are widely spaced and have unevenly toothed margins. The leaves are dark green above and slightly lighter below. Small, yellow flowerheads develop in widely branched inflorescences and have a sweet aroma (Pooley, 1998).

2.4.2.25.2 Geographical distribution

Senecio deltoideus occurs in open shrub areas and forest margins of the southern parts of the Western Cape, up north to KwaZulu-Natal, Mozambique and Malawi (Pooley, 1998).

Figure 2.26 *Senecio deltoideus* stem and leaves (photo: T. York).

2.4.2.25.3 Recorded medicinal uses

Senecio deltoideus is used to treat sore eyes by applying a leaf paste to the affected eye (Watt and Breyer-Brandwijk, 1962; Pooley, 1998).

2.4.2.25.4 Ethnobotanical information obtained from interviewees

Mix a handful of leaves with a handful of leaves from *Senecio serratuloides* and bring to boil with 1 L of water. Drink half a cup twice a day to treat chest pain, cough, fever and a runny nose. Children only take one tablespoon of the decoction twice a day.

2.4.2.26 *Senecio serratuloides* (Asteraceae)

Two day plant

2.4.2.26.1 Botanical description

This is an herbaceous perennial (Figure 2.27). With erect stems growing from a woody rootstock *S. serratuloides* can grow up to a metre high. The leaves have serrated margins and they are usually about 60 mm long. The small flowers are borne in terminal clusters and are yellow in colour (Van Wyk et al., 2009).

Figure 2.27 *Senecio serratuloides* leaves (photo: T. York).

2.4.2.26.2 Geographical distribution

Senecio serratuloides is widely distributed in South Africa's summer rainfall areas (Van Wyk et al., 2009).

2.4.2.26.3 Recorded medicinal uses

Senecio serratuloides is used to treat infected sores and burns, while a decoction is made to be taken against infections (Watt and Breyer-Brandwijk, 1962; Pooley, 1998). A decoction made from *S. serratuloide*s mixed with an unknown *Combretum* species is used to treat swollen gums and chest pains (Watt and Breyer-Brandwijk, 1962).

2.4.2.26.4 Ethnobotanical information obtained from interviewees

(a) Mix a handful of leaves with 1 L of water and bring to boil. Steam, until the decoction cools, twice a day or drink about half a cup once to three times daily to treat chest pain or fever, sore throat and a runny nose. Children are not allowed to steam, and only take one teaspoon of the decoction once to three times a day.

(b) It is used in combination with *E. grandis* and *L. javanica*, as described previously (2.4.2.12.4), to treat chest pain, cough, headache and a runny or blocked nose.

(c) Used in combination with *E. grandis*, *L. javanica* and *P. guajava*, as described previously (2.4.2.12.4), to treat cough, fever and a runny nose.

(d) It is used in combination with *S. deltoideus*, as previously described (2.4.2.25.4), to treat chest pain, cough, fever and a runny nose.

2.4.2.27 *Syzygium cordatum* (Myrtaceae)

Water berry

2.4.2.27.1 Botanical description

This is a medium-sized evergreen tree growing up to 15 metres high and having a rounded crown. The stem is often bent and it is covered in rough dark brown bark. The branchlets are 4-angled and carry bluish-green leaves. The leathery leaves are elliptic to circular and arranged in opposites close to the end of each branch. Each pair of leaves is distinctively arranged at a right angle to the next. The white to pinkish flowers are produced in branched terminal heads and contain numerous fluffy stamens. The oval fruit is a berry that turns dark purple when ripe, and

usually contains only one seed. The tip of the calyx still remains on the ripe fruit (Figure 2.28) (Van Wyk and Van Wyk., 1997; Van Wyk et al., 2009).

Figure 2.28 *Syzygium cordatum* leaves and fruits (photo: T. York).

2.4.2.27.2 Geographical distribution

This species is found in the Eastern Cape, and northwards through KwaZulu-Natal, Mpumalanga, Limpopo, Swaziland, Mozambique and tropical Africa.

2.4.2.27.3 Recorded medicinal uses

Syzygium cordatum is used to treat stomach problems, as well as respiratory ailments such as TB (Watt and Breyer-Brandwijk, 1962; Mabogo, 1990; Pooley, 2003). A root decoction is taken to treat amenorrhoea, while roots are used for headaches and a bark decoction is used to treat wounds (Arnold and Gulumian, 1984; Mabogo, 1990). A cold leaf infusion is a remedy for diarrhoea. A leaf poultice is applied to the breast of a nursing mother to enhance milk flow to the baby.

2.4.2.27.4 Ethnobotanical information obtained from interviewees

(a) Mix half a handful of chopped bark with half a handful of chopped bark from *T. sericea*, as well as the chopped up underground parts of a plant known as 'intolwane'. Add 2 L of water and boil for 30 minutes. Sieve the decoction and take one tablespoon three times a day to treat cough, sleepless nights and a runny or blocked nose. Children only take one teaspoon of this decoction three times a day.

(b) It is used in combination with *S. birrea*, as described previously (2.4.2.24.4), to treat cough, fever and a runny nose.

2.4.2.28 *Terminalia sericea* (Combretaceae)

Silver cluster-leaf

2.4.2.28.1 Botanical description

This is a small to medium deciduous tree with silver-grey leaves (Figure 2.29). The crown is rounded to flat and layered, typical of most members of this genus. The silver-haired leaves are narrowly obovate-elliptic and crowded near the branch tips (Van Wyk and Van Wyk, 1997). The pale brown to grey bark has a net-like pattern from splitting of the outer and inner bark, while the branchlets are dark brown to purplish and flaking off in strips and rings (Van Wyk and Van Wyk, 1997; Van Wyk et al., 2009). The small, cream-coloured flowers are borne in axillary spikes and have an unpleasant smell. The indehiscent fruit is surrounded by a wing and is covered in short hairs. The fruit matures to a pink to purplish red colour and dries to become rust coloured (Van Wyk and Van Wyk, 1997).

2.4.2.28.2 Geographical distribution

Terminalia sericea occurs in Mpumalanga, Limpopo, Swaziland, Mozambique, Zimbabwe and tropical Africa. It is specifically dominant in some areas in Maputaland (Pooley, 2003).

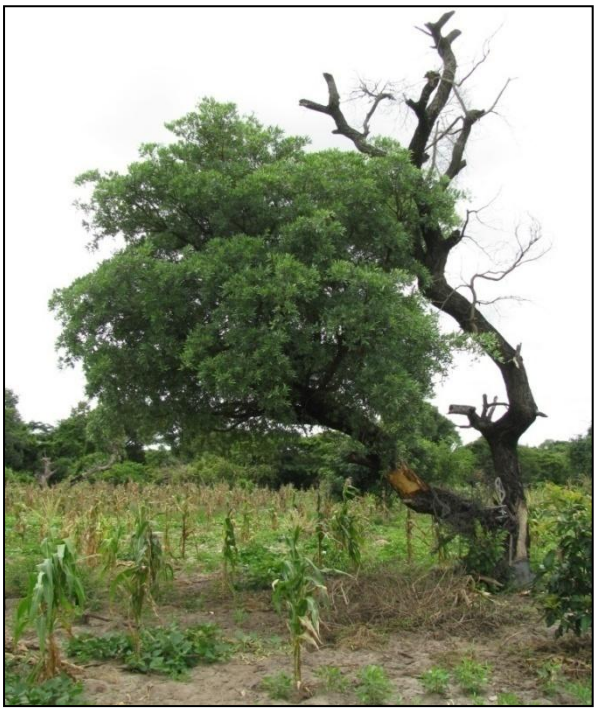


Figure 2.29 *Terminalia sericea* tree (photo: T. York).

2.4.2.28.3 Recorded medicinal uses

The bitter root decoction is taken to treat diarrhoea (Watt and Breyer-Brandwijk, 1962; Chinemana et al., 1985; Mabogo, 1990; Neuwinger, 1996; Fyhrquist et al., 2002; Palgrave, 2002; De Wet et al., 2010) and colic (Neuwinger, 1996; Palgrave, 2002). Plant parts are also used to treat stomach ailments and dysentery (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976). Roots are used as a remedy to treat infertility and venereal diseases such as syphilis and gonorrhoea (Hedberg and Hedberg, 1982; Mabogo, 1990; Neuwinger, 1996), while the leaves are used to treat wounds and menorrhagia (Mabogo, 1990). Infected eyes are treated by applying a root decoction to the affected eye (Neuwinger, 1996; Palgrave, 2002). *Terminalia sericea* is also used as a remedy for respiratory ailments such as pneumonia (Palgrave, 2002; Pooley, 2003). In Tanzania, it is used in the treatment of fever, hypertension and bacterial infections (Fyhrquist et al., 2002). Other ailments for which it is also used include diabetes (Watt and Breyer-Brandwijk, 1962) and bilharzia (Kokwaro, 1976).

2.4.2.28.4 Ethnobotanical information obtained from interviewees

(a) The bark is used in combination with *S. cordatum* and a plant known as 'intolwane', as previously described (2.4.2.27.4), to treat coughs, sleepless nights and a runny or blocked nose.

(b) The leaves are used in combination with *C. molle* and *L. javanica*, as described previously (2.4.2.8.4), to treat a chronic cough.

2.4.2.29 *Tetradenia riparia* (Lamiaceae)

Ginger bush

2.4.2.29.1 Botanical description

This is a deciduous shrub or tree, about one to three metres in height. The succulent stems are hairy and square in cross-section when young. The soft, heart shaped leaves have blunt-toothed margins (Figure 2.30). Essential oils are released from glands on both leaf surfaces, making the leaves aromatic. The flowers are borne in feathery plumes at the branch ends and are white to light purple in colour. Sexes are on different plants, with male plants having larger, more striking inflorescences. Sexes cannot be identified when the plant is not in flower (Crouch et al., 2006).

2.4.2.29.2 Geographical distribution

Tetradenia riparia occurs in southern Africa, with its distribution extending from KwaZulu-Natal through tropical eastern Africa to Ethiopia (Crouch et al., 2006).

2.4.2.29.3 Recorded medicinal uses

Tetradenia riparia is taken for stomach ache (Watt and Breyer-Brandwijk, 1962; Pooley, 1998; Scott et al., 2004; Crouch et al., 2006), nausea, ulcers, intestinal worms and diarrhoea (Crouch et al., 2006). Leaf infusions are taken to treat malaria (Watt and Breyer-Brandwijk, 1962; Pooley, 1998; Crouch et al., 2006), angina (chest pain) (Van Puyvelde et al., 1986; Scott et al., 2004; Crouch et al., 2006) or cold and flu symptoms (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Pujol, 1990; Pooley, 1998; Scott et al., 2004; Crouch et al., 2006). *Tetradenia riparia* is applied

as a topical antiseptic (Scott et al., 2004), and used for relief of oedema (abnormal accumulation of fluid under the skin) (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Scott et al., 2004).

Figure 2.30 *Tetradenia riparia* leaves (photo: T. York).

2.4.2.29.4 Ethnobotanical information obtained from interviewees

(a) Soak a handful of leaves in half to a full cup of warm water. Take two tablespoons of this decoction three times a day, or whenever feeling sick, to treat cough, fever, sore throat and a runny nose. This decoction can also be taken as an enema. Adults take a size six syringeful (180 mL) twice a day, while children take a size two (60 mL) syringeful once a day. Children only take one teaspoon of this decoction three times a day.

(b) Soak a handful of leaves (or about five leaves) in half a cup to a full cup of boiling water for about five minutes. Take one tablespoon of the decoction three times a day to treat cough, chest pain, sore throat, or a blocked nose. One interviewee mentioned that it gives immediate relief to a sore throat. Children can only take one teaspoon of this decoction twice to three times a day.

(c) Bring to boil one handful of leaves in two cups of water and drink about half a cup twice to three times a day to treat cough, chest pain, headache and a blocked or runny nose. Children take one teaspoon of this decoction twice to three times daily.

(d) Put a handful of leaves in 1 L of water and boil the mixture until the water colours. Drink half a cup of the decoction three times a day to treat chest pain and fever. Children take only one tablespoon of the decoction three times daily.

(e) Crush a handful of leaves and mix with two cups of cold water. Drink half a cup of this decoction three times a day to treat chest pain, cough, headache, shortness of breath and a runny or blocked nose. Children take one teaspoon of this infusion three times a day.

(f) It is used in combination with *E. grandis* and *L. javanica*, as described previously (2.4.2.12.4), to treat chills, cough, sleepless nights, fever and headaches.

(g) It is used in combination with *A. glabratum* and *L. javanica*, as described previously (2.4.2.1.4), to treat cough and tiredness.

(h) Used in combination with *L. javanica*, as described previously (2.4.2.17.4), to treat cough, sore throat and fever.

2.4.2.30 *Trichilia emetica* (Meliaceae)

Natal mahogany

2.4.2.30.1 Botanical description

This is a medium to large evergreen tree with sexes on separate plants. It has a dense, spreading crown. The compound leaves are glossy, dark green above and hairy below, having 9-11 leaflets per leaf. Each leaflet has broadly pointed or rounded tips with 11-18 pairs of narrowly spaced main side veins. The flowers are yellowish-green. The fruit is a dehiscent capsule, joined to the stalk by a long stipe. The black seed is almost totally enclosed by a bright red aril (Figure 2.31) (Van Wyk and Van Wyk, 1997).

2.4.2.30.2 Geographical distribution

Trichilia emetica occurs northwards from Durban (KwaZulu-Natal), along the eastern border of South Africa, extending into east tropical Africa (Van Wyk and Van Wyk, 1997).

Figure 2.31 *Trichilia emetica* fruits and leaves (photo: T. York).

2.4.2.30.3 Recorded medicinal uses

Bark decoctions are mostly taken to treat stomach and intestinal problems, including gastric ulcers and intestinal parasites (Bryant, 1966; Mabogo, 1990; Dharani, 2002; Togola et al., 2005). Other related ailments include the treatment of haemorrhoids and constipation (Togola et al., 2005). A crushed bark decoction is taken as an enema in the treatment of diarrhoea (De Wet et al., 2010). Plant parts are also used as a remedy for kidney problems and urinary infections (Mabogo, 1990; Dharani, 2002; Togola et al., 2005). In Tanzania, *T. emetica* is a remedy for epilepsy, malaria, TB, gonorrhoea and syphilis (Moshi et al., 2009), while in Mali, West Africa, uses include the treatment of jaundice, swelling, chest pain, pneumonia, fatigue, eye infections, headaches, leprosy, bilharzia, female sterility and dysmenorrhoea (Togola et al., 2005). Seed oil is applied to the affected areas in the treatment of cuts, bruises and rheumatism (Watt and Breyer-Brandwijk, 1962; Dharani, 2002). Besides using the fruit oil, leaf and fruit poultices can also be applied to bruises or problem skin (Dharani, 2002). Bark or leaf infusions are used to treat lower back pain and dysentery (Watt and Breyer-Brandwijk, 1962), while root and bark decoctions are taken for fever (Dharani, 2002).

2.4.2.30.4 Ethnobotanical information obtained from interviewees

(a) It is used in combination with *H. kraussii* and *L. javanica*, as described previously (2.4.2.14.4), to treat chest pain, cough and a blocked nose.

(b) Used in combination with *L. javanica*, as previously described (2.4.2.17.4), to treat headache, fever, cough and a runny nose.

2.4.3 An overview of plants used to treat respiratory infections in the study area

This survey found 30 species (18 plant families) as useful in managing respiratory infections by the rural people in the study area. Some species, namely *Brachylaena* spp. and *Hypoxis* sp., could not be identified to species level as flowers were not available at the time of collection. *Acanthospermum glabratum, A. marlothii, K. mosambicina, O. obovata, P. capensis* and *P. neochilus* have previously been recorded for their medicinal use, but this is the first record of use to treat respiratory infections or related symptoms. Even though the medicinal use of *P. capensis* has been recorded (Watt and Breyer-Brandwijk, 1962), there is no such information recorded on *P. capensis* subsp. *incohata*. This could be due to the fact that this subspecies is endemic to Maputaland (Van Wyk and Smith, 2001), a remote area where ethnobotanical studies are uncommon. The indigenous shrub, *L. javanica* was by far the most frequently used plant species, followed by *E. grandis* (an exotic) and *T. riparia* (indigenous). These three species all have highly aromatic leaves (Van Wyk and Wink, 2004; Van Wyk et al., 2009). Both *L. javanica* (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985; Mabogo, 1990; Roberts, 1990; Njoroge and Bussmann, 2006) and *T. riparia* (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Pujol, 1990; Pooley, 1998; Scott et al., 2004; Crouch et al., 2006; Tabuti et al., 2010) have been frequently recorded for their use against respiratory ailments. The plant part predominantly used was the leaves, while other parts used included either bark or underground parts. Eighty-seven percent of the plant species encountered in the survey are indigenous, and reports of all underground plants parts were from indigenous species. In general, the harvesting of bark and underground parts has the most detrimental influence on plant populations (Dahlberg and Trygger, 2009). This is important when looking at the sustainable use of indigenous flora.

Even though *P. guajava* has only been mentioned twice during the current survey, its use in treating respiratory diseases and fever has been recorded worldwide (Jaiarj et al., 1999; Cano and Volpato, 2004; Njoroge and Bussmann, 2006; Gautam et al., 2007; Ruysscaert et al., 2009; Mohamad et al., 2011). In the Malaysian survey *P. guajava* was recorded as one of the plant species used to treat TB-related diseases (Mohamad et al., 2011), while *P. guajava* was one of

the 67 plant species found to be used against ear-, nose- and throat infections in Kenya (Njoroge and Bussmann, 2006).

During the current survey 13 new vernacular plant names, not previously documented have been revealed for eight of the plant species. These species include *A. glabratum*, *C. brachiata*, *C. articulatus*, *E. tirucallii*, *H. kraussii*, *P. capensis*, *P. neochilus and S. deltoideus*. This, in addition to the six plant species documented for the first time in this study for their use against respiratory infections, emphasizes the importance of documenting such knowledge. Table 2.6 documents the local plant names found in the current survey as well as previously recorded local vernacular plant names (Hutchings et al., 1996; Pooley, 1998; Pooley, 2003; Von Ahlefeldt et al., 2003 and De Wet et al., 2010; De Wet et al., 2012). McGaw et al. (2008) summarized all available knowledge on South African plants used to treat TB symptoms, based on reviews written by authors including Watt and Breyer-Brandwijk, (1962), Bryant (1966) and Hutchings et al., (1996). The importance of the documentation of existing ethnobotanical knowledge was emphasized by McGaw (2008). Nine of the plant species (*C. molle*, *E. capensis*, *L. javanica*, *P. guajava*, *S. puniceus*, *S. serratulloides*, *S. cordatum*, *T. sericea* and *T. riparia*), recorded in McGaw's survey were also recorded in the current survey.

A number of different plant species can share a specific Zulu vernacular name (Hutchings et al., 1996). In the current study it was found that *P. neochilus* share its local vernacular name (*ibozane*) with *T. riparia*. The same was found with *A. glabratum*, which shares one of its mentioned vernacular names (*isihlaza*) with *K. mosambicina*. During our survey, this has made us very aware of the fact that the identity of a plant species cannot be assumed based solely on the given vernacular name. The identity of plant species were thus only based on collected plant material. For this reason, as can be seen at 2.4.2.27.4 (a), we did not identify the plant that was given the vernacular name of *intolwane*.

Table 2.6 Medicinal plants used for the treatment of respiratory infections in rural Maputaland, their Zulu vernacular names, as well as the number of occurrences at which the plant species were mentioned.

Plant scientific name	Zulu vernacular name(s)	Times mentioned
Acanthospermum glabratum	Isihlaza*; **, Nkunzana**, Inkonzana**, Isibambaphansi**, Isichathabantwana**	7
Aloe marlothii	Inhlaba, Umhlaba	1
Brachylaena discolor	Ipahla, Iphahla, Isiduli, Isiphaluga, Umduli, Umphaphla	1
Brachylaena spp.	Ipahla	3
Bridelia cathartica	Umkhawulangazi, Umnangasi, Umngwangazi, Umnwangazi, Umthundangazi, Umzilanyoni	1
Citrus limon	Lemon tree	1
Clausena anisata	Isifudu, Isifuthu, Umnukambhiba, Umnukelambiba, Umsanka, Umwashampunzi	2
Clematis brachiata	Ihlonzo leziduli, Inhlabahlanzi, Inhlongo, Umdladlatho**, Umdlandlathi, Umdlonzo, Umdlozo, Umfufuna**	3
Combretum molle	Umbondo, Umbondwe (-omhlope)	4
Cyperus articulatus	Incethe**	3
Ekebergia capensis	Isimanaye, Umathunzi wentaba, Umathunzini, Umathunzini-wezintaba, Umgwenyana wezinja, Umnyamathi, Umthoma, Usimanaye, Uvungu	1
Erythrina caffra	Umsinsi	1
Eucalyptus grandis	Gum tree, Gum bush, Impiskayihlangulwa, Umdlavusa, Umdlebe	33
Euphorbia tirucalli	Inhlonhlwane**, Umnduze, Umsululu	1
Helichrysum kraussii	Isilelevu**, Isipheshane, Isiqoqo	1
Hypoxis sp	Inkomfe, Inkomge enkula	2
Krauseola mosambicina	Isihlaza, Isihlazi	2
Lippia javanica	Umsuzwane, Umswazi	58
Ozoroa obovata	Isifice, Isifici	1

Plant scientific name	Zulu vernacular name(s)	Times mentioned
Parinari capensis	Amabhulwa, Ibulwa**	1
Plectranthus neochilus	Ibozane**	1
Psidium guajava	Uguava	2
Sansevieria hyacinthoides	Isikholokotho, Isikwendle, Isitokotoko, Isikhotakhota	1
Scadoxis puniceus	Idumbelentaba, Idumbi likanhloyile, Umphompo, Umgola, Idumbe-lika-nhloyile, Idumbe-likahlonyile, Isiphompo	1
Sclerocarya birrea	Umganu	2
Senecio deltoideus	Ulambula**	1
Senecio serratuloides	Ichazampukane, Insukumbili, Intsukumbili-uma-hanya, Umaphozisa umkutelo	6
Syzygium cordatum	Umdoni	2
Terminalia sericea	Amangwe(amanyama,-amhlophe,-amnyama), Ikonono, Inkonono	2
Tetradenia riparia	Iboza, Ibozane	10
Trichilia emetica	Ixolo, Umathunzini, Umkhuhla, Umkhuhlu, Umkhuhlwa	3

*Underlined local vernacular names were mentioned by the interviewee in the current study; **Vernacular name recorded for the first time

Plants were not only used independently but also in 24 different combinations, as shown in Table 2.7. Of the 80 homesteads studied, 49 were using plants in different combinations to treat respiratory infections. Combinations of two- (35 households), three- (11 households) and four plant species (two households) were noted. Besides these 24 different plant combinations, there was also a combination in which the roots and the leaves of the same species (*L. javanica*) were mixed. What is interesting is how frequently *L. javanica* and *E. grandis* were used in different combinations (mentioned 21 times, Table 2.7). In 85% of the times the use of *E. grandis* was mentioned, it was used in combination with *L. javanica,* and not on its own.

Table 2.7 The different plant combinations mentioned, as well as the number of occurrences at which these combinations were mentioned to treat respiratory infections by the 80 interviewees.

Plant species in the combination	**Times mentioned**
Aloe marlothii and *Hypoxis* sp. and *Scadoxis puniceus* and *Erythrina caffra*	1
Eucalyptus grandis and *Ozoroa obovata*	1
Lippia javanica and *Brachylaena* cf. *uniflora*	1
Lippia javanica and *Bridelia cathartica*	1
Lippia javanica and *Clematis brachiata*	2
Lippia javanica and *Combretum molle*	1
Lippia javanica and *Combretum molle* and *Eucalyptus grandis*	1
Lippia javanica and *Combretum molle* and *Terminalia sericea*	1
Lippia javanica and *Eucalyptus grandis*	21
Lippia javanica and *Eucalyptus grandis* and *Brachylaena* cf. *transvaalensis*	1
Lippia javanica and *Eucalyptus grandis* and *Clausena anisata*	2
Lippia javanica and *Eucalyptus grandis* and *Psidium guajava* and *Senecio serratuloides*	1
Lippia javanica and *Eucalyptus grandis* and *Senecio serratuloides*	2
Lippia javanica and *Eucalyptus grandis* and *Tetradenia riparia*	1
Lippia javanica and *Hypoxis* sp.	1
Lippia javanica and *Krauseola mosambicina*	1
Lippia javanica and *Plectranthus neochilus*	1
Lippia javanica and *Tetradenia riparia*	1
Lippia javanica and *Tetradenia riparia* and *Acanthospermum glabratum*	1
Lippia javanica and *Trichilia emetica*	2
Lippia javanica and *Trichilia emetica* and *Helichrysum kraussii*	1
Senecio deltoideus and *Senecio serratuloides*	1
Syzygium cordatum and *Terminalia sericea* and '*intolwane*'*	1
Syzygium cordatum and *Sclerocarya birrea*	1
Lippia javanica leaf and root	1

*Due to the fact that the third plant species have not been identified, further antimicrobial research focused on the combination of *S. cordatum* with *T. sericea* only.

In other ethnobotanical studies done on plants used against respiratory infections, a few mentioned the use of plant combinations (Ballabh and Chaurasia, 2007; Gautam et al., 2007; Savithramma et al., 2007; McGaw et al., 2008; Green et al., 2010), while other researchers only mentioned plant species used singularly (Caceres et al., 1991; Rojas et al., 2001; Njoroge and Bussmann, 2006). Unusually, Ballabh and Chaurasia (2007) discussed the use of plant combinations where combinations of up to seven different plant species were recorded. Minerals and rock salts formed part of most of these combinations. In the current survey, combinations comprise merely of different plant species or plant parts, and only up to four species in a combination was reported. Previous research has found that lay people don't have the knowledge

to mix remedies of more than two plant species (Dahlberg and Trygger, 2009). During the current survey it was found that 11 of the 24 different types of plant combinations (Table 2.7) are combined with three or more plant species, and that all these complex remedies were given by lay people. This demonstrates that, inspite of the complexity or education of a practitioner, multiple combinations are used. Even though both *E. grandis* (Ssegawa and Kasenene, 2007) and *L. javanica* (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985; Roberts, 1990; Purkayastha et al., 2005) have been documented for their independent use in the treatment of respiratory infections, the current survey found that these two species are frequently used in combination (Table 2.7). This supports findings by Dahlberg and Trygger (2009), where the combined use of *Eucalyptus* spp. with *L. javanica* was mentioned in the treatment of flu and headaches.

The most common way of preparing the herbal medicines documented in this survey is by taking a handful of plant material, mixed or alone, adding cold water and bringing the mixture to the boil. This decoction is then mostly taken orally while what's remaining is used for steaming. Steaming takes place until the decoction cools down. Children are often not allowed to steam at all, while in some instances steaming is allowed with parental guidance. When a decoction is taken orally, or anally, the amount given to small children is usually a little less than half the dosage given to adults. According to a study done by Dahlberg and Trygger (2009), conducted in an area close to the vicinity of the current survey area (north-western part of Mkuze wetlands, on the coastal plain of KwaZulu-Natal), the strength of a herbal preparation was considered when given to children, and the remedy was usually given in a diluted form. During the current survey it was discovered that a decoction is often prepared with warm water when applied anally (used as an enema). The enema is applied with a bulb style enema syringe (Figure 2.32). All of the interviewees mentioning the use of such a syringe referred to it as a certain size syringe. Each syringe has a different number on the bottom of its bulb, ranging from size 1 to size 10. Size one has the volume of approximately 30 mL, while size six is 180 mL and size ten 300 mL. Between the homesteads there is not much of a difference in the preparation- and administration methods of a specific plant combination. Usually the amount of water added varies a little. For example, in preparing the mixture of *L. javanica* and *E. grandis*, the method includes mixing a handful of each plant species with water and bringing to the boil. The amount of water added may vary between 1 L and 5 L.

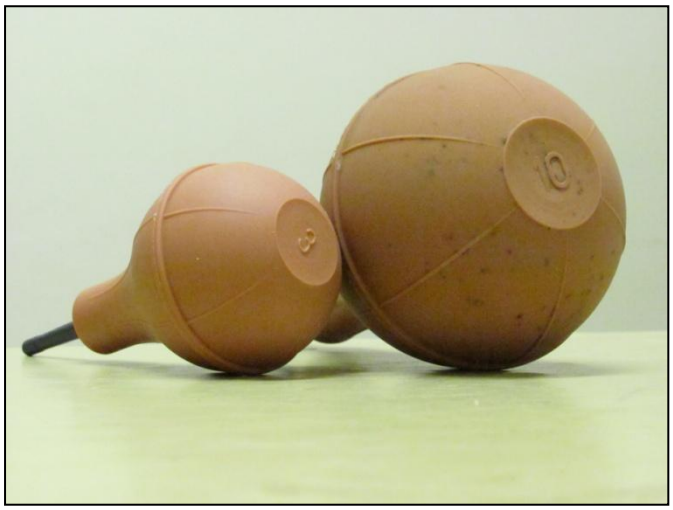


Figure 2.32 Two bulb style enema syringes (size 3 and size 10) used in anal plant administration (photo: T. York).

One interviewee specified adding sugar to her plant remedy of *L. javanica* with *P. neochilus* (Section 2.4.2.20.4). She specifically claimed that she added the sugar to enhance the taste of this decoction. Recent studies have been done on sugar as a possible natural solvent (Choi et al., 2011). These authors concluded that "natural deep eutectic solvents", such as sugars, are potential solvents for medicinal extractions and could replace conventional organic solvents. It is possible that sugar, when added to water, allows compounds that are poorly soluble in water to obtain a higher solubility (Choi et al., 2011). Thus, besides enhancing the taste of the interviewee's plant remedy, the added sugar could have had the additional benefit of extracting more active compounds from this plant mixture into the solution.

When asked whether each interviewee would choose western- or traditional medicine, the majority of the interviewees claimed to prefer using traditional medicine (Table 2.8). Many of these people would then convert to the use of western medicine if symptoms persisted. Some of these interviewees have previously used western medicine but due to the following mentioned shortcomings at their local clinics they have gone back to the use of traditional medicine; long queues, drug shortages, clinics too full or busy, clinics closed on weekends. One interviewee said that she preferred to go to the clinic, but if the health care provided by the clinic did not prove effective she would revert to the use of traditional medicine. Another interviewee claimed to have been advised by hospital staff to rather use traditional medicine due to the fact that the hospital is often too busy. Although a large proportion of interviewees claimed to choose

traditional medicine because of its better efficacy compared to western medicine (19%, Table 2.8), a reason given far more than the latter was that traditional medicine was part of the interviewee's culture and base knowledge (45%, Table 2.8). The majority of these people said that the knowledge of western medicine was either new, or that a clinic has only recently opened close to where they lived. It would seem like for many of these interviewees, that their health care choice was not necessarily an educated one. One interviewee said that she sometimes used traditional medicine, but only to show respect to her culture. Her knowledge of such traditional remedies has been passed on to her by her parents, and she shared some of this knowledge with us. Interestingly enough, the remedy that she shared (*B. cathartica;* Section 2.4.2.4.4) is unique in the current study since it was not given by any of the other interviewees. Even though these traditional plant remedies are free and easily available to these interviewees (growing in and around their homesteads), this convenience was not the main motivation toward the use of traditional medicine. There were also a few other interesting responses. Two of the interviewees stated that they found a combination of western- and traditional medicine to prove most effective. One of these interviewees specifically explained that western medicine targets the disease symptoms, while traditional medicine targets the source of the disease. Two other interviewees said that they have recently converted to the Christian religion, and because they felt that the use of traditional medicine went against this belief they no longer wanted to use this form of health care. One of these interviewees specifically admitted that this choice was also based on her fear of the spiritual connotation associated with the use of traditional medicine. During a previous survey, also conducted in rural KwaZulu-Natal, it was found that, while some church groups did not allow treatments by traditional healers due to their association with black magic, most churches allowed the use of household remedies (Dahlberg and Trygger, 2009). During this study conducted by Dahlberg and Trygger (2009) it was however found that some religious groups opposed the use of any kind of health care, believing that only God could cure disease.

Not one of the interviewees mentioned a connection between a plant's characteristics (appearance, taste or smell) and the respiratory system. The majority of interviewees did mention the use of aromatic plant species, referring to these species' strong aroma. According to the Doctrine of Signatures, the shape, colour, taste and smell of a plant indicates its healing properties (Pearce, 2008). It could be that plant species used for respiratory infections are selected based on their aromatic properties – making a connection between inhaling the strong smelling vapours of such plants, and the respiratory system. Many previous reports have

documented the use of aromatic plants in the treatment of respiratory diseases or symptoms (Hutchings et al., 1996; Dugmore and Van Wyk, 2008; Van Wyk et al., 2009).

Table 2.8 Most commonly given answers from interviewees when questioned about preference to use traditional medicine

Answers given (Often more than one answer was given by the same interviewee)	**Percentage of interviewees giving this answer (%)**
Traditional medicine is part of the interviewee's culture and it's what he/she's used to because of growing up with it	45
Traditional medicine works faster and/or is more effective than western medicine	19
Uses traditional medicine first, but if the symptoms persist he/she will go to the clinic or hospital	16
Traditional medicine is easily accessible and free	9
The interviewee have only recently been introduced to western medicine	5
Some diseases can't be cured with western medicine	4
You usually wait in long queues at the clinic, and sometimes they're out of medicine	4

Worldwide, aromatic plants have been used medicinally for thousands of years (Steflitsch and Steflitsch, 2008). Some of the plant species used by the interviewees in the current survey have strong aromatic properties, as found in *L. javanica, E. grandis, C. limon* and *T. riparia*. The aromatherapeutic potency of plants is mainly in the leaves (Pujol, 1990). This could be why, in this study area, leaves have been found to be the preferred plant part used in the treatment of respiratory ailments. In the case of the highly aromatic *L. javanica,* and also *C. brachiata*, plant parts are often rubbed between the hands and inhaled for immediate relief of symptoms. The aromatherapeutic properties of plants can also be found in one of the other plant organs, as found for example in the pseudo-bulbs of *C. articulatus* (Watt and Breyer-Brandwijk, 1962). The essential oils of such aromatic plants are produced and stored in the secretory cells, cavities, canals, epidermic cells or glandular trichomes of these various plant organs (Bakkali et al., 2008). During a survey conducted by Dahlberg and Trygger (2009) it was mentioned, though, that, while healers preferred to use more underground plant parts, lay people preferred to use more leaves. These findings correlate with results of the current survey, where leaves were the preferred plant part used by lay people. This could indicate, though, that the high percentage of leaf use found in the current survey is not necessarily due to the aromatic properties found in leaves, but owing to the fact that most of these interviewees were lay individuals.

Use of medicinal plants common to both the current survey and the antidiarrhoeal survey by De Wet et al. (2010), in the same geographical area, include; *A. glabratum*, *Brachylaena* sp., *K. mosambicina*, *L. javanica*, *P. guajava*, *S. birrea*, *S. cordatum*, *T. sericea* and *T. emetica*. De Wet et al. (2012) conducted another survey in this area, where the focus was on plants used to treat sexually transmitted infections (STI). The current survey reveals a higher incidence of plant usage to that used to treat STIs (De Wet et al., 2012) (*A. marlothii*, *B. cathartica*, *C. brachiata*, *C. molle*, *E. caffra*, *E. tirucalli*, *Hypoxis* sp., *K. mosambicina*, *S. birrea*, *S. serratuloides*, *S. cordatum* and *T. sericea*) when compared to antidiarrhoeal plants (De Wet et al., 2010). There are nine aromatic plants used in the current survey, and only two of these (*C. brachiata* and *L. javanica*) were also reported for their use in previous ethnobotanical studies done in the study region (De Wet et al., 2010, 2012). This might indicate that using aromatic plants in the treatment of respiratory conditions is the preference of the people living in this area. This use of aromatic plants for the treatment of respiratory ailments has also been found in a Kenyan study, where some of the plant species mentioned most frequently for the treatment of ear-, nose- and throat infections were aromatic (*Eucalyptus saligna* Sm., *L. javanica* and *Ocimum gratissimum* L.) (Njoroge and Bussmann, 2006). Similarly, a Ugandan survey found *Eucalyptus* spp. to be the most frequently used plant species in the treatment of TB (Tabuti et al., 2010). Many of the interviewees of the current survey prepare inhalations containing volatile substances from the aromatic plants. Njoroge and Bussmann (2006) also mentioned the frequent use of inhalations when administering aromatic plants. This is possibly because essential oils of such aromatic plants can be absorbed through the mucous membranes of the nose and lungs (Van Wyk and Wink, 2004). When inhaling the vapours from these aromatic plants, the volatile constituents come into direct contact with the lining of the respiratory tract (Van Wyk et al., 2009). A finding in the current survey that seems to contradict this is the fact that there were a few interviewees (8.8%) who preferred taking plant decoctions anally instead of orally or through inhalations. What is even more interesting is the fact that the highly aromatic *L. javanica* forms part of some of these enema decoctions. According to literature, the use of an enema in the treatment of respiratory infections is not common, but it has been documented by Watt and Breyer-Brandwijk (1962) (*Senecio* sp. applied as an enema against chest complaints). It is believed that certain plant extracts are more effective when applied through an enema. This application method is supported by the fact that the mucosa of the rectum and large bowel absorbs the active compounds of medicine very quickly (Van Wyk et al, 2009). Depending on the nature of the extract used, for better therapeutic effect it may be desirable for some herbal remedies to by-pass the stomach (Gurib-Fakim, 2006).

The current survey focused on plant use in the treatment of various respiratory problems or diseases. Such respiratory diseases could include communicable diseases such as pneumonia or otitis, or non-pathogenic diseases such as chronic obstructive pulmonary disease (COPD) or asthma. Even though the current study focused on testing the antimicrobial efficacy of the various plant remedies, interviewees using such remedies could also be suffering from non-pathogenic diseases. According to the South African Child Gauge 2009/2010 (Sanders and Bradshaw, 2010) environmental risk factors such as poorly ventilated and smoke filled living areas are risk factors in increased exposure to toxic agents and impaired immunity. When asked about the smoking habits of each homestead, only 22.5% of the homesteads had exposure to tobacco smoke, and in only one of these homesteads more than one family member was a smoker. It could be concluded that any smoke induced respiratory problems would most likely not be due to tobacco smoke, and also due to the fact that these people inhabit rural areas that are situated away from any industrial pollution, any non-pathogenic respiratory problems are probably caused by another source. In developing countries it has been found that woman spend a lot of time close to an open unvented fire when cooking meals for their families. Inhalation of wood smoke produced by such fires has effects on the health of these people (Larson and Koenig, 1994). In a clinical report done by Sandoval et al. (1993) a group of non-smoking patients with lung disease were monitored. These people had a history of living away from urban air pollution. Their smoke exposure came from wood fires used for cooking and it was suggested by the authors that these people had suffered from wood-smoke inhalation-associated lung disease (WSIALD) (Larson and Koenig, 1994).

During the current survey, where information was gathered on traditional therapies that are used against respiratory ailments, the focus was on the knowledge of lay people. Information obtained from the interviewees during the current survey has demonstrated that lay people are very knowledgeable about the use of traditional remedies for their primary health care. This supports studies done by Dahlberg and Trygger (2009) and De Wet et al (2010), as discussed in the introduction.

2.5 Summary

(a) This study documented 30 plant species which were used to treat respiratory infections by the rural people in northern Maputaland.

(b) To the best of our knowledge, *A. glabratum*, *A. marlothii*, *K. mosambicina*, *O. obovata*, *P. capensis* subsp. *incohata* and *P. neochilus* are recorded for the first time globally as medicinal plants used in treating respiratory infections or related symptoms.

(c) Twenty-four different plant combinations were mentioned, ranging from a combination of two to four plant species.

(d) The use of these complex plant combinations by lay people, in this geographical area, is new information that brings an interesting new perspective on lay people's use of traditional medicine.

(e) The preferred use of traditional medicine by the people in the current survey supports previous research on the importance of traditional medicine in the primary health of rural inhabitants.

(f) The finding of new vernacular plant names and plant uses in the current survey shows the importance of the documentation of such ethnobotanical knowledge.

Chapter 3

The antimicrobial screening of plants used to treat respiratory ailments

3.1 Introduction

Globally, there have been a number of studies focusing on the scientific validation of plants traditionally used to treat respiratory ailments, specifically testing such plants against pathogens related to respiratory diseases (Caceres et al., 1991; Rojas et al., 2001; Adeleye and Opiah, 2003; Leitão et al., 2006; Kariuki and Njoroge, 2011; Luo et al., 2011; Mohamad et al., 2011). A study on plants traditionally used in Mexico, showed that six different plant species were tested against various respiratory pathogens. It was found that 89% of these plant species exhibited antimicrobial activity against at least one of the six microorganisms they were tested against (Rojas et al., 2001). In Guatemala, Caceres et al. (1991) screened 68 plant species, used for the treatment of respiratory diseases against three Gram-positive respiratory micro-organisms and 41% of the species inhibited the growth of one or more of the selected pathogens. In a survey done in Malaysia, 70 plant species were selected from literature, as well as interviews, for their use in treating respiratory diseases, including TB. Seventy-eight extracts from these plants were tested against *M. tuberculosis*, and 32 of these extracts exhibited antituberculosis activity (Mohamad et al., 2011). In a Mozambican study, Luo et al. (2011) evaluated plant species used for the treatment of TB and other respiratory diseases for their antimycobacterial activity. Adeleye and Opiah (2003) tested the antimicrobial activity of six ingredients (five plant extracts and honey) commonly used in Nigeria as ingredients in cough mixtures. Three of the plant species tested showed a broad spectrum of antimicrobial activity. While some of these studies serve only as preliminary scientific validation for the use of plants against respiratory ailments, results from such investigations demonstrate, overall, the antimicrobial potential of traditionally used plant species in the treatment of respiratory infections.

Most of the ethnobotanical studies on South African medicinal plants used to treat respiratory illnesses have focused on plant species with possible antimycobacterial properties (Lall and Meyer, 1999; Seidel and Taylor, 2004; Mativandlela et al., 2006; Eldeen and Van Staden, 2007; Bamuamba et al., 2008; Mativandlela et al., 2008; Green et al., 2010). In one such study, conducted in the Limpopo Province, traditional healers were interviewed about treatments given to their TB patients. The 23 plant species given by the healers were tested for their

antimycobacterial activity. Many of these plants (52%) did not show antituberculosis activity against either of the mycobacterial strains tested (Green et al., 2010). In another South African study 20 plant species previously recorded for their traditional use in treating TB-related symptoms were tested for antimycobacterial activity. Fourteen plant species (acetone extracts) showed activity against *M. tuberculosis* (Lall and Meyer, 1999). Seidel and Taylor (2004) tested the extracts from two *Pelargonium* spp. used traditionally for a number of ailments, including TB, for activity against various rapidly growing mycobacterial species (including *M. smegmatis*). Only extracts obtained using the apolar solvent n-hexane showed any anti-mycobacterial activity. In another study, also on *Pelargonium* spp., Mativandlela et al. (2006) evaluated the antibacterial (including antitubercular) and antifungal activities of the root extracts. One of the two *Pelargonium* spp. showed activity against *M. tuberculosis*. Later, Mativandlela et al. (2008) selected seven plant species used traditionally in South Africa to treat tuberculosis related symptoms and tested their ethanol extracts against *M. smegmatis* and *M. tuberculosis*. Purified compounds, as well as whole plant extracts, were tested, and results demonstrated that all seven plant species had potential for their antimycobacterial properties. Eldeen and Van Staden (2007) tested the antimycobacterial activity of 78 extracts obtained from 10 trees used in South African traditional medicine, of which 30% inhibited the growth of *Mycobacterium aurum*. Besides these antimycobacterial studies, few South African studies specifically aimed to validate the efficacy against other pathogens related to respiratory diseases. One study specifically aimed at respiratory pathogens was that of Suliman et al. (2010), in which the aim was to validate the antimicrobial activity of combined indigenous essential oils to treat various respiratory ailments. Three essential oil combinations (from four aromatic plant species) were analysed against four test pathogens. The individual oils displayed mostly moderate antimicrobial activity, while their combinations interactive mostly additively (Suliman et al., 2010). In Venda, 10 plant species traditionally used for the treatment of respiratory conditions were tested against six respiratory pathogens for their antimicrobial activity. Four of the plant species possessed noteworthy antimicrobial activity against at least one of the pathogens (Pallant and Steenkamp, 2008). Although Buwa and Afolayan (2009) mainly focused on the anti-mycobacterial properties of selected plant species, traditionally used plants were also antimicrobially tested against various other pathogens associated with respiratory diseases. In this present study, not only is the documenting of plants used to treat various respiratory diseases in northern Maputaland examined, but the antimicrobial evaluation against a range of pathogens associated with respiratory infections is also undertaken.

3.2 Materials and methods

3.2.1 Plant extractions

Collected plant material (Chapter 2, Table 2.5) was dried at room temperature and ground to a fine powder using a Scientec RSA Hammer Mill. Two types of plant extracts were prepared. One, a 1:1 combination of dichloromethane and methanol (CH_2Cl_2:MeOH) (Merck)- and two, an aqueous extract for each plant species sampled. For the organic extracts, approximately 10 g of ground material of each plant sample was added to 50-100 mL of CH_2Cl_2:MeOH. After thoroughly mixing the plant material with the organic solvent, it was left over night (24 hr) at ambient temperature and filtered through 90 mm Grade 3hw filter paper.

When visiting the 80 homesteads, it was found that the plant remedies used were obtained by using various different aqueous extraction methods (Table 3.2). The most popular way of preparing the medicinal plant for consumption was by adding cold water to the plant part(s) and bringing to the boil. This method of preparation was mentioned by 80 % of the interviewees, with 26 of the 35 plant parts (from 30 plant species) used, prepared in this way (Chapter 2, Section 2.4.2). Another commonly used method was that of adding boiling water to the plant (most frequently used with six of the plant parts). The two less frequently used methods were that of adding cold water (used twice), or adding warm water (most popular method used with the leaves of *K. mosambicina*). As part of the current study the aqueous extracts were prepared in the laboratory to mimic the actual preparation in the homestead. An average of 25 g of ground plant material (depending on availability of plant material) was weighed and soaked in approximately 100 mL sterile distilled water sufficient to just cover the powder. Where applicable, plant material was soaked in sterile boiling water (boiling water extracts), warm water (warm water extract) or cold water (cold water extracts). In the case of the boiling water extracts the plant material was soaked in sterile cold water and brought to the boil. This plant-water mixture was then removed as soon as it started boiling. All plant-water mixtures were left over night (for 24 hr) at ambient temperature and thereafter plant extracts were filtered through sterile cotton wool. The extracts were stored at -80 °C for 48 before lyophilisation. All aqueous extracts were left in the lyophiliser (Bergbron) for up to seven days until totally freeze dried.

Leaves and/or stems of aromatic plants (*C. limon*, *E. grandis*, *H. kraussii*, *L. javanica* and *T. riparia*) were hydro-distilled in a Clevenger-type apparatus. The round bottomed flask of this

apparatus was, depending on availability, packed with between 146 g and 661 g plant material (Table 3.3) and then heated. The essential oils condensed in a cooling system and were collected after three hours to allow for the complete removal of all volatile constituents from the plant material. The samples collected were stored in amber vials and refrigerated for minimum inhibitory concentration (MIC) screening.

After extraction and distillation, the total mass of the CH_2Cl_2:MeOH plant extracts (hereafter referred to as organic extracts), aqueous extracts and essential oil distillates were measured and the percentage yield was calculated (Table 3.1, 3.2 and 3.3 respectively).

3.2.2 Antimicrobial screening

3.2.2.1 Test organisms

The following ATCC cultures responsible for respiratory infections were used: *C. neoformans* (ATCC 14116), *K. pneumoniae* (ATCC 13883), *M. catarrhalis* (ATCC 23246), *M. smegmatis* (ATCC 14468) and *S. aureus* (ATCC 6538). Although many of the pathogens tested during the current study are not always the predominant causes of respiratory infections, most of them are known to cause respiratory infections specifically in people with compromised immune systems (Kerkering et al., 1981; Aberg et al., 1999; Nester et al., 2001).

Freeze dried stock cultures were stored at -20 °C. These stock cultures were, depending on the organism used, rehydrated with approximately 1 mL of Tryptone Soya broth (TSB) or ADC (Bovine albumin fraction V 2.5, Dextrose and Catalase) enriched Mycobacterial media, and inoculated onto agar. Tryptone Soya broth or Tryptone Soya (TS) agar was the medium prepared for the growth of *C. neoformans* and all bacterial cultures except *M. smegmatis*, while ADC enriched media was prepared for the growth of *M. smegmatis.* The inoculated TS or ADC enriched agar plates were then incubated at 37 °C for 24 h (bacterial pathogens) or 48 h (*C. neoformans*). For each microorganism a single colony was then reinocculated into 5 mL of each test organism's corresponding broth type and incubated. Turbidity was adjusted to the equivalent of that of a McFarland's No. 1, which is $1x10^6$ colony forming units (CFU)/mL, for the MIC studies.

Cryptococcus neoformans is encapsulated yeast, and is the causative agent of cryptococcal meningoencephalitis - an infection of the meninges and brain. Pulmonary cryptococcosis is established when *C. neoformans* enters the body by inhalation of dust particles containing the yeast or its spores. The organism is initially established as an infection in the lung. In most people this infection causes mild or no symptoms, but causes severe infection in immunocompromised individuals (Nester et al., 2001). Pulmonary cryptococcosis leads to pleural effusions, causing symptoms such as coughing and chest pain (Núñez et al., 2000; Lindell et al., 2005). People who have weakened cell-mediated immunity, particularly AIDS-patients, are at high risk (Nester et al., 2001; Rittershaus et al., 2006).

Klebsiella pneumoniae is a Gram-negative bacterium and is a member of the family Enterobacteriaceae. *Klebsiella pneumoniae* resides mainly in the intestinal tract of humans and animals (Nester et al., 2001). Enterobacteria such as *Klebsiella* spp. can cause pneumonia, especially in immunocompromised people. This can include the very young, the very old, as well as HIV/AIDS patients. Pneumonia continues to be an important community-acquired infection in South Africa, with *K. pneumoniae* still being the associated pathogen in Africa and Asia (Ko et al., 2002). These infected patients show most of the symptoms caused by pneumococcal pneumonia, but can tend to die sooner than other pneumonia patients - having mortality rates of up to 80% (Nester et al., 2001).

Moraxella catarrhalis is a Gram-negative bacterium forming part of the normal flora of the nasopharynx. Several bacterial species colonize the nasal cavity, nasopharynx and pharynx, and although generally harmless, these organisms are opportunists able to cause disease in immunocompromised individuals (Nester et al., 2001). *Moraxella catarrhalis* has recently emerged as a true pathogen only in the past 20 to 30 years. It is now considered to be an important cause of upper respiratory tract infections in healthy children and elderly people, while it causes severe infections in immunocompromised hosts. Such infections can include pneumonia, endocarditis, septicaemia as well as meningitis. Furthermore, *M. catarrhalis* causes lower respiratory tract infections predominantly in adults suffering from COPD (Verduin et al., 2002).

Mycobacterium smegmatis is a rapid-growing, non-pathogenic *Mycobacterium* species. Since it shares many biosynthetic pathways with *M. tuberculosis*, and is sensitive (*in vitro*) to the effect of many conventional antitubercular anti-infectives, it may serve as a good model system in investigating sensitivity patterns against *M. tuberculosis* (Mitscher and Baker, 1998; Ojha et al., 2000). *Mycobacterium tuberculosis* serves as an immense threat to humanity, with a large proportion of the world's population asymptomatically carrying the tubercle bacillus. The latent bacilli are opportunistic and can reactivate themselves as soon as the host becomes immunocompromised (Ojha et al., 2000). Most cases of TB are caused by *M. tuberculosis*, a slender rod-shaped, acid-fast bacterium. Tuberculosis is a chronic illness usually contracted by inhaling airborne organisms from a person suffering from tuberculosis (Nester et al., 2001).

Staphylococcus aureus is a Gram-positive bacterium causing a variety of diseases, including skin and wound infections, as well as food poisoning. The nasal cavities are the preferred habitat of these Staphylococci bacteria, and virtually everyone carries this commensal organism in their nostrils at one time or another (Nester et al., 2001; Gibbons, 2004). Touching the affected area with your hands can spread the organism to other parts of the body, where it can be the causative agent of wound infections. *Staphylococcus aureus* survives well outside a host, and when inanimate objects are touched indirect transmission can occur. *Staphylococcus aureus* can cause diseases such as otitis media, and is also known to cause secondary infection in influenza patients - subsequently causing death (Nester et al., 2001). Hospital acquired *S. aureus* infections often occur, and this Gram-positive bacterium's ability to acquire resistance to many common antibiotics causes for great concern (Gibbons, 2004).

3.2.2.2 Culture media

Tryptone Soya broth was prepared by adding 1 L of distilled water to 30 g of TSB powder (Merck). The mixture was dissolved and autoclaved for 15 minutes at 121 °C. Tryptone Soya agar was prepared by adding 1 L of distilled water to 38 g of TS agar powder (Meck). The mixture was dissolved and autoclaved for 15 minutes at 121 °C. Shortly before solidifying, the liquid agar was poured into 90 mm petri dishes (Merck). For the growth of *M. smegmatis*, ADC (Bovine albumin fraction V 2.5, Dextrose and Catalase) enriched Mycobacterial media (Fluka Analytical) was prepared. The broth was prepared by adding 180 mL of distilled water to 0.94 g Middlebrook 7H9 Broth Base (Fluka Analytical), and autoclaved for 15 minutes at 121 °C. After autoclaving the media, 20 mL M0553 Middlebrook ADC Growth Supplement (Fluka Analytical)

was added, and thoroughly mixed into the media. Solid media was prepared by adding 2.4 g of Middlebrook Agar (Merck) before autoclaving the mixture. The supplement was then added just before the mixture started to solidify (± 45 °C) and poured into 65 mm petri dishes (Merck).

3.2.3 Minimum inhibitory concentration (MIC) assays

A conventional technique often used in microbial assays is the agar diffusion assay, but this method has been found to be problematic as certain extracts can lead to false positive or false negative results (Eloff, 1998). The technique described in Eloff (1998) involves the use of a 96-well microplate and requires a small amount of sample. When compared to the previously mentioned method, the inhibition of microbial growth can be detected at much lower concentrations, making it a highly sensitive method. Eldeen et al. (2005) found that extracts not showing activity with the agar diffusion method inhibited microbial growth when using the micro-dilution assay. The Clinical and Laboratory Standards Institute (CLSI/NCCLS, 2003) methodology and guidelines as well as Eloff (1998) were used to determine the minimum inhibitory concentration (MIC) of the plant extracts in the current study.

The plant extracts and essential oils were redissolved in acetone (organic extracts and essential oils) or sterile distilled water (aqueous extracts) to a final concentration of 64 mg/mL. A few organic extracts could not be redissolved adequately in acetone only, and were therefore dissolved in either a 1:1 combination of dimethyl sulphoxide (DMSO) (Merck) and acetone (*C. anisata*, *K. mosambicina*, *S. hyacinthoides*, *S. cordatum* and *T. emetica*), or in 100% DMSO (*C. brachiata*, *Hypoxis* sp., *L. javanica* root, *P. capensis* and *S. puniceus*).

Sterile 96-well microtitre plates were prepared aseptically, with 100 µL sterile distilled water added to each well. A 100 µL of plant extract or oil was added to the top row of wells of each microtitre plate. The following controls were added (100 µL) when each MIC assay was performed; culture control, antimicrobial control and solvent control(s). Each test organism's corresponding growth medium (Section 3.2.2.2) was added to all MIC assays as culture controls to confirm the presence of microbial growth in the absence of antimicrobials. The antimicrobial controls, ciprofloxacin (bacterial strains) or amphotericin B (*C. neoformans*), were added at an initial concentration of 0.01 mg/mL respectively. These conventional antimicrobials served as positive controls and were added to each MIC assay to confirm the antimicrobial susceptibility of the test pathogen. Each plant extract/essential oil's respective solvent type (acetone, DMSO or water) was made up to a concentration of 64 mg/mL and added to each MIC assay to serve as a

solvent control by allowing the investigation of the possible inhibitory effect that each solvent might have on the growth of the test micro-organisms. Due to the fact that the plant organic extracts were redissolved in different solvents, each MIC assay of the organic plant extracts included three solvents as controls (100% acetone, a 1:1 combination of acetone with DMSO and 100% DMSO). Serial dilutions were performed by transferring 100 µL aliquots, and 100 µL microbial culture (innoculum size of $1x10^6$ CFU/mL) was added to all wells. Each microtitre plate was then sealed with a sterile sealing film and incubated according to each test organism's required growth conditions. Essential oil evaporation is minimized by sealing the plates before incubation (Suliman, 2011). In order to verify the purity of the culture used, a streak plate of each pathogen was undertaken to ensure pure colonies were evident.

After incubation, 40 µL Iodonitrotetrazolium chloride (INT) (Fluka BioChemika) was added to each well. The INT solution was prepared by adding 0.08 g of INT to 200 mL of distilled water (0.04%) and mixing well. Iodonitrotetrazolium chloride is added to the microtitre plates to detect microbial growth, where INT changes to a red colour in the presence of actively metabolizing organisms (Eloff, 1998). Each microtitre plate was investigated for microbial growth as soon as efficient colour change took place for each test organism's corresponding culture control. For efficient colour change to take place, MIC plates had to be left at room temperature for 3h (*K. pneumoniae*), 5 h (*M. catarrhalis* and *S. aureus*), 6 h (*M. smegmatis*) or 24 h (*C. neoformans*) - depending on the time it took for their culture controls to indicate growth. This was done in order to confirm the presence of microbial growth in the absence of antimicrobials. The MIC value was reported as the lowest concentration of extract having no indication of microbial growth. The concentration range of the extract in each well ranged from 16 mg/mL in the top row, to 0.125 mg/mL in the last row of wells. In some cases there was no detection of growth for a specific plant sample (i.e. MIC value ≤ 0.125). The extract was then further diluted before repeating the MIC assay. Each extract was tested in triplicate against each microorganism. A difference of no more than one dilution factor was accepted within these three repetitions.

3.3 MIC results and discussion

The interpretations of the MIC results were as follows; MIC values < 1.00 mg/mL were considered noteworthy (Fabry et al., 1998; Gibbons, 2004; Ríos and Recio, 2005). For essential oils MIC values ≤ 2.00 mg/mL were considered to be noteworthy (Van Vuuren, 2008).

The average MIC results for organic (Table 3.1) and aqueous (Table 3.2) extracts, as well as the five essential oil distillates (Table 3.3), against each of the five test organisms, were determined. Where the MIC values of plant samples were greater or equal to the measured MIC value of its corresponding solvent (acetone, 50% DMSO or 100% DMSO), such values were recorded as not susceptible (NS) and not considered, as activity may be as a result of solvent rather than plant extract. MIC values obtained for the positive controls (ciprofloxacin and amphotericin B) against the test organisms used in the study are shown in Table 3.1 and 3.2.

Of the 35 organic plant extracts tested, 28 (from 24 species) were found to have noteworthy activity against one or more of the test organisms, with all 28 of these extracts displaying noteworthy antifungal activity against *C. neoformans* (MIC values from 0.03 - 0.87 mg/mL) - making this fungal pathogen the most sensitive test organism (Table 3.1). Eleven of the organic extracts (from 10 species) showed noteworthy activity against three or more of the five test organisms (Table 3.1). The mature bark extract of *S. birrea* demonstrated the highest broad-spectrum activity, with an average MIC value of 0.52 mg/mL. Extracts from this tree species displayed noteworthy MIC values against four of the five test organisms (MIC values from 0.08 mg/mL - 0.67 mg/mL). During a previous study the acetone extracts of *S. birrea* bark were tested against *S. aureus* and three other bacterial pathogens. The extracts inhibed the growth of all four test organisms (MIC values ranging from 0.15 to 2.43 mg/mL), thus validating the ethnobotanical use of *S. birrea* in the treatment of bacteria-related diseases (Eloff, 2001).

P. capensis subsp. *incohata* and *T. riparia* showed the overall strongest antimicrobial activity, having the lowest MIC values (0.03 mg/ml) against *C. neoformans* and *S. aureus* respectively. Although not extracted in this way by the interviewees, the organic extract of *T. riparia* did have noteworthy MIC values against three of the test organisms (Table 3.1). Boily and Van Puyvelde (1986) found *T. riparia* methanolic extract to exhibit antimicrobial activity against *S. aureus* at a concentration of 1 mg/mL. These same authors also tested this extract (using the agar dilution streak method) against *M. smegmatis* and found it to inhibit the growth of this microorganism. The organic extract of the endemic *P. capensis* subsp. *incohata* showed noteworthy MIC values against three of the five test organisms used in this study (0.03 - 0.50 mg/ml). To the best of our knowledge, this is the first time that the antimicrobial efficacy of *P. capensis* subsp. *incohata* has been recorded.

Table 3.1. The yield and mean MIC values (mg/mL) of the organic extracts (CH_2Cl_2:MeOH) against five respiratory pathogens.

Plant species	Plant part used	% Yield	Pathogens				
			C. neoformans **ATCC 14116**	***K. pneumoniae*** **ATCC 13883**	***M. catarrhalis*** **ATCC 23246**	***M. smegmatis*** **ATCC 14468**	***S. aureus*** **ATCC 6538**
A. glabratum	W	5.94	**0.30**	3.33	4.00	**0.67**	2.00
A. marlothii	Lf	8.85	1.00	2.67	6.67	3.33	4.00
B. discolor	Lf	4.28	**0.83**	4.00	2.67	**0.90**	1.33
Brachylaena cf. *uniflora*	Lf	10.90	**0.50**	3.00	2.00	1.33	**0.67**
Brachylaena cf. *transvaalensis*	Lf	8.74	**0.40**	1.33	1.33	**0.67**	**0.50**
B. cathartica	Lf	6.78	**0.67**	4.00	**0.50**	**0.67**	2.67
C. limon	Lf	4.46	**0.50**	2.67	2.67	2.00	5.33
C. anisata	Lf*	8.12	**0.23**	1.67	2.00	1.67	3.33
C. brachiata	L+S**	3.34	1.00	2.00	3.33	2.00	6.67
C. molle	Lf	5.56	1.00	6.00	2.00	1.33	2.67
C. articulatus	R	4.63	**0.50**	2.67	4.00	NS	1.00
E. capensis	Lf	13.94	**0.40**	1.33	2.00	NS	13.33
E. caffra	Lf	2.56	**0.50**	8.00	**0.10**	NS	**0.15**
E. grandis	Lf	8.93	**0.10**	2.00	**0.83**	2.00	**0.13**
E. tirucallii	S	7.61	**0.83**	6.67	8.00	NS	3.33
H. kraussii	L+S	7.07	**0.83**	1.33	4.00	2.00	2.00
Hypoxis cf. *acuminata*	C**	9.75	**0.87**	1.67	2.00	NS	4.00
K. mosambicina	L+S*	5.20	1.00	3.00	12.00	1.00	6.67
L. javanica	Lf	6.90	**0.25**	1.67	3.33	3.00	2.00
	R**	4.62	1.00	1.50	6.00	2.67	1.33
O. obovata	Lf	5.12	**0.83**	4.00	4.00	2.00	1.33
P. capensis	R**	2.11	**0.03**	1.50	**0.50**	2.67	**0.25**
P. neochilus	Lf	8.42	**0.42**	2.00	**0.67**	1.00	**0.67**

Plant species	Plant part used	% Yield	Pathogens				
			***C. neoformans* ATCC 14116**	***K. pneumoniae* ATCC 13883**	***M. catarrhalis* ATCC 23246**	***M. smegmatis* ATCC 14468**	***S. aureus* ATCC 6538**
P. guajava	Lf	14.61	**0.50**	1.00	1.00	2.00	**0.50**
S. hyacinthoides	Lf*	8.14	1.67	4.00	10.67	NS	4.00
S. puniceus	Bu**	3.30	2.00	3.00	NS	2.67	8.00
S. birrea	B,y	7.37	**0.08**	**0.67**	**0.25**	1.33	**0.25**
	B,m	8.69	**0.04**	1.00	**0.36**	2.00	**0.42**
S. deltoideus	Lf	12.71	**0.83**	1.33	4.00	1.33	3.00
S. serratuloides	Lf	10.25	**0.83**	1.33	4.00	1.33	2.67
S. cordatum	B*	6.90	**0.42**	1.00	**0.83**	1.33	**0.38**
T. sericea	B	5.16	**0.33**	**0.67**	2.00	**0.67**	**0.50**
	Lf	5.14	**0.67**	1.50	1.33	2.00	1.33
T. riparia	Lf	3.56	**0.60**	1.75	**0.10**	1.00	**0.03**
T. emetica	Lf*	4.92	**0.27**	1.67	1.40	2.67	**0.83**
Acetone	NA	NA	>16	>16	>16	4.00	16.00
DMSO 50%	NA	NA	8.00	16.00	16.00	4.00	16.00
DMSO 100%	NA	NA	4.00	8.00	8.00	4.00	16.00
Ciprofloxacin control (µg/ml)	NA	NA	NA.	0.08	0.42	0.08	0.31
AmphotericinB control (µg/ml)	NA	NA	1.25	NA	NA.	NA	NA.

NS = not susceptible at highest concentration tested; NA = not applicable control; B = bark; B,y = young bark; B,m = mature bark; Bu = bulb; C = corm; Lf = leaf; L+S = leaf and stem; R = root; S = stem; W = whole; Noteworthy activities are given in bold; *Plant parts dissolved in a 1:1 combination of DMSO and acetone; **Plant parts dissolved in 100% DMSO.

Klebsiella pneumoniae was the most sensitive test organism against the aqueous extracts (Table 3.2). In fact, more of the aqueous extracts showed noteworthy MIC results (six extracts) against this Gram-negative organism than the organic extracts (only two of the extracts having noteworthy MIC values). Conversely, Buwa and Afolayan (2009) found the MIC values of plant aqueous extracts to be especially high against Gram-negative organisms, and in a study done by Lall and Meyer (2000) aqueous extracts did not inhibit the growth of any of the Gram-negative bacteria tested. Other studies have found that most plant extracts (aqueous or organic) display, when compared to Gram-negative bacteria, better antimicrobial activity against Gram-positive bacteria (Taylor et al., 2001; Matu and Van Staden, 2003). Gram-positive organisms are, in fact, more sensitive to many antimicrobials than Gram-negative bacteria (Nester et al., 2001).

The most active aqueous extracts were that of the rarely mentioned *O. obovata* and *S. birrea* young bark (0.10 mg/ml against *C. neoformans*). While the antimicrobial activity of *S. birrea* extracts has been investigated (Eloff, 2001; Ojewole et al., 2010; Moyo et al., 2011), no antimicrobial analysis has been conducted on *O. obovata*. The aqueous extracts of *S. birrea* bark (both young and mature) showed noteworthy MIC values against three of the five test organisms (*C. neoformans*, *M. catarrhalis* and *S. aureus*). As described in Chapter 2, Section 2.4.2.24.4, the difference between the two types of *S. birrea* bark tested is that the mature bark was chopped off the trunk of an *S. birrea* tree, while the young bark was pulled off the branchlets. These two methods of bark preparation were not given by the same interviewee, thus having these two types of bark not harvested from the same tree. Although there seems to be no difference between the activities of the young- and mature bark of *S. birrea* (aqueous extracts, Table 3.2), when looking at the organic extracts, the young bark has better overall antimicrobial activity than the mature bark (Table 3.1). Results found by Moyo et al. (2011) is highly comparable to activities found during the current study. As can be seen in Table 3.1, *S. birrea* young bark showed noteworthy MIC values against both *K. pneumoniae* (MIC value = 0.67 mg/mL) and *S. aureus* (MIC value = 0.25 mg/mL), which is very similar to values found by Moyo et al. (2011) for the ethanolic extracts of *S. birrea* twigs. The same authors found *S. birrea* ethanolic leaf extracts to also have noteworthy MIC values against both *K. pneumoniae* and *S. aureus*.

Even though the aqueous extract preparation aimed to mimic the traditional use, very little noteworthy antimicrobial activity was observed with the aqueous extracts when compared to the organic extracts. This difference in activity between these two extract types was, in fact, found

with most plant species analysed in this study. Results obtained during the current study demonstrated that the organic plant extracts generally displayed higher inhibitory activity (Table 3.1) when compared with the aqueous extracts (Table 3.2). Of the 35 aqueous extracts tested, only 11 (from nine plant species) showed noteworthy antimicrobial activity against one or more of the five test organisms (Table 3.2). These results correlate with results found during previous antimicrobial studies done on medicinally used plants (Shale et al., 1999; Bamuamba et al., 2008; Van Vuuren and Naidoo, 2010; Kariuki and Njoroge, 2011; Ncube et al., 2012), where aqueous extracts showed low or no antimicrobial activity. Shale et al. (1999) specifically suggested that water is not the most effective solvent for extracting the active compounds from plants. Water is too polar to sufficiently dissolve the majority of organics that are associated with components derived from plants (Ong et al., 2006). Germano et al. (2005) tested the root extracts of *T. emetica* against *S. aureus*, *Streptococcus pyogenes*, *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* and found the organic extract (ethyl ether) to exhibit good activity against these organisms, recording noteworthy activity specifically against *S. aureus*. In contrast to this, the aqueous extract showed low activity against all five of these test organisms. These results correspond with our findings, with respect to the fact that, even though the organic extract (CH_2Cl_2:MeOH) of *T. emetica* leaves showed some antimicrobial activity against all five of the test organisms, the aqueous extracts also had poor activity against all of these microorganisms (aqueous extract's MIC values = 6.67 - 8.00 mg/mL).

The aqueous extracts of two of the plant species (*C. limon* and *S. hyacinthoides*) displayed some of the poorest antimicrobial activities recorded during this study - displaying MIC values > 16.00 mg/mL against all five of the test organisms (Table 3.2). Aromatic plants such as *C. limon* could be used for the physical relief experienced when inhaling its aromatic vapours. *Citrus limon* leaves were not administered through steam inhalation, but suggested to be taken orally in a tea (Chapter 2, Section 2.4.2.5.4). *Citrus limon* leaves contain ascorbic acid (Vitamin C) which acts as a mild antihistamine and stimulates the body's antibody response (Kalra et al., 2011). The interviewee mentioned taking this decoction to relieve a blocked nose (Chapter 2, Section 2.4.2.5.4), and the fact that these antihistamines have the ability to reduce inflammation, and the secretion of mucus in the airways, could support this traditional use. A study done by Valnet (1990) have also pointed towards the possible immune enhancing properties of *C. limon* (Steflitsch and Steflitsch, 2008), which would aid in combating any disease - including respiratory ailments. *Sansevieria hyacinthoides* contain various sapogenins, including ruscogenin, which is a proven anti-inflammatory agent (Kou et al., 2005). *Sansevieria*

hyacinthoides is commonly used in the treatment of haemorrhoids as well as ear infection. The anti-inflammatory properties of this plant could thus explain the extensive use of this plant in these inflammatory related diseases. (Van Wyk et al., 2009). *Erythrina caffra*, during the current survey (Chapter 2, Section 2.4.2.11), was mentioned to only be used in combination with other plant species. Even though the organic extracts gave noteworthy MIC values against *C. neoformans* (MIC value = 0.50 mg/mL), *M. catarrhalis* (MIC value = 0.10 mg/mL) and *S. aureus* (MIC value = 0.15 mg/mL), the aqueous extract also gave MIC values > 16.00 mg/mL against all five of the test organisms (Table 3.2). In a survey conducted by Pillay et al. (2001), five different *Erythrina* spp. were tested against various bacteria, including *S. aureus* and *K. pneumoniae*. No noteworthy activity was found for *E. caffra* aqueous extracts (leaf or bark) against either *K. pneumoniae* or *S. aureus*, while the organic bark extracts showed activity against *S. aureus*. This previous study showed that the aqueous extract of *E. caffra* bark had good antimicrobial activity. Nevertheless, the bacteria that were susceptible to *E. caffra* aqueous extracts are all commonly associated with skin infections and not respiratory illnesses (Nester et al., 2001; Pillay et al., 2001). Another plant that showed very weak antimicrobial activity with its aqueous extract, even though never used in combination with other plant species for enhanced efficacy, is *E. tirucalli*. *Euphorbia tirucalli* aqueous extract did however show some activity against *K. pneumoniae* (MIC value = 2.67 mg/mL). According to the very early studies of Karel and Roach (1951) *E. tirucalli* have given positive antibiotic results against *S. aureus*. However, our recent study showed weak activity against *S. aureus* (MIC = 8.00 mg/mL). All of these plants (*C. limon*, *E. caffra*, *E. tirucalli* and *S. hyacinthoides*), however, have only been mentioned once during our entire survey, so the fact that the antimicrobial activities of their aqueous extracts were weak was not totally unexpected.

The aqueous extract of the highly aromatic *T. riparia* (mentioned ten times during this survey; Chapter 2, Table 2.6) did not show noteworthy antimicrobial activity against either of the five test organisms, although it showed moderate antimicrobial activity against four of the test organisms, including *S. aureus* (MIC value = 2.00 mg/mL). During a study done by Scott et al. (2004) it was found that *T. riparia* aqueous extract showed no activity against *S. aureus*. The reason for this difference in outcome might be that, unlike the MIC analysis technique used in the current study, these authors used the disk assay method. In the current study the *T. riparia* aqueous extract was prepared in a slightly different way, though, (add cold water and bring to boil) from the method used in Scott et al. (2004) (add boiling water). It could be possible that the method used by the interviewees in the current study is more sufficient in releasing active

compounds from this plant species. Harvesting from a different geographical region may also have yielded a different chemical profile.

Even though the use of *S. serratuloides* was often mentioned during this survey (mentioned six times, Chapter 2, Table 2.6), the aqueous extract showed weak antimicrobial activity against all test organisms (MIC ranged between 2.00 mg/mL and 16.00 mg/mL, Table 3.2). This may be due to the fact that *S. serratuloides* was never used on its own, but always in combination with one or more other plant species (Chapter 2, Section 2.4.2.26.4). Another explanation could be that *Senecio* spp. contain the macrocyclic pyrrolizidine alkaloid - platyphylline, which is a known antispasmodic (Bull et al., 1968; Van Wyk et al., 2009). Phytomedicines that are topically applied to treat bronchitis (a viral, bacterial or fungal infection of the bronchial mucosa characterised by swelling, increased mucus production, cough and shortness of breath) may have spasmolytic properties (Van Wyk and Wink, 2004). The majority of the interviewees mentioned using *S. serratuloides* to treat, amongst other symptoms, a cough (Chapter 2, Section 2.4.2.26.4), which could be a symptom of bronchitis. The MIC value found against *M. smegmatis* for *S. serratuloides* aqueous extract (MIC value = 6.67 mg/mL) correlates with results found by Lall and Meyer (1999) when testing this extract against *M. tuberculosis* (MIC value = 5.00 mg/mL).

None of the aqueous extracts displayed noteworthy activity against *M. smegmatis* (Table 3.2). When looking at the ethnobotanical results in Chapter 2, it is important to note though that only one person specifically mentioned using a plant species (*P. capensis* subsp. *incohata*) for treating TB (Chapter 2, Section 2.4.2.19.4). However, this ethnobotanical result was not totally unexpected, seeing as the interviewees were specifically asked to describe the respiratory symptoms, and not the disease, that a specific plant remedy was used for.

Table 3.2. The yield and mean MIC values (mg/mL) of the aqueous extracts against five respiratory pathogens.

Plant species	Plant part used	Method	% Yield	Pathogens				
				C. neoformans **ATCC 14116**	***K. pneumoniae*** **ATCC 13883**	***M. catarrhalis*** **ATCC 23246**	***M. smegmatis*** **ATCC 14468**	***S. aureus*** **ATCC 6538**
A. glabratum	W	Boil	10.08	>16.00	10.67	>16.00	8.00	16.00
A. marlothii	Lf	Boil	13.93	16.00	4.00	16.00	8.00	16.00
B. discolor	Lf	C-B	6.79	10.67	6.67	16.00	8.00	2.67
Brachylaena cf. *uniflora*	Lf	C-B	6.29	16.00	4.00	8.00	16.00	1.00
Brachylaena cf. *transvaalensis*	Lf	C-B	8.95	16.00	5.33	13.33	8.00	4.00
B. cathartica	Lf	C-B	7.63	8.00	**0.67**	8.00	16.00	1.00
C. limon	Lf	C-B	8.26	>16.00	>16.00	>16.00	>16.00	>16.00
C. anisata	Lf	C-B	11.50	>16.00	16.00	>16.00	>16.00	>16.00
C. brachiata	L+S	C-B	8.32	4.00	16.00	>16.00	16.00	8.00
C. molle	Lf	C-B	7.16	4.00	**0.67**	4.00	4.00	1.33
C. articulatus	R	Cold	16.18	13.33	16.00	>16.00	>16.00	8.00
E. capensis	Lf	Boil	12.65	16.00	13.33	16.00	16.00	10.67
E. caffra	Lf	Boil	8.61	>16.00	>16.00	>16.00	>16.00	>16.00
E. grandis	Lf	C-B	6.04	3.33	**0.67**	4.00	4.00	1.33
E. tirucallii	S	C-B	9.43	>16.00	2.67	16.00	16.00	8.00
H. kraussii	L+S	C-B	8.41	16.00	2.67	13.33	8.00	4.00
Hypoxis cf. *acuminata*	C	C-B	9.52	1.67	4.00	8.00	5.33	8.00
K. mosambicina	L+S	Warm	6.92	3.33	16.00	>16.00	16.00	16.00
L. javanica	Lf	C-B	8.86	6.67	1.33	10.67	8.00	2.00
	R	Boil	6.04	16.00	8.00	10.67	13.33	8.00
O. obovata	Lf	C-B	10.80	**0.10**	1.67	1.00	4.00	1.00
P. capensis	R	C-B	1.46	8.00	3.33	1.00	16.00	**0.50**
P. neochilus	Lf	C-B	12.54	16.00	1.33	8.00	8.00	2.00

Plant species	Plant part used	Method	% Yield	Pathogens				
				C. neoformans **ATCC 14116**	***K. pneumoniae*** **ATCC 13883**	***M. catarrhalis*** **ATCC 23246**	***M. smegmatis*** **ATCC 14468**	***S. aureus*** **ATCC 6538**
P. guajava	Lf	C-B	3.48	6.00	**0.67**	**0.67**	4.00	**0.50**
S. hyacinthoides	Lf	C-B	3.69	>16.00	>16.00	>16.00	>16.00	>16.00
S. puniceus	Bu	Boil	7.29	10.67	>16.00	>16.00	>16.00	>16.00
S. birrea	B,y	C-B	2.36	**0.10**	1.00	**0.50**	2.00	**0.25**
	B,m	Cold	7.05	**0.23**	1.00	**0.42**	2.00	**0.67**
S. deltoideus	Lf	C-B	25.68	6.67	1.67	8.00	8.00	2.00
S. serratuloides	Lf	C-B	10.79	16.00	2.00	13.33	6.67	4.00
S. cordatum	B	C-B	7.50	**0.17**	**0.50**	**0.50**	2.00	**0.25**
T. sericea	B	C-B	2.40	1.00	**0.83**	2.00	2.67	**0.50**
	Lf	C-B	9.73	**0.23**	1.00	2.00	3.33	1.00
T. riparia	Lf	C-B	6.70	1.00	2.00	8.00	6.67	2.00
T. emetica	Lf	C-B	7.40	6.67	8.00	8.00	8.00	8.00
Water	NA	NA	NA	>16.00	>16.00	>16.00	>16.00	>16.00
Ciprofloxacin control (µg/ml)	NA	NA	NA	NA	0.08	0.42	0.08	0.31
AmphotericinB control (µg/ml)	NA	NA	NA	1.25	NA	NA	NA	NA

NA = not applicable; B = bark; B,y = young bark; B,m = mature bark; Bu = bulb; C = corm; Lf = leaf; L+S = leaf and stem; R = root; S = stem; W = whole; Boil = add boiling water; C-B = add cold water and bring to boil; Cold = add cold water; Warm = add warm water; Noteworthy activities are given in bold.

Despite the fact that *L. javanica* leaves were the most frequently used plant part (Chapter 2, Table 2.6), the aqueous leaf extract of this plant species did not have noteworthy MIC values against any of the test organisms (Table 3.2). The organic extract of *L. javanica* demonstrated noteworthy activity against *C. neoformans* (Table 3.1, MIC value = 0.25 mg/mL). The interviewees prepared this plant using water and thus one would expect some noteworthy activity with the aqueous extracts. This was not evident. It is, however, important to note that *L. javanica* leaves were mostly used in combination with other plant species, and secondly, this plant's leaves are mostly taken through steam/vapour inhalation (Chapter 2, Section 2.4.2.17.4). The reason for the inhalation of the *L. javanica* leaf vapours is possibly because essential oils of such aromatic plants can be absorbed through the mucous membranes of the nose and lungs (Van Wyk and Wink, 2004). One would thus expect the essential oils from *L. javanica* leaves to show noteworthy antimicrobial activity, but overall *L. javanica* essential oils did not show good activity - having noteworthy activity only against *C. neoformans* (MIC value = 0.25 mg/mL, Table 3.3). Studies done by Viljoen et al. (2005) on *L. javanica* demonstrated the time kill rate of this essential oil (0.5%) against *C. neoformans* within one hour. This result supports results found in the current study, where *L. javanica* inhibited the growth of *C. neoformans* at a low essential oil concentration (MIC value = 0.25 mg/mL). *Lippia javanica* leaves are highly aromatic, containing various monoterpenoids (Van Wyk et al., 2009). Monoterpenes from such essential oils can generate a sensation of cooling, relieving the discomfort of cold symptoms (Van Wyk and Wink, 2004). The traditional use of this plant, in spite of the low antimicrobial activity found against the test organisms, can also be supported by the fact that *L. javanica* volatile oils have decongestant properties (Van Wyk et al., 2009). Olivier et al. (2010) found verbascoside and isoverbascoside (secondary plant metabolites) to be present in the aerial parts of *L. javanica*. Verbascoside have been shown to possess *in vitro* antibiotic activity, while its analgesic properties have been proven in animal studies (Bajaj, 1995).

Although displaying overall weak antimicrobial activity, the MIC value of *L. javanica* essential oil distillate against *C. neoformans* (MIC = 0.25 mg/mL, Table 3.3) was the lowest obtained by any of the 5 essential oils analysed. All the plant essential oils that have been tested in this study showed noteworthy activity against *C. neoformans*. A similar finding has been documented by Suliman et al. (2010), where *C. neoformans* was the most sensitive pathogen against all of the essential oils that were tested. Viollon and Chaumont (1994) also found that many essential oils provided strong *in vitro* antifungal properties against *C. neoformans*. These same authors suggested that such essential oils could, due to their volatility, enter the infected lungs of a

pulmonary cryptococcosis patient with ease to aid in combating this disease. Besides the MIC values displayed against *C. neoformans*, the essential oil distillates of the majority of the aromatic plants did not have noteworthy MIC values against any of the bacterial test organisms. Suliman et al. (2010) observed similar results, where the essential oils tested showed moderate activity against most of the organisms, including *K. pneumoniae* and *M. catarrhalis*. Monoterpenes are molecules that make up 90% of essential oils (Bakkali et al., 2008), and these aromatic plant remedies could also be used for the physical relief experienced due to the cooling sensation generated by the monoterpenes forming part of their volatile fractions. According to Van Wyk and Wink (2004) some essential oils might help heal an ailment due to the positive effect it has on a patient's mind and mood. Anxiety reduction has been demonstrated in patients who have received aromatherapy massages (Steflitch and Steflitch, 2008). Thus therapeutic steaming could induce a positive emotional response to treatment.

The *E. grandis* essential oil was the only essential oil that showed noteworthy antimicrobial activity against any of the bacterial organisms, with an MIC value = 1.67 mg/mL against *M. smegmatis*. *Eucalyptus grandis* has been extensively used during this survey (Chapter 2, Table 2.6) - especially in combination with *L. javanica* (Chapter 2, Table 2.7). All *Eucalyptus* species yield a volatile oil, and the chemical composition of the oil varies between species (Watt and Breyer-Brandwijk, 1962). Although this variation in chemical composition could be the cause of inter-species differences in the antimicrobial activity of *Eucalyptus* essential oils, species sharing certain chemical compounds in their oils could share a similar antimicrobial profile (Elaissi et al., 2011). Previously, Suliman et al. (2010) examined the antimicrobial activity of the essential oil of *Eucalyptus globulus* Labill., another *Eucalyptus* sp., and also tested against *M. catarrhalis* and *K. pneumoniae* - giving results that are in agreement with our findings. *Eucalyptus globulus* leaves have been proven to have antiviral activity, where the leaf aqueous extract inhibited the replication of influenza virus A. Inhalation of the essential oil have been proven to relieve coughing in guinea-pigs (Kalra et al., 2011). Although all plant extracts were tested for antibacterial and anti-yeast activity, many of these plants could possess antiviral properties and could relieve the interviewees' cold and flu symptoms through antiviral action. More than 90% of respiratory tract disorders are caused by viruses that destroy the mucosal cells, often resulting in secondary bacterial infections (Van Wyk and Wink, 2004). Although much research has been conducted on the antimicrobial efficacy of various *Eucalyptus* spp. (Watt and Breyer-Brandwijk, 1962; Oyedeji et al., 1999; Gilles et al., 2010), and especially *E. globulus* (Schelz et al., 2006; Mulyaningsih et al., 2010; Suliman et al., 2010; Tyagi and

Malik, 2011), hardly any research has been conducted on the antimicrobial abilities of *E. grandis* (Elaissi et al., 2011) - especially against respiratory pathogens.

Aromatic plant extracts have also often been proven to display stronger antimicrobial activity in comparison to their essential oils only (Van Vuuren and Viljoen, 2011). Of the five aromatic plants in Table 3.3, three (*C. limon*, *H. kraussii* and *T. riparia*), displayed better antimicrobial activity against all five test organisms when their organic extracts were analysed instead of their essential oils only. Both the organic extracts of *L. javanica* and *E. grandis* displayed better antimicrobial activity than their corresponding essential oils against four of the five test organisms (Table 3.1 and 3.3).

Table 3.3. The yield and mean MIC values (mg/mL) of the essential oil distillates against five respiratory pathogens.

Plant	**Plant part used**	**% Yield**	**Pathogens**				
			***C. neoformans* ATCC 14116**	***K. pneumoniae* ATCC 13883**	***M. catarrhalis* ATCC 23246**	***M. smegmatis* ATCC 14468**	***S. aureus* ATCC 6538**
C. limon	Lf	0.46	**0.83**	16.00	13.33	NS	6.67
E. grandis	Lf	0.60	**0.67**	8.00	4.00	**1.67**	2.67
H. kraussii	L+S	0.08	**1.00**	3.00	5.33	NS	>16.00
L. javanica	Lf	1.27	**0.25**	6.67	5.33	NS	5.33
T. riparia	Lf	0.17	**0.83**	4.00	5.33	NS	8.00
Acetone	NA	NA	>16.00	>16.00	>16.00	4.00	16.00
Ciprofloxacin control (µg/ml)	NA	NA	NA.	0.08	0.42	0.08	0.31
Amphotericin B control (µg/ml)	NA	NA	1.25	NA	NA.	NA	NA.

NS = not susceptible at highest concentration tested; NA = Not applicable control; Lf = leaf; L+S = leaf and stem; Noteworthy activities are given in bold.

The test organism that was least sensitive to most of the plant species tested (both of the extracts and essential oil distillates) was *M. smegmatis*. *Mycobacterium smegmatis* is often used as a test organism in the initial screening of plants with possible activity against TB. Compared to *M. tuberculosis*, this test organism is much safer to work with, and grows more rapidly. Even though correlations are often found between the antimicrobial results against *M. tuberculosis* and *M. smegmatis*, this is not always the case (Mitscher and Baker 1998; Mativandlela et al., 2008; McGaw et al., 2008). It has been found, for example, that the acetone extracts of *E. capensis* bark showed strong antimycobacterial activity (MIC value = 0.50 mg/mL; MIC value = 0.10

mg/mL) against *M. tuberculosis* (Lall and Meyer, 1999; Van Wyk et al., 2009), while the organic leaf extract tested during this survey showed very weak antimycobacterial activity (not susceptible, Table 3.4). Even though testing against *M. smegmatis* can be a suitable preliminary test for anti-tuberculosis activity, this is not always the case (Mativandlela et al., 2008; McGaw et al., 2008). In an attempt to determine how accurately *M. smegmatis* reveals a plant extract's activity against *M. tuberculosis*, Mitscher and Baker (1998) screened 33 plant extracts against both *M. smegmatis* and *M. tuberculosis*. Even though 25% of extracts were shown to be active against both *Mycobacterium* species, 25% were active against *M. tuberculosis* and not *M. smegmatis*. The differing antimycobacterial results obtained for *E. capensis* could also indicate that, although this plant contains antimycobacterial properties, those properties could be plant organ specific. There is often a difference in the active ingredients of different plant parts, and for that reason the whole plant is rarely used medicinally (Van Wyk et al., 2009). This difference in antimycobacterial activity could be due to geographical variation as well (Taylor et al., 2001).

During the current study, *P. capensis* subsp. *incohata* was the only plant species mentioned to be used for the treatment of TB (Chapter 2, Section 2.4.2.19.4), yet it did not show any noteworthy activity against *M. smegmatis* (MIC value, aqueous extract = 16.00 mg/mL; MIC value, organic extract = 2.67 mg/mL). When a person has TB, medicinal plants might often be used to treat the symptoms of this disease, rather than the disease itself (Green et al., 2010). *Moraxella catarrhalis* is a Gram-negative bacterium that can be indirectly responsible for secondary infections in a person suffering from TB or bronchitis (Mativandlela et al., 2006). Seeing as *P. capensis* showed good antimicrobial activity against *M. catarrhalis* (MIC value, organic extract = 0.50 mg/mL; MIC value, aqueous extract = 1.00 mg/mL), this could indicate that, in this case, when a patient suffered from TB, *P. capensis* was actually used to treat the secondary infection caused by *M. catarrhalis*. Plant extracts that did show noteworthy activity against *M. smegmatis* during the current study include the organic extracts of *A. glabratum*, *Brachylaena* spp., *B. cathartica* and *T. sericea* bark. The latter is in agreement with results found by Green et al. (2010). During that survey, *T. sericea* was highlighted as important in further antituberculosis research. One of the interviewees claimed to use *T. sericea* leaves (combined with other plant species) to treat a chronic cough (Chapter 2, Section 2.4.2.28.4), which could possibly indicate this plant's use in the treatment of TB (Nester et al., 2001) in rural Maputaland, KwaZulu-Natal. According to our knowledge this is the first documentation, globally, of the antimicrobial activity of *A. glabratum* against *M. smegmatis*.

While Watt and Breyer-Brandwijk (1962) recorded the use of *S. cordatum* as a TB remedy by the Zulus, our ethnobotanical survey only documented symptoms such as chest pain, cough and fever. Although these symptoms can be associated with TB, it can also be associated with other respiratory diseases such as pneumonia (Nester et al., 2001). The *S. cordatum* aqueous extract showed only moderate activity against *M. smegmatis* (MIC value = 2.00 mg/mL). Mativandlela et al. (2008) also tested the bark extracts (ethanol extract) of *S. cordatum* against *M. smegmatis*, and a slightly higher MIC value of 6.25 mg/mL was obtained. While *S. cordatum* bark aqueous extract only displayed intermediate antimicrobial activity against *M. smegmatis*, noteworthy MIC values were obtained for this extract against all other test organisms (MIC values ranging from 0.17 - 0.50 mg/mL). The MIC value displayed against *S. aureus* (MIC value = 0.25 mg/mL) correlates with a previous MIC result that was also recorded for the aqueous bark extracts of *S. cordatum* (MIC value = 0.50 mg/mL) (Pallant and Steenkamp, 2008). According to Pallant and Steenkamp (2008), *S. cordatum* aqueous bark extract also inhibited the growth of another respiratory pathogen, *H. influenzae* (MIC value = 1.00 mg/mL).

Much research has been done on the antimicrobial activities of species from the Combretaceae family (Eloff, 1999; McGaw et al., 2001; Fyhrquist et al., 2002; Masoko et al., 2007; Eloff et al., 2008). Some of these studies focus specifically on *Combretum* and/or *Terminalia* spp. (Fyhrquist et al., 2002; Masoko et al., 2007). Eloff et al. (2008) and Eldeen et al. (2005) found *T. sericea* extracts to be active against both Gram-positive and Gram-negative bacteria, which coincides with results found in this study, where *T. sericea* bark extract showed significant MIC values against both *K. pneumoniae* (Gram-negative) and *S. aureus* (Gram-positive). Fyhrquist et al. (2002) also reported the leaf extract of *T. sericea* to show activity against *S. aureus*, which correlates with *T. sericea* leaf extract results found in the current study (MIC value, organic = 1.33 mg/mL; MIC value, aqueous = 1.00 mg/mL). During the current study the bark extracts (both organic and aqueous) showed noteworthy activity against *S. aureus* (MIC value = 0.50 mg/mL). Steenkamp et al. (2007) found the root extract (methanol or aqueous) of *T. sericea* to have some activity against *S. aureus.* Results of the current study, as well as findings in these previous reports, could thus indicate that, although differing in antimicrobial strength, all plant parts of *T. sericea* have at least some activity against *S. aureus*. When looking at the organic extract of *T. sericea,* the fact that the bark extract has noteworthy MIC values against four of the five test organisms (*C. neoformans, K. pneumoniae, M. smegmatis* and *S. aureus*), while the leaf extract only shows noteworthy activity against *C. neoformans*, is quite prominent in indicating better antimicrobial activity in the bark of this species (when compared to the leaves only). It has

been found that, due to the unequal distribution of constituents throughout a plant, there is a difference in the antifungal or antibacterial activity of different plant parts (Skalli et al., 2007; Mahesh and Satish, 2008). When looking at the MIC values of *C. molle* leaf extracts, the only noteworthy MIC value was obtained for its aqueous extract against *K. pneumoniae* (MIC value = 0.67 mg/mL). All the other MIC values were in the range of indicating that *C. molle* leaves posessed only some antimicrobial activity against the test organisms. A previous finding that could explain the traditional use of *C. molle* leaves is the detection of high anti-inflammatory activity in the leaf extracts (McGaw et al., 2001). Several diseases (including respiratory illnesses) could be perpetuated by inflammatory reactions (Iwalewa et al., 2007). It is also important to mention here that *C. molle* was used on its own only once out of the four times its use was mentioned during this survey (Chapter 2, Section 2.4.2.8.4). The value of this plant's antimicrobial properties could thus only be fully experienced when it is taken in combination with other species (as mentioned during the current survey).

Many of these medicinally used plants might be efficient in the treatment of respiratory symptoms due to their anti-inflammatory properties. Several of the plant species tested in the current study (*L. javanica*, *S. hyacinthoides*, *S. birrea*, *T. sericea*, *T. riparia* and *T. emetica*) have also been used in treating inflammatory and pain disorders (Iwalewa et al., 2007; Van Wyk et al., 2009). This could be one of the many explanations for the medicinal use of some species whose aqueous extracts show very low or no antimicrobial activity. Inflammation may be the basis underlining many ailments. Inflammation is a localized protective reaction of the body's cells or tissues to injury, irritation (chemical or allergic), or infections. Some secondary plant metabolites capable of anti-inflammatory action include flavonoids, tannins, chalcone, coumarins and essential oils, and because many of these herbal medicines contain some of these metabolites, they are involved in the biochemical pathway of the inflammation process (Watt and Breyer-Brandwijk, 1962; Iwalewa et al., 2007; Van Wyk et al., 2009; Fawole et al., 2010). Even though, for example, *T. emetica* aqueous extract showed weak or no antimicrobial activity against all five test organisms (MIC values = 6.67 - 8.00 mg/mL), it does have some anti-inflammatory activity (Iwalewa et al., 2007). As was found during our ethnobotanical survey (Table 3.1, *S. puniceus*), the bulbous plants usually contribute only a small percentage of plant species used medicinally in South Africa. Most of these bulbous plant remedies are in fact used for their anti-inflammatory purposes (Louw et al., 2002). *Scadoxis puniceus* belong to the family Amaryllidaceae which contain anti-inflammatory compounds, including flavanoids. Although the aqueous extract from the bulb of *S. puniceus* produced very low antimicrobial activities

against all five test organisms (MIC values ranging from 10.67 to > 16.00 mg/mL), this plant species could be used for its anti-inflammatory properties. According to Piwowarski et al. (2011) the use of tannin-rich plant material in the treatment of inflammatory-related diseases can be justified due to their proven influence on extracellular matrix (ECM) components. The ECM is a complex mixture of molecules that support cell and tissue structure, and are often involved in inflammatory responses (Adair-Kirk and Senior, 2008).

Various *Terminalia* spp. produce tannins, while *S. birrea* gum and *P. guajava* leaves are rich in tannins (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 2009). *Sclerocarya birrea* can contain between 10-20% tannins (Roodt, 1998), and both the aqueous and methanolic stem-bark extracts of this species have produced anti-inflammatory effects in rats (Ojewole, 2003). When testing both the aqueous and organic extracts, all three of these species showed noteworthy antimicrobial activity against at least two of the five test organisms (Tables 3.1 and 3.2), justifying the use of these species in the treatment of respiratory ailments. In addition to its anti-inflammatory properties, the antimicrobial toxicity of tannins has also been well documented (Scalbert, 1991; Van Wyk et al., 2009). While the presence of tannins could be the main source of antimicrobial activity, many other plant molecules have antibacterial or antifungal activities. Some of these botanical compounds include flavonoids, quinones and triterpenoids. *Terminalia sericea* is rich in triterpenoids (Van Wyk et al., 2009), while *P. guajava* leaves contain flavonoids, triterpenoids and derivatives of quercetin. The two triterpenoids (guavanoic acid and guavacoumaric acid) have been isolated from guava leaves (Gutiérrez et al., 2008).

Psidium guajava is an extensively studied medicinal plant (Oliver-Bever, 1983; Cáceres et al., 1993; Jaiajr et al., 1999; Lutterodt et al., 1999; Nascimento et al., 2000; Abdelrahim et al., 2002; Arima and Danno, 2002; Qa'dan et al., 2005; Sanches et al., 2005; Gutiérrez et al., 2008; Mohamad et al., 2011). Antibacterial compounds have been detected in the leaves of *P. guajava* (Gutiérrez et al., 2008). Results from the current study indicate that *P. guajava* aqueous leaf extract is active against both Gram-positive and gram-negative bacteria. These findings are in agreement with previous reports (Oliver-Bever, 1983). Lutterodt et al. (1999) found *P. guajava* methanol leaf extract to inhibit the growth of *S. aureus* at an MIC value of 1.00 mg/mL, which is close to the value obtained for the *P. guajava* organic extract in the current study (MIC value = 0.50 mg/mL). An MIC value = 0.50 mg/mL that was recorded by Sanches et al. (2005) for the aqueous leaf extract of *P. guajava*, against the same test organism, is identical to our result. In a study by Jaiarj et al. (1999), the extract of *P. guajava* leaves inhibited the growth of various

S. aureus strains, which correlates with our results - where both the aqueous and the organic extracts had noteworthy MIC values against this pathogen. *Staphylococcus aureus* strains have been known to be susceptible to tannins (Scalbert, 1991), and the high level of tannins present in *P. guajava* could be responsible for this activity (Van Wyk et al., 2009). Jaiarj et al. (1999) also did *in vivo* studies where *P. guajava* aqueous leaf extracts indicated a marked anticough activity. During a study by Mohamad et al. (2011) methanol extracts of *P. guajava* didn't show inhibition of *M. tuberculosis* growth at the highest extract concentration of 1.60 mg/mL. In contrast to this, Antoun et al. (2001) found that *P. guajava* extracts showed strong activity against *M. tuberculosis*. The current study found *P. guajava* leaf extract to have some antimicrobial activity against *M. smegmatis* (MIC value, aqueous extract = 4.00 mg/mL; MIC value, organic extract = 2.00 mg/mL). Even though the use of *P. guajava* was only mentioned twice during the current study, the aqueous extract demonstrated one of the best antimicrobial activities (noteworthy MIC values against three of the five test organisms). In addition to this, a previous study has found *P. guajava* leaf extracts to also inhibit the growth of *S. pneumoniae* (Caceres et al., 1991).

Even though many of the once mentioned plant species' aqueous extracts display some of the highest MIC values, frequency of use does not necessarily correlate with antimicrobial efficacy. The aqueous extracts of some of the most frequently used plant species (*L. javanica*, *T. riparia* and *S. serratuloides*) did not give noteworthy MIC values against any of the five test organisms. Despite being only mentioned twice (Chapter 2, Table 2.6), the bark of *S. birrea* and *S. cordatum* both show broad antimicrobial activity in the current study, with their organic and aqueous extracts having noteworthy MIC values against most of the test organisms (Tables 3.1 and 3.2). The use of *S. birrea* young bark was mentioned only once (Chapter 2, Table 2.6), but its aqueous extract (as well as that of *O. obovata*) displayed the lowest MIC values of all the aqueous extracts tested (Table 3.2), while the aqueous extract of the most frequently mentioned *L. javanica*, showed mostly moderate antimicrobial activity. A similar incidence has been found in a previous southern African survey (Van Vuuren and Naidoo, 2010), where results could demonstrate that frequency of use do not necessarily correlate with high antimicrobial efficacy. However, as can be seen in Table 3.1, the frequently used *T. riparia* showed noteworthy antimicrobial activity against the majority of the microorganisms tested. It is only the organic extracts of *T. riparia* that gave any noteworthy results, though, and seeing as it is the aqueous extract that is being used by the interviewees (Chapter 2, Section 2.4.2.29.4) one would expect this extract type to have displayed better antimicrobial activity during this study. Thus, even

though *T. riparia* is the third most frequently used plant species, only its organic extract displayed good antimicrobial activity (MIC values ranging from 0.03 - 1.75 mg/mL).

All of these plant extracts and essential oils have only been tested against the five test organisms mentioned, and where no antimicrobial activity was noted, such extracts may in fact be active against other respiratory pathogens not tested during this survey (Shale et al., 1999). Secondly, when weak activity has been demonstrated *in vitro*, it does not necessarily imply weak activity *in vivo* (Houghton et al., 2007). Due to the metabolic transformation of some plant components into highly active intermediates, some of these plant extracts may be more potent *in vivo* (Ngemenya et al., 2006). When conducting *in vitro* analysis, medicinal plants should also not only be tested for a single biological activity, such as antimicrobial activity, but should be screened in different *in vitro* assays such as anti-inflammatory, immune-stimulating or the investigation of antioxidative activities (Houghton et al., 2007; Fawole et al., 2010).

3.4 Summary

(a) The organic extracts of *P. capensis* subsp. *incohata* (MIC value = 0.03 mg/mL against *C. neoformans*) and *T. riparia* (MIC value = 0.03 mg/mL against *S. aureus*) displayed the lowest overall MIC values (strongest activity).
(b) The most active aqueous extracts were that of *O. obovata* and *S. birrea* (MIC values = 0.10 mg/mL against *C. neoformans*).
(c) Of the five aromatic plants tested for their essential oils, *L. javanica* leaf essential oil demonstrated the strongest antimicrobial activity (MIC value = 0.25 mg/mL against *C. neoformans*).
(d) Results demonstrated that frequency of use does not necessarily always correlate with high antimicrobial efficacy.
(e) Thirty-one percent of the aqueous extracts and 80% of the organic extracts displayed noteworthy activity.

Chapter 4

Combination Studies

4.1 Introduction

Plant remedies have been used since the existence of mankind (Hedberg and Staugård, 1989), and, as is typical in traditional Chinese medicine, are often given in fixed mixtures of different herbs or plant parts (Van Wyk and Wink, 2004). African traditional healers, like Chinese herbalists, also combine different plant parts or plant species to achieve the most favourable outcome, and rarely use only one plant in treating an ailment (Van Vuuren and Viljoen, 2011). Synergy assessment has become important in the quest toward finding a scientific rationale for the traditional use of multidrug combinations - especially since these combined remedies are often preferred over single constituents (Wagner, 2011). Synergy is observed when a combination of different components gives a better therapeutic outcome than would be expected from simply adding together. Combining substances thus enhances their therapeutic effect (Williamson, 2001; Keith et al., 2005). Such interactions can, of course, also be detected within a single plant remedy, as each plant extract can contain multiple components - able to interact synergistically (Li and Zhang, 2008). Examples of such single plant remedies include *Ginkgo biloba* L., *Valeriana officinalis* L. and *Zingiber officinale* Roscoe. Within these mono-extracts, as with some plant combinations, synergy has been verified according to the method by Berenbaum (1978, 1989) (Van Vuuren and Viljoen, 2011; Wagner, 2011).

Many plant compounds have proven, *in vitro*, to reduce MIC values of antibiotics against resistant organisms (Aiyegoro et al., 2010). Many *in vitro* antimicrobial studies have been taken on to determine synergy when combining traditionally used plant species with conventional antimicrobials (Lachowicz et al., 1998; Nascimento et al., 2000; Aburjai et al., 2001; Shin and Kang, 2003; Aqil et al., 2005; Filoche et al., 2005; Betoni et al., 2006; Adwan and Mhanna, 2008; Aiyegoro et al., 2009). However, less attention has been given to combinations consisting of plants only. In many ethnobotanical studies it is often found that certain plant species are used in combination with one or more other plant species to treat infectious diseases (Cano and Volpato, 2004; Mahishi et al., 2005; Togola et al., 2005; Maregesi et al., 2007; Ssegawa and Kasenene, 2007; Dahlberg and Trygger, 2009; Moshi et al., 2009; Ruysschaert et al., 2009; De Wet et al., 2012). Although the use of combined species has been mentioned in previous studies,

validation of such combined uses is seldom explored. Gathirwa et al. (2011) conducted an ethnopharmacological survey in Kenya, identifying plant species used by traditional healers to treat malaria. Although none of these plant species were reported to be used in combination, the study also attempted to evaluate combination effects of these plants according to the method by Berenbaum (1978). Synergism was revealed when combining some of the extracts, while one combination demonstrated antagonism. Some research have been conducted towards the antimicrobial validation of synergy in traditionally used plant combinations. These include surveys done by Azas et al. (2002), Al-Heali and Rahemo (2006), Kamatou et al. (2006) and Suliman et al. (2010). Other, similar studies, surveyed interactions between the volatile and non-volatile fractions from one plant species (Van Vuuren and Viljoen, 2009), different extract types from the same plant species (Ncube et al., 2012) or extracts from different parts of one plant species (Van Vuuren and Viljoen, 2008). Based on information found during previous ethnobotanical surveys in Mali, Azas et al. (2002) further investigated four plants found to be commonly used in the treatment of febrifuge and malaria. Using standard isobologram analysis, the *in vitro* antimalarial investigations of interactions of the combined extracts of these plants were evaluated. Three of the combinations tested showed strong *in vitro* synergistic inhibition of plasmodial development, and justify their use in traditional medicine. In another survey, the anti-bacterial activity of two plants (*Salvia chamelaeagnea* K. Bergius and *Leonotis leonurus* (L.) R.Br), often used in combination, was determined singularly and in combination (Kamatou et al., 2006). The study was based on the fact that various *Salvia* spp. are, according to literature, often used in combination with *L. leonorus* to treat infections, including influenza and pulmonary TB (Watt and Breyer-Brandwijk, 1962). Synergistic actions were observed against the Gram-positive bacteria for nearly all ratios, while diverse interactions were observed when testing the different ratios of this combination against the Gram-negative bacteria (Kamatou et al., 2006). Few such studies, though, have focused specifically on plants used in treating respiratory ailments (Van Vuuren and Viljoen, 2009; Suliman et al., 2010). In an antimicrobial study conducted by Suliman et al. (2010) the oils of three selected aromatic plants (*Agathosma betulina* (P.J. Bergius) Pillans, *E. globulus* and *Osmitopsis asteriscoides* (P.J. Bergius) Less.) were evaluated for possible antimicrobial synergistic interactions when combined with *Artemisia afra* Jacq. Ex Willd. - one of the most widely used medicinal plant species in southern Africa. These aromatic plant species are known to be used in combination with *A. afra* for the treatment of various respiratory ailments (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 2009). Except for one combination showing synergy at one ratio, the MIC results for all the different ratios of each of the three *A. afra* combinations demonstrated mostly additive effects against the four respiratory test pathogens (Suliman et al., 2010). Van Vuuren and Viljoen (2009) evaluated

the antimicrobial efficacies of the non-volatile and volatile fractions of *Tarchonanthus camphoratus* L. This South African plant species has been used in the treatment of respiratory complaints such as bronchitis and sinusitis (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 2009). Using MIC analysis and the isobologram method of Berenbaum (1978) respectively, the volatile and non-volatile fractions of this plant species have been tested singularly as well as in combination. With synergistic interactions observed at combinations with higher volatile content, this study demonstrated clearly that the volatile (essential oil) and non-volatile constituents of this plant species need to be combined for enhanced antimicrobial efficacy.

As described in Chapter 2 (Table 2.7), 23 of the 30 plant species documented during the current survey were combined (in combinations of two-, three- or four plant species) to produce the 24 different plant combinations. Besides these 24 different plant combinations, there was also a combination in which the roots and the leaves of the same species (*L. javanica*) were mixed. One of the main objectives of this study was to determine whether the plant remedies documented showed any antimicrobial efficacy against respiratory pathogens. While all 30 plant species have been evaluated independently, interactive antimicrobial plant combination studies has to be performed on these 25 combinations as well. The traditional method of combining plant species for better efficacy is supported by very little scientific evidence, and priority should be given to further studies regarding the medicinal use of plant combinations (Ríos and Recio, 2005; Van Vuuren and Viljoen, 2008). One of the most experimentally feasible methods for proving synergy in phytomedicine seems to be the isobole method of Berenbaum (1989) (Wagner and Ulrich-Merzenich, 2009). Understanding the synergy involved in traditional medicine could validate their use in combination therapy.

4.2 Materials and methods

The 25 different plant combinations (1:1, 1:1:1 or 1:1:1:1) (Chapter 2, Table 2.7) have been prepared, respectively, from both aqueous- and organic plant extract stock solutions (64 mg/mL) to a total volume of 100 µl. For the 1:1 test combinations, equal aliquots of 50 µl each of the two extracts were mixed to make up to a volume of 100 µl while 1:1:1 and 1:1:1:1 combinations had each extract contributing 33.3 µl and 25 µl respectively to make up 100 µl in the first wells of a 96-well microtitre plate. In the case of the organic extracts, combinations have also been prepared where the aromatic plants' (*E. grandis, L. javanica* and *T. riparia*) extracts have been replaced by their essential oil derivatives. Using MIC assays (Chapter 3, Section 3.2.3), the antimicrobial activity has been determined for these combinations to establish whether any

interaction is evident when plants in this ethnobotanical study are combined. The following methods have been followed to validate antimicrobial plant interactions: The sum of the fractional inhibitory concentration index (∑FIC) and isobologram studies (Berenbaum, 1978; Van Vuuren and Viljoen, 2011).

4.2.1 Fractional inhibitory concentrations (FIC)

In order to measure the activities of combinations, FIC indices were calculated for the 1:1, 1:1:1 and the 1:1:1:1 combinations. The ΣFIC was calculated from MIC values obtained from plants investigated independently (Chapter 3, Section 3.2.3) and for each combination. The ∑FIC expresses the interaction of each plant extract in combination as a fraction of the effect when it is used independently (Van Vuuren and Viljoen, 2011). The following calculations were used by Berenbaum (1978) in order to determine the FIC index:

$\sum FIC = FIC_A + FIC_B$

$$FIC_A = \frac{MIC_A \text{ combined with } MIC_B}{MIC_A \text{ independently}} \qquad FIC_B = \frac{MIC_B \text{ combined with } MIC_A}{MIC_B \text{ independently}}$$

During the current study, this calculation has been used, bringing into account the amount of plant species forming part of each combination. In order to determine if a combination is synergistic, additive, non-interactive or antagonistic, the method by Berenbaum (1978) have been adjusted by Van Vuuren and Viljoen (2011), where each ratio (represented by an isobologram data point) was interpreted as having synergistic (≤ 0.5), additive (> 0.5 - 1.0), non-interactive (> 1.0 - ≤ 4.0) or antagonistic (> 4.0) interaction (Figure 4.1). The interpretation by Van Vuuren and Viljoen (2011) is a more conservative analysis of interpreting synergism.

Conventional antimicrobials have been included in all repetitions, as described in Chapter 3, Section 3.2.3. The MIC results for the combined extracts and essential oils (where applicable) were recorded in triplicate, against each of the five test organisms, and the average MIC result for each combination was tabulated, along with their sum fractional inhibitory concentration (∑FIC) values (Table 4.1 and 4.2). A difference of no more than one dilution factor was accepted within these repetitions.

4.2.2 Isobologram studies

The isobologram ratio method (Berenbaum, 1978) has been used to determine if synergistic interactions are optimal at varied ratios. This involves combining the plant combinations in nine ratios, i.e. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. The MIC values have been determined for all ratios of the plant samples as well as plant samples independently. The MIC values (mg/mL) of plant samples in combination, relative to the independent MIC values, were plotted on an isobologram as a ratio. The isobologram can then be interpreted by examining the data points of the different ratios in relation to the 1:1 line (independent MIC values), the 0.5:0.5 line and the 4.0:4.0 line (Figure 4.1). Synergy is depicted when a data point falls in the area closest to, or below the 0.5:0.5 line, while antagonism is portrayed by a data point falling in the area above the 4.0:4.0 line (Van Vuuren and Viljoen, 2011). Conventional antimicrobials have been included in all repetitions, as described in Chapter 3, Section 3.2.3. All tests have been undertaken in duplicate, and the mean values have then been plotted on the isobologram.

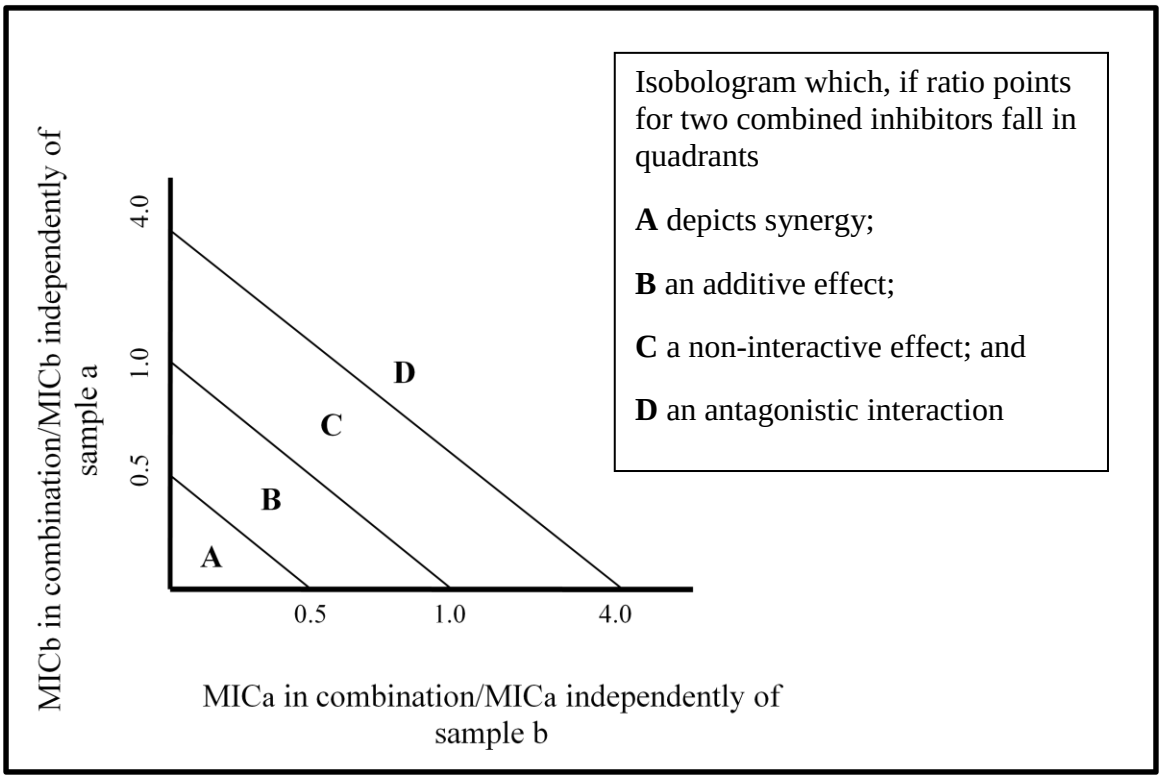


Figure 4.1 An isobologram indicating synergy, an additive effect, non-interaction and antagonism (Van Vuuren and Viljoen, 2011).

4.3 Results and discussion

4.3.1 MIC and ∑FIC values of organic extracts and essential oils

Eighty percent of the organic extract combinations (without essential oils) and 91% of the 21 combinations containing essential oils, displayed noteworthy activity against at least one of the five test organisms respectively (Table 4.1). Ten of the 25 plant combinations depicted synergy against at least one of the five test organisms. Nine of these combinations contained aromatic plants as one or more of the plant species, and seven of these nine combinations only displayed synergy where the aromatic plant's organic extract has been replaced by its essential oil derivative. Essential oils are able to enhance the penetration of antimicrobials into bacterial cells (Solórzano-Santos and Miranda-Novales, 2011; Van Vuuren and Viljoen, 2011). Five of the six combinations depicting synergy against *M. smegmatis* only showed synergistic interaction against this test organism when the aromatic plant's (*L. javanica*, *H. kraussii* and/or *T. riparia*) organic extract has been replaced by its essential oil derivative. As *Mycobacterium smegmatis* was not susceptible to the individual essential oils of either *L. javanica*, *H. kraussii* or *T. riparia* (Chapter 3, Table 3.3), these ∑FIC values (Table 4.1) support the preferred traditional use of these aromatic plants species whithin their different combinations (Chapter 2, Section 2.4.2). Van Vuuren and Viljoen (2009) evaluated the efficacy of the volatile and non-volatile fractions of *T. camphoratus*. During their study, they also found that by combining the non-volatile components with the essential oils (volatile components) of this plant species, the antimicrobial efficacy was enhanced. These authors concluded that essential oils could enhance the antimicrobial activity of a combination by contributing a different mode of antimicrobial action to that of the non-volatiles. Aromatic compounds are able to disrupt the different layers of the bacterial cell wall and cell membrane thus, enabling the enhanced penetration of antimicrobials into such bacterial cells (Bakkali et al., 2008; Solórzano-Santos and Miranda-Novales, 2011).

The mean MIC (mg/mL) and the calculated ∑FIC values of organic and/or essential oils in 1:1, 1:1:1 or 1:1:1:1 combinations against five respiratory pathogens are given in Table 4.1. The lowest MIC value (highest antimicrobial activity) displayed by the organic extract and/or essential oil plant combinations was that of *L. javanica* essential oil with *Brachylaena* cf. *uniflora* against *C. neoformans* (MIC value = 0.06 mg/mL, ∑FIC value = 0.18, Table 4.1). The combined use of these two plant species was only mentioned once during the current ethnobotanical survey. The interviewee mentioning this plant mixture, specifically suggested using it as a remedy to treat flu, mentioning symptoms such as chills, coughs and a blocked nose

(Chapter 2, Section 2.4.2.3.4). The main causative agent of flu is the influenza virus (influenza A, B or C), and symptoms include headache, fever, muscle pain, and a dry, persisting cough (Nester et al., 2001). As the majority of the interviewees were lay people, the perceived belief that the patient is infected with the influenza virus may be incorrect. Infection could have been from any of the many respiratory infections, including pulmonary cryptococcosis. Pulmonary cryptococcosis is caused by *C. neoformans* in people with weak immune systems (Kerkering et al., 1981; Núñez et al., 2000). Symptoms include, amongst others, coughing (Núñez et al., 2000; Lindell et al., 2005). Due to the fact that *Brachylaena* cf. *uniflora* has not been identified to species level, it is possible that it is *B. discolor*. After our ethnobotanical survey it was not possible to identify the medicinally used *Brachyleana* spp. to species level if they were not in flower. One can assume that the lay people using such species would then also not be able to differentiate between different *Brachylaena* species, and that different species could be used for the same purpose. *Brachylaena discolor* is known as a decongestant (Pujol, 1990), and due to the fact that the plant mixture of *Brachylaena* cf. *uniflora* with *L. javanica* is also taken to treat a blocked nose, it is possible that this *Brachylaena* sp plays a role in aiding decongestion.

Van Vuuren and Viljoen (2011) highlighted the importance of also discussing antagonistic effects due to the fact that such combinations would suggest that the plant samples forming part of that mixture have opposing effects. While ten of the combinations (Table 4.1) depicted synergy, nine of the plant mixtures displayed antagonism against one or more of the test organisms. Eight of these combinations consisted of organic extracts only, three were combined from organic extracts and essential oils, and one combination consisted of essential oils only. While three of these plant organic extract-only combinations (the 1:1:1 combination of *L. javanica* with *C. molle* and *T. sericea*, the 1:1 combination of *L. javanica* with *Hypoxis* cf. *acuminata*, and the 1:1 combination of *L. javanica* with *T. riparia*) also displayed antagonism when the combinations' aromatic plant species' organic extracts were replaced by their essential oil derivatives, four of the combinations containing aromatic plants didn't display antagonism when the aromatic plant species' organic extracts were replaced by their essential oil derivatives. Including a plant species' essential oil derivative in a combination thus does not always imply improved antimicrobial interaction against a specific test organism. The combination of *L. javanica* with *T. riparia* interacted highly antagonistically (∑FIC value = 19.80) against *M. smegmatis* when the organic extracts were combined, but when the essential oils were combined no marked effect was noted (∑FIC value = 1.50). Contrary to this, though, while a non-interactive result (∑FIC value = 2.83) was displayed against *C. neoformans* when only the

organic extracts of *L. javanica* and *T. riparia* were combined, the essential oil combination displayed antagonism (∑FIC value = 5.20). When the combined aqueous extracts of *L. javanica* and *T. riparia* were analysed for antimicrobial interaction, a non-interactive interaction (∑FIC value = 1.15) was obtained against this same test organism (Table 4.2). This supports the traditional way of preparing this plant mixture by using water. When using water as opposed to organic solvents as the extractant, less of the active compounds can be released into solution (Shale et al., 1999; Ong et al., 2006). It is possible that, by releasing extra compounds into solution (by using an organic solvent), an antagonistic interaction takes place between such compounds not normally present in the aqueous extracts.

The ∑FIC values calculated from the frequently used *L. javanica* with *E. grandis* (Chapter 2, Table 2.7) was non-interactive (∑FIC values ranging from 1.11-2.25) against four of the five test organisms, while an ∑FIC value of 0.73 against *K. pneumoniae* indicates an additive interaction for this pathogen. Also found during the current ethnobotanical survey, when medicinally applying plant remedies to treat respiratory ailments, such remedies are often administered through inhalation (Caceres et al., 1991; Van Vuuren and Viljoen, 2009). The aromatic plant mixture of *E. grandis* with *L. javanica* was mostly (90% of the times it was used) administered through steam inhalation (Chapter 2, Section 2.4.2.12.4). One would thus expect the essential oil combination to show at least an additive interaction against one or more of the test organisms, but the combined essential oil distillates of this frequently used plant mixture did not show any positive antimicrobial interaction against any of the five test organisms (∑FIC values ranging between 1.10 and 2.33). While this aromatic plant combination was mostly applied through steam inhalation, during 56% of the times steaming was applied, the decoction was taken orally as well. For the combination of *L. javanica* with *E. grandis*, the combined use of drinking and steaming could thus enhance antimicrobial activity through the combination of both the volatile and non volatile fractions of these plant species (Van Vuuren and Viljoen, 2009).

During the current study *L. javanica* essential oil was also analysed for antimicrobial interaction in combination with *T. riparia* essential oil, and in this case synergy was displayed against *M. smegmatis* (∑FIC value = 0.49). Unlike the frequently mentioned combination of *E. grandis* with *L. javanica*, this combination was only mentioned by one interviewee (Chapter 2, Table 2.7). The combined use of *S. cordatum* and *T. sericea* bark was only mentioned once for the treatment of respiratory infections, but the combination (organic extracts) proved to be

synergistic against both *C. neoformans* (∑FIC value = 0.20) and *K. pneumoniae* (∑FIC value = 0.31). Masoko et al. (2005) analysed the organic extracts of six *Terminalia* spp. against five different fungal pathogens (including *C. neoformans*), and found *T. sericea* to be the most active against nearly all organisms tested - further supporting the antifungal properties of this plant species. Although the antimicrobial activities of both *T. sericea* and *S. cordatum* have been previously reported against organisms associated with respiratory infections (Fyhrquist et al., 2002; Steenkamp et al., 2007; Eloff et al., 2008; Mativandlela et al., 2008; McGaw et al., 2008; Pallant and Steenkamp, 2008; Green et al., 2010), the combined antimicrobial efficacy has not been analysed before. Another combination only mentioned once was that of *E. grandis*- with *O. obovata* leaves (Chapter 2, Table 2.7). In spite of this low frequency of use, the combination of *E. grandis* essential oil with *O. obovata* organic extract showed strong synergistic interactions against *C. neoformans* (∑FIC value = 0.34) and *K. pneumoniae* (∑FIC value = 0.28). While some medicinal information has been documented on *O. obovata* (Verzár and Petri, 1987; Bruschi et al., 2011) this study is, according to our knowledge, the first documentation of the combined use and antimicrobial interaction of this species in combination with *E. grandis*.

Table 4.1 The mean MIC values (mg/mL) and the ∑FIC values of the organic extracts (CH_2Cl_2:MeOH) and/or essential oil (EO) distillates used in combination (1:1, 1:1:1 or 1:1:1:1) against five respiratory pathogens.

***Plant combinations**	**Pathogens**									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
E. grandis, *O. obovata*	**0.23**	1.31	1.67	0.63	1.33	0.97	2.00	1.00	**0.25**	1.09
E. grandis EO, *O.obovata*	**0.25**	**0.34**	1.50	**0.28**	4.00	1.00	1.33	0.73	2.00	1.13
Hypoxis cf. *acuminata*, *S. puniceus*, *A. marlothii*, *E. caffra*	1.33	1.55	2.67	0.96	2.67	6.89	NS	1.75	1.67	2.95
L. javanica leaf, *E. grandis*, *C. anisata*	**0.25**	1.52	1.67	0.94	1.67	1.11	2.67	1.27	2.00	5.82
L. javanica EO, *E. grandis* EO, *C. anisata*	**0.20**	0.65	2.00	0.58	6.67	2.08	2.00	0.90	2.67	0.77

*Plant combinations	Pathogens									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
L. javanica leaf, *E. grandis*	**0.22**	1.52	1.33	0.73	3.00	2.25	2.67	1.11	**0.42**	1.76
L. javanica EO, *E. grandis* EO	**0.42**	1.15	8.00	1.10	10.67	2.33	NS	1.50	4.00	1.13
L. javanica leaf, *E. grandis*, *S. serratuloides*	**0.25**	1.27	1.33	0.82	2.67	1.56	1.33	0.70	**0.83**	2.45
L. javanica EO, *E. grandis* EO, *S. serratuloides*	**0.25**	0.56	2.00	0.68	4.00	0.92	1.33	0.67	2.00	0.63
L. javanica leaf, *E. grandis*, *T. riparia*	**0.60**	3.13	**0.75**	**0.42**	**0.70**	2.59	1.33	0.81	**0.07**	1.08
L. javanica EO, *E. grandis* EO, *T. riparia* EO	**0.83**	1.86	5.33	0.93	10.67	2.22	3.33	0.94	4.00	0.92
L. javanica leaf, *T. emetica*	**0.33**	1.29	1.50	0.90	2.67	1.35	1.33	**0.47**	3.33	2.83
L. javanica EO, *T. emetica*	**0.25**	0.97	4.00	1.50	4.00	1.80	2.67	0.70	2.67	1.85
L. javanica leaf**, *H. kraussii*, *T. emetica*	**0.27**	0.80	2.00	1.30	2.67	1.12	2.00	0.81	3.33	2.44
L. javanica EO, *H. kraussii* EO, *T. emetica*	**0.33**	0.87	2.00	0.72	4.00	1.54	2.00	**0.39**	4.00	1.93
L.javanica leaf, *Brachylaena* cf. *uniflora*	**0.25**	0.75	2.67	1.24	4.00	1.60	1.33	0.72	1.00	1.00
L. javanica EO, *Brachylaena* cf. *uniflora*	**0.06**	**0.18**	4.00	0.97	4.00	1.38	1.00	**0.45**	1.33	1.13
L. javanica leaf, *Hypoxis* cf. *acuminata*	3.33	8.59	2.00	1.20	5.33	2.13	NS	2.04	2.00	0.75
L. javanica EO, *Hypoxis* cf. *acuminata*	1.67	4.29	4.00	1.50	6.67	2.29	2.00	**0.40**	4.00	0.88

*Plant combinations	Pathogens									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
L. javanica leaf, *B. cathartica*	**0.43**	1.19	3.33	1.42	1.33	1.53	1.33	1.22	2.00	0.88
L. javanica EO, *B. cathartica*	**0.33**	0.92	2.67	0.53	8.00	8.75	2.00	1.65	2.67	0.75
L. javanica leaf, *C. molle*, *T. sericea* leaf	**0.25**	0.54	1.67	0.80	8.00	4.13	1.33	0.70	1.67	0.90
L. javanica EO, *C. molle*, *T. sericea* leaf	**0.25**	1.21	1.50	0.64	8.00	4.83	2.00	1.13	2.67	1.50
L. javanica leaf, *C. molle*, *E. grandis*	**0.27**	1.33	1.50	0.63	2.67	1.78	1.00	0.53	**0.63**	1.84
L. javanica EO, *C. molle*, *E. grandis* EO	**0.47**	1.01	4.00	0.59	4.00	1.25	1.33	0.67	3.33	1.04
L. javanica leaf, *C. molle*	3.33	8.33	2.67	1.02	5.33	2.13	1.50	0.81	2.00	0.88
L. javanica EO, *C. molle*	**0.25**	0.63	4.00	0.63	8.00	2.75	2.00	0.90	6.67	1.88
L. javanica leaf, *K.mosambicina*	2.00	5.00	2.67	1.24	8.00	1.53	1.50	1.00	4.00	1.30
L. javanica EO, *K.mosambicina*	**0.25**	0.63	2.67	0.64	8.00	1.08	2.67	1.53	13.33	2.25
L. javanica leaf, *C. brachiata*	**0.25**	0.63	3.33	1.83	8.00	2.40	1.50	0.63	2.67	0.87
L. javanica EO, *C.brachiata*	**0.20**	**0.50**	2.67	0.87	8.00	1.95	NS	1.30	4.00	0.68
L. javanica leaf, *T. riparia*, *A. glabratum*	**0.35**	1.05	2.67	1.31	1.33	4.51	1.50	1.42	**0.19**	2.54
L. javanica EO, *T. riparia* EO, *A. glabratum*	**0.20**	0.57	4.00	0.93	4.00	0.83	**0.47**	**0.27**	2.00	0.54
L. javanica leaf, *E. grandis*, *Brachylaena* cf. *transvaalensis*	**0.25**	1.38	2.67	1.64	2.67	2.00	1.67	1.30	**0.50**	1.74

*Plant combinations	Pathogens									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
L. javanica EO, *E. grandis* EO, *Brachylaena* cf. *transvaalensis*	**0.28**	0.76	2.67	0.91	4.00	1.58	1.00	0.75	2.00	1.71
L. javanica leaf, *P. neochilus*	**0.18**	0.59	2.00	1.10	2.67	2.40	**0.83**	0.56	**0.83**	0.83
L. javanica EO, *P. neochilus*	**0.25**	0.80	2.00	0.65	4.00	1.13	1.67	0.96	1.00	0.84
L. javanica leaf, *S. serratuloides*, *E. grandis*, *P. guajava*	**0.15**	0.65	1.67	1.19	2.00	1.38	1.00	0.52	**0.67**	1.80
L. javanica EO, *S. serratuloides*, *E. grandis* EO, *P. guajava*	**0.25**	0.54	2.67	1.35	2.67	3.38	2.00	1.00	2.00	1.47
S. serratuloides, *S. deltoides*	1.00	1.20	2.00	1.50	5.33	1.33	2.00	1.50	4.00	1.42
S. cordatum, *T. sericea* bark	**0.08**	**0.20**	**0.25**	**0.31**	**0.75**	0.64	1.00	1.13	**0.70**	1.63
S. cordatum, *S. birrea* mature bark	**0.25**	3.30	**0.83**	0.83	**0.38**	0.75	1.67	1.04	**0.23**	0.57
L. javanica leaf, *L. javanica* root	**0.67**	1.67	1.00	0.63	2.67	0.62	2.00	0.71	3.33	2.08
L. javanica leaf EO, *L. javanica* root	**0.83**	2.08	3.33	1.36	5.33	0.94	NS	1.05	4.00	1.88
L. javanica leaf, *T. riparia*	1.00	2.83	2.00	1.17	4.00	19.80	2.00	1.33	**0.25**	5.06
L. javanica EO, *T. riparia* EO	**2.00**	5.20	4.00	0.80	8.00	1.50	NS	**0.49**	8.00	1.25

*All plant extracts are organic, with the exception of the essential oils (indicated with EO); **For plant species where different plant parts were used, the plant part used is specified. Where plant parts are not specified, parts used are as indicated in Chapter 3, Table 3.1.; NS = not susceptible; Noteworthy- and synergistic activities are given in bold; Antagonistic activities are underlined.

4.3.2 MIC and ∑FIC values of aqueous extracts

All plant remedies analysed during this study have been traditionally prepared using water, and aqueous extracts have been prepared and analysed in order to mimic the traditional method of extraction. Thirteen of the 25 combinations gave ∑FIC values > 1.00 for four or more of the five test organisms. For five of these combinations no antimicrobial activity could be detected against some or all of the test organisms, even at the highest concentration tested, thus making the calculation of these ∑FIC values not possible (NE, Table 4.2). Even though only two (8%) of the aqueous extract combinations (the 1:1 combinations of *S. cordatum* with *T. sericea*, and *L. javanica* with *T. emetica*) displayed synergism, the majority of these combinations (68%) displayed additive interaction against at least one of the five test organisms -lending some credibility to the combined traditional use of some of the plant species in treating respiratory infections. Besides the five combinations without calculated ∑FIC values, only two of the aqueous extract combinations displayed antagonism. When comparing these results to the ∑FIC values of the organic extracts, where eight combinations displayed antagonism (Table 4.1), results support the traditional use of water to extract plant material.

The only combinations displaying noteworthy antimicrobial activity were the 1:1:1combination of *L. javanica* with *T. riparia* and *A. glabratum* (MIC value = 0.33 mg/mL against *C. neoformans*), the 1:1 combination of *E. grandis* with *O. obovata* (MIC value = 0.30 mg/mL against *C. neoformans*), the 1:1 combination of *S. cordatum* with *T. sericea* bark, and the 1:1 combination of *S. cordatum* with *S. birrea* bark (Table 4.2). Both these bark combinations have each only been mentioned once during the ethnobotanical survey (Chapter 2, Table 2.7), but displayed strong antimicrobial activity against three of the five test organisms (with MIC values ranging from 0.10 mg/mL to 0.50 mg/mL). Fourteen of the other combinations (including the frequently used *L. javanica* with *E. grandis*) showed some antifungal activity against *C. neoformans*, with MIC values ranging from 2.00 - 6.67 mg/mL, but no synergy was depicted.

When combining *S. cordatum* and *T. sericea* bark, noteworthy antimicrobial activity was shown against *C. neoformans* (MIC value = 0.10 mg/mL), *K. pneumoniae* (MIC value = 0.50 mg/mL) and *S. aureus* (MIC value = 0.25 mg/mL). The combination of *S. cordatum* with *S. birrea* showed noteworthy activity against *C. neoformans* (MIC value = 0.20 mg/mL), *M. catarrhalis* (MIC value = 0.50 mg/mL) and *S. aureus* (MIC value = 0.25 mg/mL). *Syzygium cordatum* forms

part of both these bark remedies, and even though both combinations show broad-spectrum antimicrobial activity, synergy was only depicted when *S. cordatum* was combined with *T. sericea* (∑FIC value = 0.35 against *C. neoformans*). While the combined effect of *S. cordatum* with *T. sericea* bark proved to be synergistic against *C. neoformans*, additive interaction was displayed against *K. pneumoniae* (∑FIC value = 0.80), *M. smegmatis* (∑FIC value = 0.88) and *S. aureus* (∑FIC value = 0.75). The only other combination, besides that of *S. cordatum* with *T. sericea*, depicting synergy is that of *L. javanica* with *T. emetica*. Despite showing poor antimicrobial activity against all five test organisms (MIC values = 4.00 - 13.33 mg/mL), this leaf combination displayed synergistic interaction against *M. smegmatis* (∑FIC value = 0.50). Both these combinations (*L. javanica* with *T. emetica*, and *S. cordatum* with *T. sericeae*) also displayed synergistic interaction against the same test organisms when testing their organic extracts (Table 4.1).

When testing *L. javanica* with *E. grandis*, their 1:1 combination proved to be additive against both *M. catarrhalis* (∑FIC value = 0.69) and *S. aureus* (∑FIC value = 0.83), but non-interactive against *C. neoformans*, *K. pneumoniae* and *M. smegmatis* (∑FIC values from 1.20-1.50). Besides the combination of *E. grandis* with *L. javanica* (mentioned at 26% of the homesteads), all of the combinations (Chapter 2, Table 2.7) have only been mentioned once or twice. In 85% of the times the use of *E. grandis* was mentioned by the interviewees, it was used in combination with *L. javanica*, and not on its own. While the fact that only one of these less frequently mentioned combinations' aqueous extracts displayed synergy was not totally unexpected, one would expect the combination of *E. grandis* with *L. javanica* to show synergy to validate its frequent use. However, in Chapter 3 it was concluded that frequency of use do not necessarily always correlate with high antimicrobial efficacy. Results obtained for some of the combined remedies thus further supports this conclusion. In an ethnobotanical survey conducted by Dahlberg and Trygger (2009) the authors discussed *Eucalyptus* spp for the treatment of headaches, flu, coughs and fever. Dahlberg and Trygger (2009) also found the interviewees to have a strong affinity towards the use of *L. javanica* and *Eucalyptus* spp. in combination to treat flu and headaches. The survey done by Dahlberg and Trygger (2009) was conducted in an area (in KwaZulu-Natal, South Africa) close to the vicinity of the current study area, and thus supports the common use of these two species in treating respiratory infections in northern KwaZulu-Natal. Both these plant species occur in abundance in this study area (Van Wyk et al., 2009; Dahlberg and Trygger, 2009) and are both highly aromatic (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 2009), which possibly explains their popularity in the treatment of

respiratory ailments. The aroma-therapeutic potency of plants is mainly in the leaves (Pujol, 1990), and for both of these aromatic plants their leaves were the preferred plant part used to treat various respiratory problems (Chapter 2, Section 2.4.2.12.4 and 2.4.2.17.4).

Instead of displaying antimicrobial synergy, many of these combinations could also exert beneficial effects to their users through their possible synergistic action on targeting sites associated with a physiological process such as inflammation or immunity (Okigbo et al., 2009). In addition, Shale et al. (1999) mentioned that traditional plant remedies are often administered to adults in large dosages, which could cause for an efficient antimicrobial effect from aqueous extracts having intermediate or low antimicrobial activities. Many of the remedies tested in this study are traditionally taken in high dosages (Chapter 2, Section 2.4.2). To demonstrate the amount of plant extracts that can be consumed per day, the most frequently taken *L. javanica* is used here as an example. According to Chapter 2, Section 2.4.2.17.4 (e), a handful of *L. javanica* leaves (averaged to 40 g) are brought to the boil in two litres of water (i.e 20 mg plant material/mL). Taking into consideration the average yield of *L. javanica* leaf extract gained from aqueous extraction (yield = 8.86%, Chapter 3, Table 3.2), 1.77 mg/mL of *L. javanica* extract will be taken as a remedy. One tablespoon (15 mL) of this aqueous extract is then taken once or twice daily. A total of 26.58 mg (15 mL x 1.77 mg/mL) will then be taken once or twice a day. One should consider this when analysing aqueous extracts' MIC results from this and other plant remedies.

Table 4.2 The mean MIC values (mg/mL) and the sum fractional inhibitory concentration (∑FIC) values of the aqueous extracts used in combination (1:1, 1:1:1 or 1:1:1:1) against five respiratory pathogens.

Plant species in combinations	**Pathogens**									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
E. grandis, *O. obovata*	**0.30**	1.55	1.67	1.75	2.67	1.67	4.00	1.00	1.00	0.88
Hypoxis cf. *acuminata*, *S. puniceus*, *A. marlothii*, *E. caffra*	6.67	NE	8.00	NE	16.00	NE	10.67	NE	16.00	NE

Plant species in combinations	Pathogens									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
L. javanica leaf*, *E. grandis*, *C. anisata*	4.00	NE	2.67	2.06	6.67	NE	8.00	NE	3.33	NE
L. javanica leaf, *E. grandis*	5.33	1.20	1.33	1.50	4.00	0.69	8.00	1.50	1.33	0.83
L. javanica leaf, *E. grandis*, *S. serratuloides*	13.33	2.28	1.33	1.22	6.67	0.93	8.00	1.40	2.00	1.00
L. javanica leaf, *E. grandis*, *T. riparia*	3.00	1.45	1.33	1.22	6.67	1.04	8.00	1.40	2.00	1.17
L. javanica leaf, *T. emetica*	8.00	1.20	4.00	1.75	13.33	1.46	4.00	**0.50**	4.00	1.25
L. javanica leaf, *T. emetic*, *H. kraussii*	6.67	0.81	3.33	1.39	16.00	1.57	8.00	1.00	4.00	1.17
L. javanica leaf, *Brachylaena* cf. *uniflora*	13.33	1.42	2.00	1.00	13.33	1.46	8.00	0.75	2.00	1.50
L. javanica leaf, *Hypoxis* cf. *acuminata*	6.67	2.50	3.33	1.67	8.00	0.88	4.00	0.63	4.00	1.25
L. javanica leaf, *B. cathartica*	5.33	0.73	1.33	1.50	8.00	0.88	8.00	0.75	2.00	1.50
L. javanica leaf, *C. molle*, *T. sericea* leaf	6.67	10.77	1.33	1.44	8.00	2.25	4.00	0.90	1.00	0.75
L. javanica leaf, *C. molle*, *E. grandis*	6.67	1.56	1.33	1.67	6.67	1.32	6.67	1.39	1.00	0.67
L. javanica leaf, *C. molle*	5.33	1.07	1.00	1.13	6.67	1.15	8.00	1.50	1.33	0.83
L. javanica leaf, *K. mosambicina*	10.67	2.40	5.33	2.17	>16.00	NE	16.00	1.50	8.00	2.25
L. javanica leaf, *C. brachiata*	4.00	0.80	8.00	3.25	>16.00	NE	13.33	1.25	16.00	5.00
L. javanica leaf, *T. riparia*, A. glabratum	**0.33**	NE	5.33	2.39	>16.00	NE	13.33	1.78	3.33	1.18
L. javanica leaf, *E. grandis*, *Brachylaena* cf. *transvaalensis*	8.00	1.37	2.00	1.63	8.00	1.12	8.00	1.33	2.00	1.00

Plant species in combinations	Pathogens									
	***C. neoformans* ATCC 14116**		***K. pneumoniae* ATCC 13883**		***M. catarrhalis* ATCC 23246**		***M. smegmatis* ATCC 14468**		***S. aureus* ATCC 6538**	
	MIC values	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**	**MIC values**	**∑FIC values**
L. javanica leaf, *P. neochilus*	5.33	0.57	2.00	1.50	8.00	0.88	8.00	1.00	2.00	1.00
L. javanica leaf, *S. serratuloides*, *E. grandis*, *P. guajava*	4.00	0.68	1.33	1.42	4.00	1.92	5.33	1.03	1.00	0.88
S. serratuloides, *S. deltoideus*	10.67	1.13	2.00	1.10	8.00	0.80	13.33	1.83	4.00	1.50
S. cordatum, *T. sericea* bark	**0.10**	**0.35**	**0.50**	0.80	1.00	1.25	2.00	0.88	**0.25**	0.75
S. cordatum, *S. birrea* mature bark	**0.20**	1.03	1.00	1.50	**0.50**	1.10	2.00	1.00	**0.25**	0.69
L. javanica leaf, *L. javanica* root	16.00	1.70	6.67	2.92	16.00	1.50	16.00	1.60	8.00	2.50
L. javanica leaf, *T. riparia*	2.00	1.15	2.00	1.25	8.00	0.88	16.00	2.20	4.00	2.00

*For plant species where different plant parts were used, the plant part used is specified. Where plant parts are not specified, parts used are as indicated in Chapter 3, Table 3.1.; Noteworthy- and synergistic activities are given in bold; Antagonistic activities are underlined; NE = The MIC value of at least one of the plant samples had no endpoint i.e >16.00 mg/mL.

4.3.3 In depth analysis of 1:1 combinations

When looking at a combination of two plant species, the ∑FIC method assumes that 50% of the antimicrobial effect observed would have been provided by half the extract concentration. Even though this might sometimes be the case, it cannot always be assumed. When determining combination effects one should take into account that various ratios of the two combined plants may yield different interactive efficacies. This is what the isobologram method of analysis aims to do (Van Vuuren and Viljoen, 2011).

The combinations further analysed through the isobologram method were selected as follows;
(a) *Eucalyptus grandis* with *L. javanica* (organic and aqueous extracts, as well as the essential oils) - The combination has an extremely high frequency of use by the interviewees.

(b) *Eucalyptus grandis* essential oil with *O. obovata* organic extract; *S. cordatum* with *T. sericea* (organic and aqueous extracts) - These combinations showed synergistic ∑FIC-results (∑FIC ≤ 0.5) against more than one test organism.
(c) *Lippia javanica* with *T. emetica* (organic and aqueous extracts, as well as the essential oil of *L. javanica*) - The combination showed synergistic ∑FIC-results (∑FIC ≤ 0.5) against *M. smegmatis* which could indicate the possible antimycobacterial activity of this combination.

4.3.3.1 *Eucalyptus grandis* with *Lippia javanica*

Regardless of the fact that all the 1:1 combinations of *E. grandis* with *L. javanica* showed no synergistic interaction against any of the test organisms, ratio combinations were further evaluated due to the extremely high frequency of use by the interviewees. In an attempt to validate this highly preferred traditional remedy, further research into varied ratio analysis was undertaken. This in depth analysis was conducted with the hypothesis that if the two plant species were combined in different ratios a more favourable interaction would be evident. The combined essential oils, and aqueous- and organic extracts of *E. grandis* and *L. javanica* were consequently tested at different ratios against all five test organisms.

Despite the display of mostly non-interactive results against three of the five test organisms, the isobolograms of the organic extracts displayed varied interactions between the five test organisms (Figure 4.2). Ratios containing a larger percentage of *E. grandis* organic extract proved to be mostly non-interactive against *K. pneumoniae*, but one of these ratios with a higher amount of *E. grandis* (6:4) displayed, when compared to all other ratios, interaction closest to synergy. The combination gave non-interactive results against both *M. catarrhalis* and *S. aureus* for all ratios but two. These two ratios were the 9:1 ratio (a), where the *E. grandis* ratio was higher, as well as the 1:9 ratio (b), where the *L. javanica* ratio was higher. These results further highlight the fact that the efficacies of combined drugs rely greatly on the specific ratio in which they are combined, as well as the test organism they are used against. The combination showed additive interactions (on the 1:1 line) against *M. smegmatis* for all ratios tested. The isobologram profile displayed by the organic extracts against *C. neoformans* showed all but two ratios to be non-interactive. These two ratios (2:8 and 1:9) each contained a higher ratio of *L. javanica* organic extract.

When looking at the combined aqueous extracts of *E. grandis* and *L. javanica* a definite pattern was notable against both *K. pneumoniae* and *M. smegmatis*. While the other ratios were non-interactive, only ratios containing a higher amount of *E. grandis* extract displayed an additive interaction (Figure 4.3). When testing the aqueous extracts of this combination against both *M. catarrhalis* and *S. aureus*, most ratios depicted a non-interactive profile against these test organisms. In the case of *S. aureus*, all but two of the ratios lay within the non-interactive range. These two ratios were both additive, where one ratio (9:1) (a) contained a higher ratio of *E. grandis*, while the other (3:7) (c) contained a higher ratio of *L. javanica*. This latter result is similar to the results displayed by the organic extracts against both *M. catarrhalis* and *S. aureus* (Figure 4.2).

While both the organic and aqueous extracts displayed non-interactive predominant profiles, the combination of *E. grandis* with *L. javanica* essential oil demonstrated mainly additive interactions (Figure 4.4). Three of the five test organisms (*C. neoformans*, *K. pneumoniae* and *M. catarrhalis*) displayed similar additive susceptibility to all ratios of combining *E. grandis* with *L. javanica* essential oils. The essential oil combinations with a higher ratio of *E. grandis* (Figure 4.4) proved to be additive against *S. aureus*, while the ratio containing the highest percentage of *E. grandis* essential oil was close to synergistic against *M. smegmatis*.

Although 20 of the 80 homesteads claimed to use *E. grandis* and *L. javanica* in a 1:1 combination, one interviewee specifically stated that she combined these two plant species in a 2:1 (drinking) or a 3:1 (steaming) combination - where *E. grandis* is the higher ratio (Chapter 2, Section 2.4.2.12.4). This correlates with isobologram results found for the combined aqueous extracts against *K. pneumoniae* and *M. smegmatis* (Figure 4.3). A similar pattern was noticeable for the isobolograms of the essential oils against *M. smegmatis* and *S. aureus*. Steaming releases the volatile essential oils into the lungs (Van Wyk et al., 2009), and could explain the essential oils combination profile against *S. aureus* (a Gram-positive bacterium able to cause lower respiratory tract infections) and *M. smegmatis* (a test organism for the causative agent of TB) (Mitscher and Baker, 1998; Moise and Schentag, 2000). While these aqueous extract- and essential oil results prove that ratios with a higher percentage of *E. grandis* display enhanced antimicrobial activity against certain microorganisms, the opposite is true for the organic extracts (Figure 4.2). Ratios containing a higher percentage of *L. javanica* showed improved antimicrobial activity against both *C. neoformans* and *K. pneumoniae*.

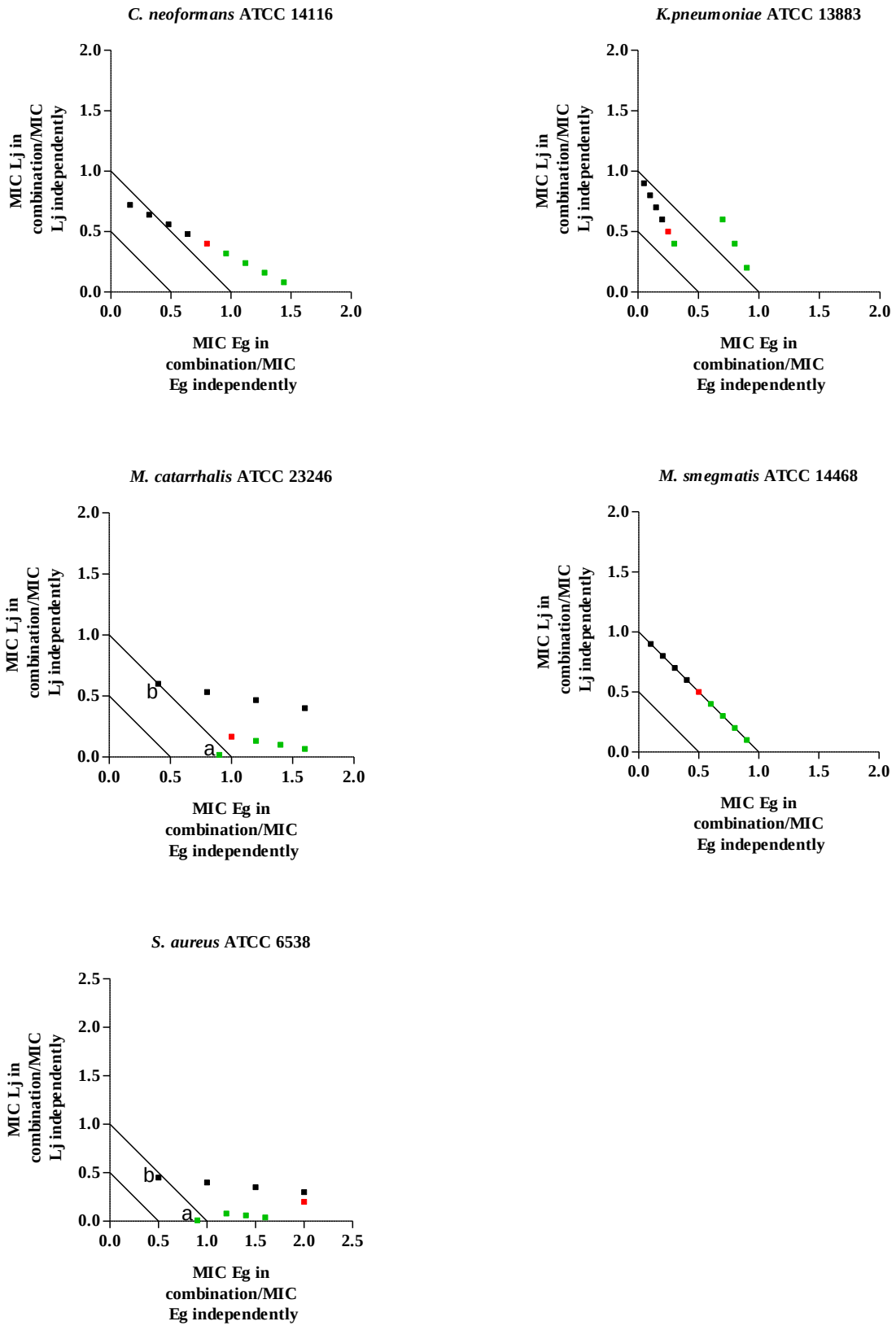


Fig 4.2 Isobologram representation of the combination of *E. grandis* (Eg) with *L. javanica* (Lj) organic extracts against five pathogens. Combinations containing more *E. grandis* are depicted with a green square (■), while combinations containing more *L. javanica* are depicted with a black square (■).The 1:1 ratios are depicted with a red square (■).

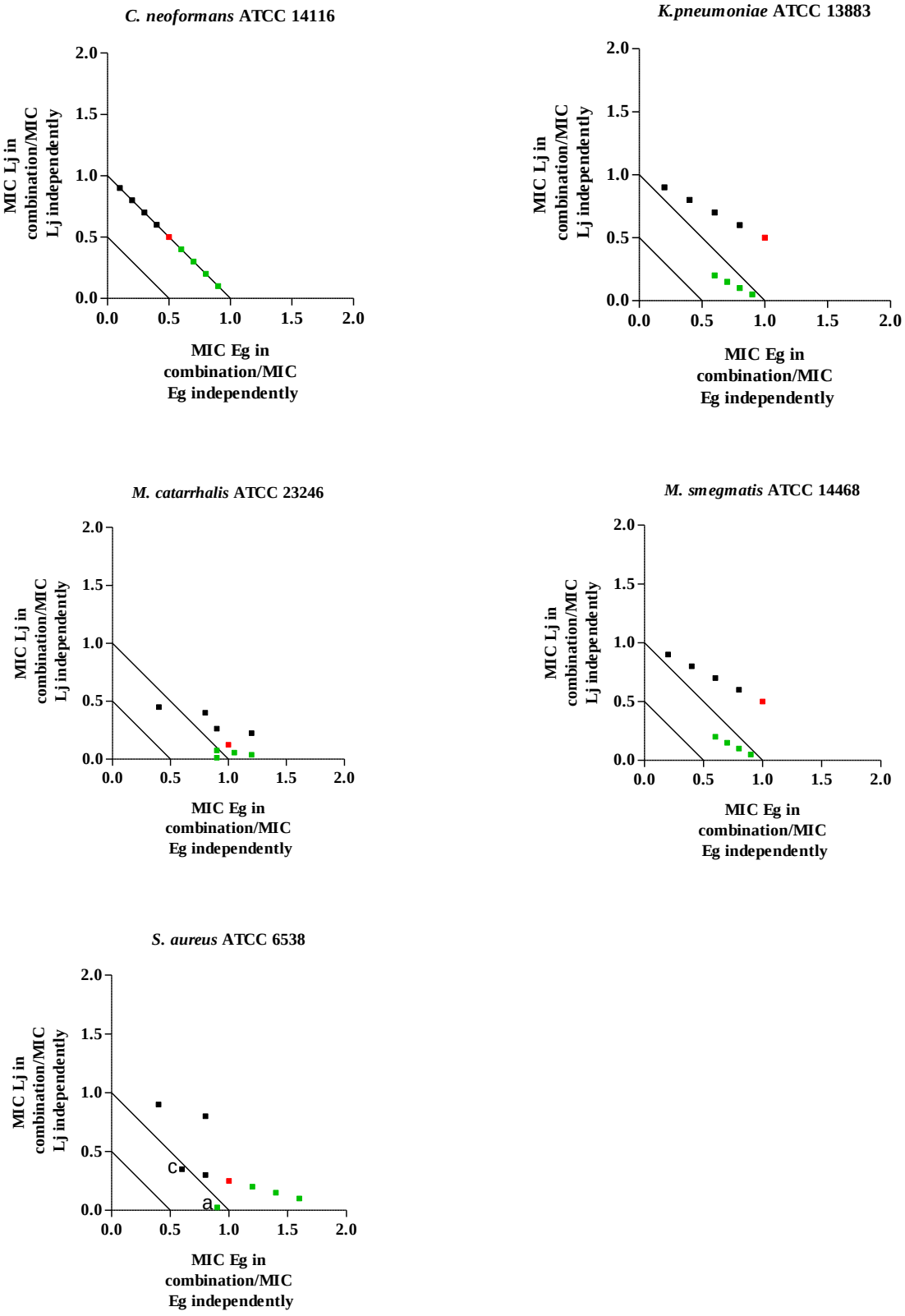


Fig 4.3 Isobologram representation of the combination of *E. grandis* (Eg) with *L. javanica* (Lj) aqueous extracts against five pathogens. Combinations containing more *E. grandis* are depicted with a green square (■), while combinations containing more *L. javanica* are depicted with a black square (■).The 1:1 ratios are depicted with a red square (■).

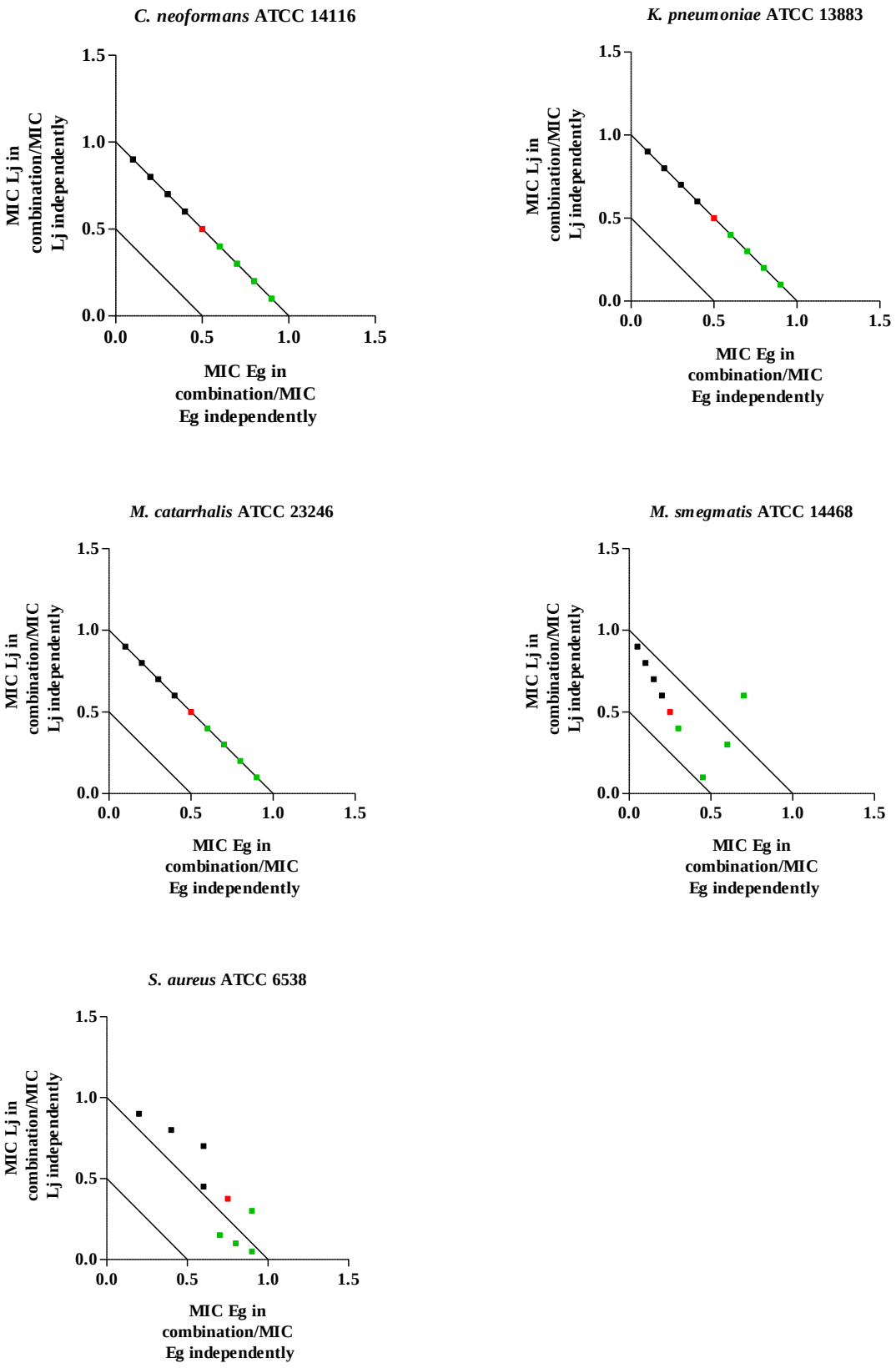


Fig 4.4 Isobologram representation of the combination of *E. grandis* (Eg) with *L. javanica* (Lj) essential oils against five pathogens. Combinations containing more *E. grandis* are depicted with a green square (■), while combinations containing more *L. javanica* are depicted with a black square (■).The 1:1 ratios are depicted with a red square (■).

This further supports the traditional use of water extraction for enhanced activity when using a higher percentage of *E. grandis* in this remedy. It also brings into consideration the importance of documenting combination studies in all possible ratios. While synergy could be depicted by one ratio of combining two substances, antagonism can be displayed by combining these same two substances in a different ratio (Williamson, 2001). It should be noted, though, that during the current study, no antagonism was noted against both the organic and aqueous extract combinations, as well as the combined essential oils of *E. grandis* and *L. javanica*.

4.3.3.2 *Eucalyptus grandis* with *Ozoroa obovata*

The display of synergy against more than one test organism, motivated the in depth analysis of the following two combinations; *E. grandis* essential oil with *O. obovata* organic extract, as well as *S. cordatum* with *T. sericea* (aqueous and organic extracts). A third combination (*L. javanica* essential oil with *Brachylaena* cf. *uniflora*) also interacted synergistically against two of the test organisms (Table 4.1). Due to the fact that this *Brachylaena* sp. was unidentified, we have decided to exclude this third combination from further analysis.

When combining *E. grandis* essential oil with *O. obovata* organic extract (1:1), synergy was displayed against both *C. neoformans* (∑FIC value = 0.34) and *K. pneumoniae* (∑FIC value = 0.28) (Table 4.1). Due to this strong display of synergy, an in depth investigation has been conducted on this combination against these two test organisms. When analysing the different ratios against *C. neoformans*, all ratios having a higher percentage of *E. grandis* essential oil were in the additive profile range, while all ratios with equal amounts of *O. obovata* and *E. grandis*, or a higher percentage of *O.obovata* organic extract displayed synergy. As discussed in Section 4.3.1, many of the 1:1 combinations containing aromatic plants only displayed synergy when the aromatic plant's essential oil formed part of the combination, implying that the presence of essential oil(s) enhances the antimicrobial interaction of some plant combinations. *Ozoroa obovata* organic extract displayed, when tested on its own, noteworthy antimicrobial interaction against *C. neoformans* (Chapter 3, Table 3.1, MIC value = 0.83 mg/mL), and although *E. grandis* essential oil seems to enhance antimicrobial interaction, a high enough percentage of the antimicrobially active *O. obovata* extract needs to be present in this combination for increased interaction to take place. Against *K. pneumoniae* (Figure 4.5) the combination of *E. grandis* essential oil with *O. obovata* organic extract yielded synergistic

interactions for all ratios tested. One interviewee specifically reported combining the leaves from *O. obovata* and *E. grandis* in a 1:1 ratio and applying it through steam inhalation to treat chest pain, fever and cough (Chapter 2, Section 2.4.2.12.4). As with most respiratory infections, all three of these symptoms are present when a patient is suffering from *Klebsiella* pneumonia - a respiratory disease caused by *K. pneumoniae* (Nester et al., 2001).

No antagonism was displayed for any of the combined ratios of *O. obovata* with *E. grandis*, when tested against *C. neoformans* and *K. pneumoniae*

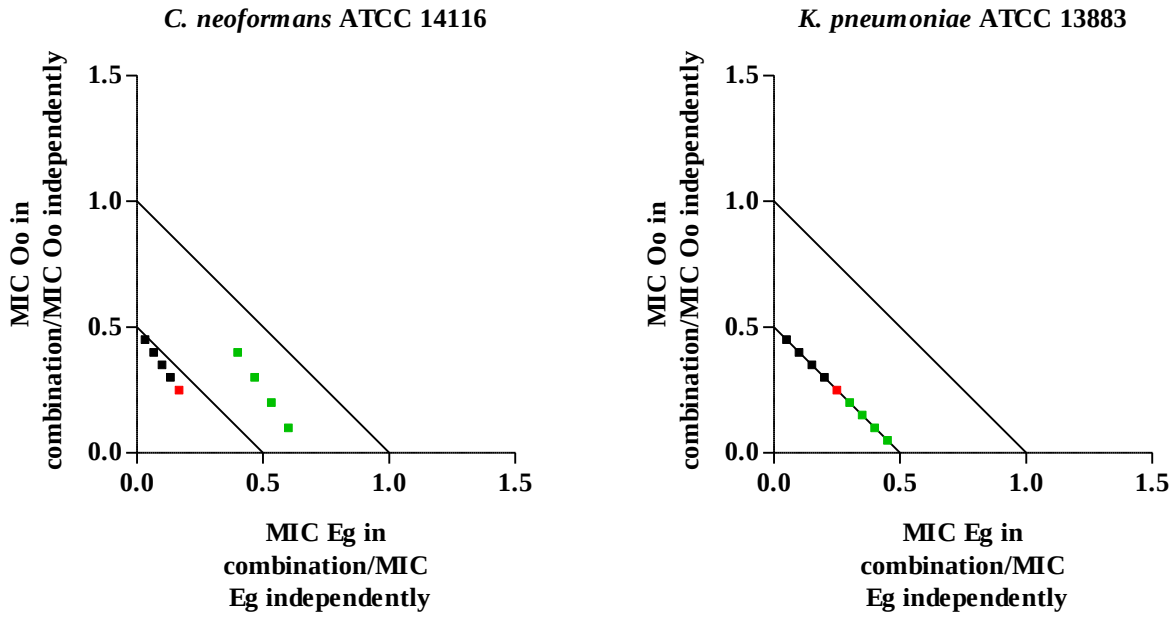
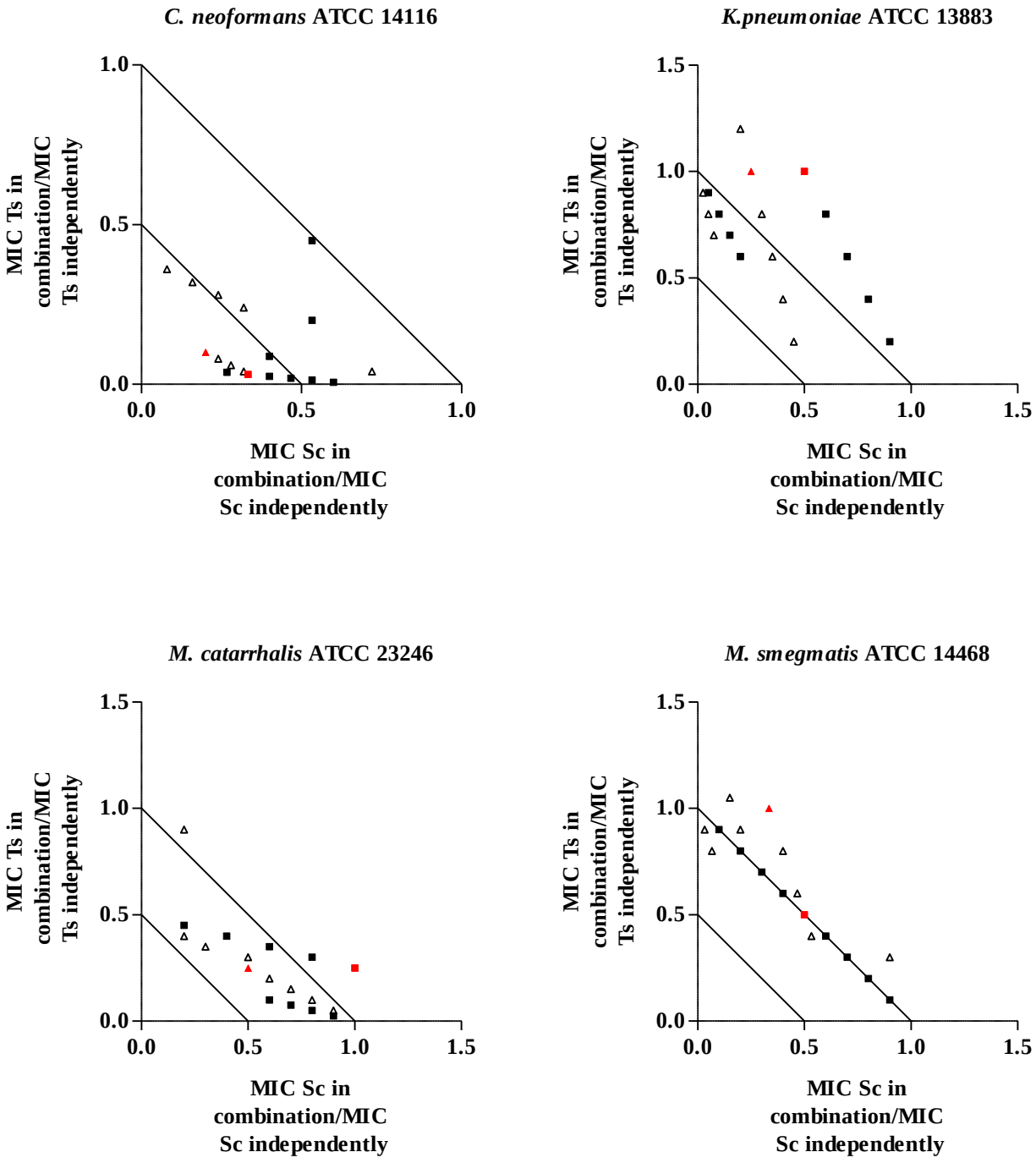


Fig 4.5 Isobologram representation of the combination of *E. grandis* (Eg) essential oil with *O. obovata* (Oo) organic extract against *C. neoformans* and *K. pneumoniae*. Combinations containing more *E. grandis* are depicted with a green square (■), while combinations containing more *O. obovata* are depicted with a black square (■). The 1:1 ratios are depicted with a red square (■).

4.3.3.3 *Syzygium cordatum* with *Terminalia sericea*

Although the combination of *S. cordatum* and *T. sericea* consists of bark only, and was tested in this way, it was in fact not documented as a bark-only combination (Chapter 2, Section 2.4.2.27.4). When analysing the 1:1 combination of *S. cordatum* with *T. sericea*, very strong

synergy was displayed against both *C. neoformans* and *K. pneumoniae* (Table 4.1). Bark is often the preferred plant part to be used medicinally due to the fact that it contains high concentrations of active ingredients (Van Wyk et al., 2009). This is surprising, seeing as 68% of the combinations mentioned in this study were combined from leaves only (Chapter2, Table 2.7). The only plant combination that was mentioned by the interviewees as a bark-only remedy was that of *S. cordatum* with *S. birrea*. This combination, though, did not display any synergism (Table 4.2).



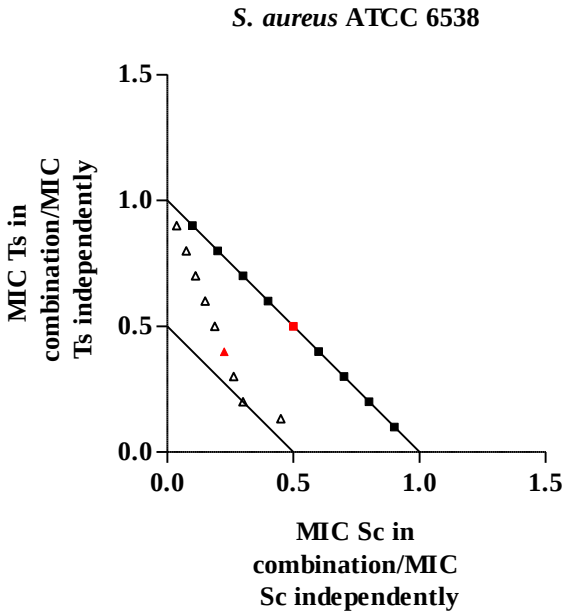


Fig 4.6 Isobologram representation of the combination of *S. cordatum* (Sc) with *T. sericea* (Ts) organic (Δ) and aqueous extracts (■) against five pathogens. The 1:1 ratios are depicted in red.

In the isobologram representation of *S. cordatum* with *T. sericeae* against *C. neoformans* (Figure 4.6), the scale has been amended to make all data points visible. For both the organic and aqueous extract combination most ratios interacted synergistically when tested against *C. neoformans* (Figure 4.6). When analysed against *K. pneumoniae*, the aqueous extract combination with ratios having more *T. sericea* displayed additive effects, while the remaining ratios (including the 1:1 combination) fell into the non-interactive profile range. When assayed independently, both the *T. sericea* (MIC value = 0.83 mg/mL) and *S. cordatum* (MIC value = 0.50 mg/mL) aqueous bark extracts displayed noteworthy antimicrobial activity against *K. pneumoniae* (Chapter 3, Table 3.2). When testing the organic extract combination against this Gram-negative organism, most of the ratios fell within the additive profile range. Only three ratios (6:4, 1:1 and 4:6) were non-interactive, implying that ratios containing equal- or close to equal percentages of each plant species are less interactive against *K. pneumoniae*. This further supports the importance of also testing combinations in ratios other than the 1:1. Most (or all) of the ratios for both extract types fell into the additive range when analysed against both *M. catarrhalis* and *S. aureus*. Against *M. catarrhalis* only two ratios of the aqueous extract combinations were non-interactive (4:6, where *T. sericea* was present in a higher percentage, and 1:1). When testing the organic extracts against *S. aureus*, additivity was displayed by all ratios but one (8:2, where *S. cordatum* was present in a higher ratio) where synergy was observed. The

aqueous extract combination had additive profiles against both *S. aureus* and *M. smegmatis*, where all ratios fell on the 1:1 line. During a study conducted by Green et al. (2010) *T. sericea* was highlighted as important in further anti-tuberculosis research. In the current survey one interviewee mentioned using *T. sericea* bark (in combination with *S. cordatum* bark) to treat, amongst other symptoms, cough and sleepless nights. Although neither of these two species' aqueous bark extracts showed noteworthy MIC values against *M. smegmatis*, their combination gave an additive effect against this test organism at all ratios tested (Figure 4.6). With the organic extracts, though, most ratios are non-interactive against *M. smegmatis* - further supporting the traditional method (using water to extract) of preparing this combination.

4.3.3.4 *Lippia javanica* with *Trichilia emetica*

The combination of *L. javanica* with *T. emetica* has been mentioned twice during our ethnobotanical survey (Chapter 2, Table 2.7). The two interviewees mentioning this remedy lived in two different localities (Mbazwana and Mabibi, Chapter 2, Figure 2.1), and even though these two regions are quite isolated from each other both these remedies have been prepared and used in a very similar way (Chapter 2, Section 2.4.2.17.4) -supporting the traditional use. This combination showed synergistic ∑FIC-results against *M. smegmatis*, which could indicate its possible antimycobacterial activity. This encouraged further analysis of this combination through isobolograms (Figure 4.7). When the organic extracts of both plant species were tested in combination against *M. smegmatis* (Δ), most ratios depicted synergy, but when *L. javanica* organic extract was replaced by its essential oil derivative, all ratios displayed additivity (●). An opposite profile was visible when looking at the aqueous extract combination (■) vs. the aqueous extract-essential oil combination (o). In this instance, only where the combination contained *L. javanica* essential oil, instead of its aqueous extract, was synergy displayed. While the two ratios where the percentage of *T. emetica* aqueous extract was the highest were non-interactive against *M. smegmatis*, the three ratios containing the highest ratio of *L. javanica* essential oil were the only synergistic ratios for the combination of *L. javanica* essential oil with *T. emetica* aqueous extract. The fact that both these interviewees prepared and took this mixture through steaming could support this specific traditional use. As mentioned in Chapter 3, Section 3.4, steam inhalation allows the mucous membranes of the nose and lungs to absorb the essential oils present in aromatic plants (Van Wyk and Wink, 2004), which could support the fact that ratios with a higher percentage of essential oil did in fact display synergy (Figure 4.7).

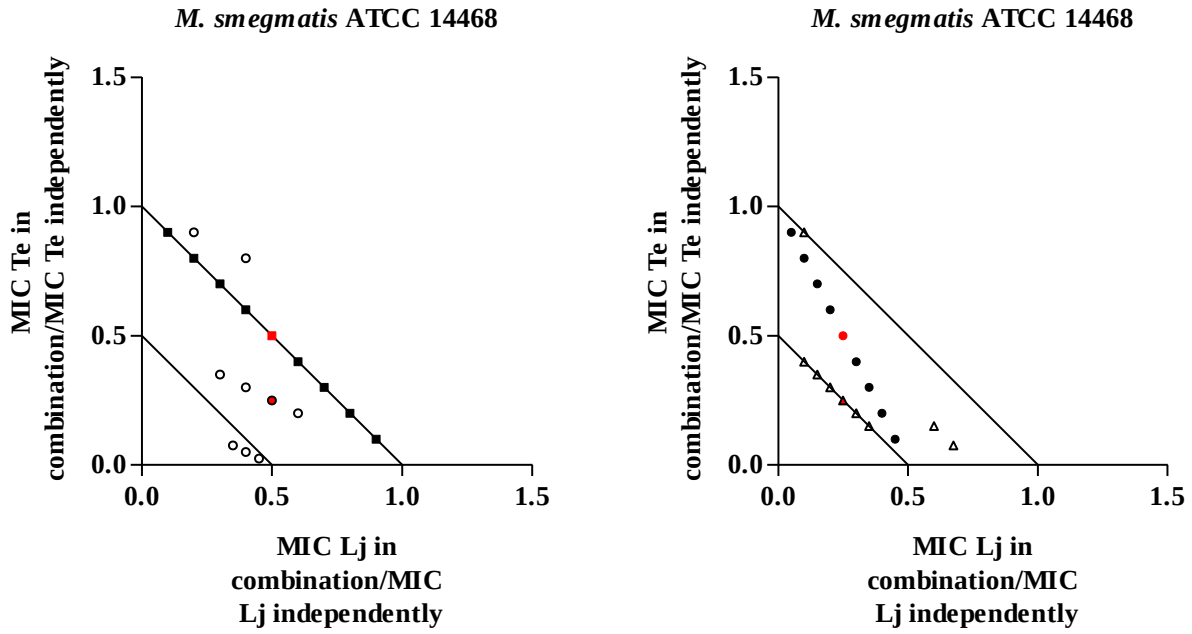


Fig 4.7 Isobologram representation of the combination of *L. javanica* aqueous extract with *T. emetica* aqueous extract (■); *L. javanica* EO with *T. emetica* aqueous extract (o); *L. javanica* organic extract with *T. emetica* organic extract (Δ) and *L. javanica* EO with *T. emetica* organic extract (●) against *M. smegmatis*. The 1:1 ratios are depicted in red.

Even though our FIC and isobologram results both depicted possible antimycobacterial activity (Table 4.2 and Figure 4.7), the interviewees used this remedy to relieve what seems like symptoms of the common cold (cough, fever, runny- or blocked nose) (Nester et al., 2001). Besides the antimicrobial activity of this combination, the physiological contribution of both these plant species towards this plant mixture's successful use as a respiratory ailment remedy is also a possibility. The essential oils inhaled from the vapours of aromatic plants such as *L. javanica* could relieve the discomfort of cold symptoms (Van Wyk and Wink, 2004). While *L. javanica* has been proven to have analgesic and decongestant properties, *T. emetica* has antipyretic properties (Sanogo et al., 2001). One interviewee specifically mentioned using this combination to treat, amongst others, fever, while the other interviewee suggested that this remedy was used to (besides other respiratory related symptoms) treat headaches and a blocked nose. These biological properties thus further validate the traditional use of these two plant species in combination. Thus, besides using the combination studies used in the current study, the traditional use of this remedy could also be supported by understanding the healing mechanism contributed by each plant species in this combination.

When further analysing the combinations of *L. javanica* with *E. grandis*, *E. grandis* with *O. obovata*, *S. cordatum* with *T. sericea* and *L. javanica* with *T. emetica*, the isobologram results of most of these combinations' 1:1 ratios correlated with their corresponding ∑FIC values (Table 4.1 and Table 4.2). While the majority of the 1:1 ratios displayed similar interaction when comparing isobologram results to ∑FIC results, for some combinations, the displayed interaction differed between these two methods. For example, the isobologram results for the 1:1 combination of the organic extracts of *S. cordatum* with *T. sericea* against both *K. pneumoniae* and *S. aureus* (Figure 4.6) did not correlate with the corresponding ∑FIC values (Table 4.1). During combination studies conducted by Suliman (2011) a similar phenomenon was found, where there was a difference, when comparing ∑FIC values with isobologram plots, in the displayed interaction of a 1:1 plant combination. Suliman (2011) suggested that this difference in results could be simply due to the fact that different methods of analysis had been used.

4.4 Summary

(a) The 1:1 combination of *L. javanica* essential oil with *Brachylaena* cf. *uniflora* organic extract displayed the lowest ∑FIC value of 0.18, against *C. neoformans*.

(b) Other combinations displaying synergism, include *E. grandis* essential oil with *O. obovata* organic extract (∑FIC value = 0.28 against *K. pneumoniae*). Also, *S. cordatum* with *Terminalia sericea* organic extracts (∑FIC value = 0.20 against *C. neoformans* and 0.31 against *K. pneumoniae*) and *L. javanica* with *Trichilia emetica* organic extracts (∑FIC value = 0.47 against *M. smegmatis*).

(c) For combinations containing aromatic plants, replacing these plants' extracts with their essential oils seem to enhance the antimicrobial interaction of many such plant combinations. These combinations include the 1:1 combination of *E. grandis* essential oil with *O.obovata* organic extract, the 1:1:1 combination of *L. javanica* and *H. kraussii* essential oils with *T. emetica* organic extract, the 1:1 combination of *L. javanica* essential oil with *Brachylaena* cf. *uniflora* organic extract, the 1:1 combination of *L. javanica* essential oil with *Hypoxis* cf. *acuminata* organic extract, the 1:1 combination of *L. javanica* essential oil with *C. brachiata* organic extract, the 1:1:1 combination of *L. javanica* and *T. riparia* essential oils with *A. glabratum* organic extract and the essential oil combination of *L. javanica* with *T. riparia*.

(d) Even though only two of the aqueous extract combinations displayed synergism, the majority of these combinations displayed additive interaction against at least one of the five test

organisms -lending credibility to the combined traditional use of some of the plant species in treating respiratory infections.

(e) When using the isobologram method, the 1:1 organic extract combination of *S. cordatum* with *T. sericea* displayed the strongest synergistic interaction against *C. neoformans*.

Chapter5

Summary and conclusions

5.1 Significance of the ethnobotanical survey

The first objective of this study was to perform an ethnobotanical survey, focusing on lay people's knowledge on plants used for treating respiratory infections in the study area of northern Maputaland. Although six of the 80 interviewees were healers, the current study focused on lay knowledge, which makes the information recorded during the current survey invaluable. During the ethnobotanical survey, 30 plant species were documented for their use in the treatment of respiratory infections by the interviewees. Six plant species (*A. glabratum*, *A. marlothii*, *K. mosambicina*, *O. obovata*, *P. capensis* subsp. *incohata* and *P. neochilus*) were recorded for the first time globally as medicinal plants used in the treatment of respiratory infections or related symptoms. Besides the use of single plant remedies, the preference was often to use plants of different species in combination. Twenty-five different plant combinations were documented. Compared to previous research, such complex combinations are usually prepared by healers, which makes current information novel. The use of these complex herbal mixtures by lay people brings an interesting new perspective on the way lay people use traditional remedies. Besides the use of these complex and novel plant remedies, for eight of the plant species used, new vernacular names were given by the interviewees. The finding of new vernacular plant names and plant uses in the current survey shows the importance of the documentation of such ethnobotanical knowledge.

Furthermore, this study explored how medicinal knowledge of lay people is used in their primary health care. Information obtained from the interviewees during the current survey has demonstrated that lay people are very knowledgeable about the use of traditional remedies for their primary health care. The ethnobotanical research conducted here thus contributes towards the quality of primary health of the people living in rural Maputaland.

5.2 Antimicrobial validation

The second objective of this study was to investigate *in vitro* antimicrobial activity of these herbal remedies against respiratory pathogens. The current antimicrobial study validates some of the plant remedies used to treat respiratory ailments in northern Maputaland. Two of the six medicinal plants mentioned for the first time to be used against respiratory ailments (*O. obovata* and *P. capensis* subsp. *incohata*) demonstrated strong MIC values (MIC values = 0.10 mg/mL and 0.03 mg/mL respectively). Eleven of the 35 aqueous extracts tested displayed noteworthy antimicrobial activity against at least one of the five test organisms (Chapter 3, Table 3.2). Antimicrobial activity of the plant organic extracts validated the antimicrobial efficacy of most single plant remedies tested (28 of the 35 extracts tested displayed noteworthy antimicrobial activity against at least one of the five test organisms, Chapter 3, Table 3.1).

Even though only two of the aqueous extract combinations showed synergism (*L. javanica* with *T. emetica*, ∑FIC value = 0.50; *S. cordatum* with *T. sericea*, ∑FIC value = 0.35, Chapter 4, Table 4.2), the majority of these combinations displayed additive interaction against at least one of the five test organisms. This lends credibility to the combined traditional use of some of the plant species in treating respiratory infections. While the traditional method of preparing these plant remedies (using water) was not supported for the use of most of the single plant remedies, this method was supported for the extraction of most combined remedies.

As demonstrated with the combination of *L. javanica* with *E. grandis*, other combinations that did not show strong antimicrobial interaction could display better interaction when combined in different ratios in stead. The importance of also testing the antimicrobial interaction of plant combinations in different combined ratios was demonstrated.

5.3 Correlation between traditional use and antimicrobial screening

Antimicrobial results (single and combined) could demonstrate that frequency of use do not necessarily always correlate with high antimicrobial efficacy. Although *L. javanica* (mentioned 58 times) was by far the most frequently used plant species, followed by *E. grandis* (mentioned 33 times), the most active aqueous extracts were that of the rarely mentioned *O. obovata* and *S. birrea* (MIC values = 0.10 mg/mL), while both *P. capensis* subsp. *incohata* (mentioned once) and *T. riparia* (mentioned ten times) had the most active organic extracts (MIC values = 0.03 mg/mL). While these less frequently used plant species displayed the lowest MIC values, the antimicrobial activities of *L. javanica* extracts proved overall to be mostly only moderate. In

addition, the rarely mentioned *S. birrea* (MIC values, aqueous extract, ranged from 0.10 - 2.00 mg/mL) and *S. cordatum* (MIC values, aqueous extract, ranged from 0.17 - 2.00 mg/mL) displayed the broadest antimicrobial activities. When looking at the combined plant remedies, the most frequently mentioned combination of *L. javanica* with *E. grandis* did not show any synergy, while the once mentioned combination of *E. grandis* with *O. obovata* displayed synergy against both *C. neoformans* (∑FIC value = 0.34) and *K. pneumoniae* (∑FIC value = 0.28). Also, while the aqueous extracts of the most frequently mentioned 1:1 combination of *L. javanica* with *E. grandis* were non-interactive against most test organisms, less frequently used ratios (where the percentage of *E. grandis* was higher) displayed additive antimicrobial interaction.

As the two most frequently used plant species (*L. javanica* and *E. grandis*) are both highly aromatic, it would seem that, in the study area, aromatic plant species are preferencial in the treatment of respiratory ailments. Less frequently used plant species showing the strongest- (*O. obovata*, *P. capensis* subsp. *incohata* and *S. birrea*), or the overall broadest antimicrobial activities (*S. birrea* and *S. cordatum*) were the non-aromatics, while the most frequently used species were aromatic, but often displayed only moderate antimicrobial activities. This could indicate that, in the case of plant use against respiratory infections, the focus is not so much on plants with the ability to heal an infection, but on plants that are able to relieve the symptoms associated with such infections i.e aromatic plants. Many of the aromatic plant containing remedies have in fact been applied through steaming. The essential oils of such aromatic plants can be absorbed through the mucous membranes of the respiratory tract, and generate a sensation of physical relief of some symptoms commonly associated with respiratory infections.

5.4 Limitations and future recommendations

Plant remedies that displayed antimicrobial activity during the current study may owe their effectiveness against respiratory ailments at least in part to their antimicrobial efficacy. Instead of displaying antimicrobial activity, many of the plant remedies studied here could exert beneficial effects to their users through their possible action on targeting sites associated with physiological processes. Botanicals used for respiratory diseases can be used as diaphoretics (to promote sweating and the release of toxins), expectorants (to expel phlegm), febrifuges and immune stimulants (to increase antibody production and stimulate white blood cells). Thus, plant remedies used in the current study should also be screened for these possible functions. Plants

with weak antimicrobial activities could also be used because of their physiological relief of respiratory symptoms due to their anti-inflammatory, antispasmodic or analgesic properties.

Some of these plant extracts displayed poor activity against the five test organisms used in this study. This could imply that these remedies are possibly active against other respiratory pathogens (including cold and flu viruses) not analysed here. More than 90% of respiratory tract disorders are caused by viruses (such as influenza A, B or C), while the most common bacterial causes of respiratory infections include *H. influenzae* and *S. pneumoniae*. These pathogens could not be analysed due to the availability of limited resources in the laboratory. During the current study, easily culturable organisms were used. Furthermore, *M. smegmatis* was used as a test organism in the initial screening of plants to determine their possible activity against TB. Even though testing against *M. smegmatis* can be a suitable preliminary test for anti-tuberculosis activity, this is not always the case, and further analysis of these plant remedies should include screening against *M. tuberculosis*. The endemic *P. capensis* subsp. *incohata* was the only plant species mentioned for its use to treat TB, and even though MIC results were not noteworthy against *M. smegmatis* this endemic plant in particular should be tested further for anti-TB activity against *M. tuberculosis*. In addition to *P. capensis* subsp. *incohata*, a plant remedy that has in fact shown promising results against *M. smegmatis* is the combination of *L. javanica* with *T. emetica* (∑FIC value, aqueous extract, = 0.50), and this mixed remedy should also be further analysed for possible synergy against *M. tuberculosis*. Although many of the pathogens tested during the current study are not common causes of respiratory infections, most of them are known to cause respiratory infections specifically in people with compromised immune systems.

These antimicrobial screenings should be followed by the identification and separate analysis of the active compounds. In the case of the mixed remedies, further combination studies should involve combining these plants' active compounds with each other, and analysing the antimicrobial interaction between such compounds. Essential oils, for example, can contain over 173 compounds in any given plant, and any of these compounds could interact to reduce or enhance pharmacological effects (Van Vuuren and Viljoen, 2011).

While this study supports the traditional use of some of these plant remedies, we cannot imply they are safe to use. Herbal remedies have been shown to sometimes have unfavourable effects, which could be caused by various elements, including the misidentification of plant species, incorrect dosages, and consumption of plants containing toxic compounds (Skalli et al., 2007).The use of herbal medicines can cause severe toxicity, and even death (Steenkamp et al.,

2000; Vahid et al., 2006; Malangu and Ogunbanjo, 2009). Herbal medicines are associated with a wide array of toxicities, including nephrotoxicity (kidneys), hepatotoxicity (liver), anaphylaxis and cardiac toxicity, to name a few (Vahid et al., 2006). It has been found that many child deaths in local hospitals have been due to a condition known as "herbal intoxication" (Steenkamp et al., 2000). Malangu and Ogunbanjo (2009) conducted a study on cases of acute poisoning in eight South African hospitals, of which four were situated in KwaZulu-Natal. From the 423 cases reviewed, 100 (2.4%) were due to traditional medicines, and the majority of those poisoned were female black Africans. Seeing as most people living in rural areas, including the current study area, depend on traditional medicine for their primary health care (Appidi, 2008), such claims of toxicity should cause for concern. Thus, even if most of the plant species used against respiratory diseases display strong antimicrobial activity against pathogens associated with respiratory infections, it is important that further studies should also involve toxicity tests. There have been some reports on the toxicity of the plant species reported during the current study (Appendix C), but for some plants no information could be found on their possible toxicity (*A. glabratum*, *B. cathartica*, *E. capensis*, *K. mosambicina*, *P. capensis* subsp. *incohata*, *P. neochilus*, *S. hyacinthoides*, *S. deltoideus*, *S. serratuloides*, *S. cordatum* and *T. riparia*). Some of the plant species used have in fact been reported to be either potentially toxic (*C. anisata*), or have dosage dependent toxicity (*C. brachiata*, *C. articulatus*, *L. javanica*), while deaths from the ingestion of other species (*E. tirucalli* and *S. puniceus*) have been reported. In the case of plant species with recorded toxicity, it is important to note though that not all parts of even the most poisonous plant are necessarily toxic. Toxicity levels in plants can also vary, depending on the growth season, locality, or the plant's age (Munday, 1988). It is thus important to harvest these medicinally used plants from the area where they are used, and to conduct toxicity tests on the specific plant organ used. Due to the fact that the oral, inhalation and dermal LD_{50} (the lethal dose for 50% of a certain group of animals) results may differ greatly, such tests should also be conducted, specifically focusing on the method of application (Van Wyk et al., 2002). This study only provided *in vitro* evidence of the antimicrobial potential of traditionally used plants and their combinations. Before recommending the use of the effective remedies in the treatment of respiratory ailments, toxicity tests should also be carried out on plant species with unknown toxicity.

Additional studies on plant remedies displaying strong *in vitro* antimicrobial properties should involve the investigation of such remedies in clinical studies. When weak activity has been demonstrated *in vitro*, it does not necessarily imply weak activity *in vivo*, and vice versa

(Ngemenya et al., 2006). In addition, herbal medicines are often taken in conjunction with conventional drugs, and health professionals should be made aware of the possible side effects caused by plant-drug interaction (Skalli et al., 2007). Studies involving the analysis of plant-drug interactions should thus also be conducted. While results obtained during the current study is an invaluable contribution toward the primary health care of the inhabitants of rural Maputaland, the *in vitro* scientific validation of these plant remedies is only an initial step toward validating the use of such remedies.

Finally, it is important to go back to these rural communities in northern Maputaland, and educate them on the scientific findings associated with these remedies. At this moment in time conducting such a visit would be premature, as the knowledge obtained by the *in vitro* studies here need to be supported by *in vivo*, toxicity and anti-inflammatory studies. Nevertheless, the information attained in this study remains invaluable as the first step in understanding the traditional healing practices of the lay people living in northern Maputaland.

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APPENDIX A

QUESTIONNAIRE

Date:_____ Questionnaire no:

Name of the interviewee:_____

Particulars of area:

GPS reading:_____

Name of area:_____

Name of Sub-location/Sub-area:_____

Name of Village (exact place):_____

Sociodemographic data:

Age:	
Gender:	
Educational background:	

Plant information: Plant number:____

Zulu name:_____

English name:_____

Botanical name:_____

Source:

Collected from wild	Cultivated at home	Both

Part(s) used:

Underground parts	Leaves	Bark	Fruit	Stem	Whole plant

Other:_____

Preparation method:

a.) Method(s) of administration:		
b.) Amount of plant material harvested for 1x usage:		
c.) Quantify this amount (e.g.: 1 Handful=1 Cup):		
d.) Is material used dry or fresh?		
e.) If dry material is used, is everything used, or only some? (Specify exact amount):		
f.) Preparation before taken (e.g.: Add 1cup of boiling water to handful of plant material)		

Dosage:

How much of the medicine (as mentioned at (e)) will be taken every time?

_____

How many times a day will this amount be taken? _____

If/when this medicine is given to babies or small children, is there a difference in dosage? If yes, describe the difference(s)? _____

_____

Symptoms:

Abdominal pain	Chest pain	Chills	Cough, dry	Cough, chronic	Ear ache	Ear, pus from-	Eyes, red
Fatigue	Fever	Headache	Muscles, aching	Neck, swollen	Night sweats	Nose, runny	Shortness of breath
Sinusitis	Sputum, yellow or greenish	Sputum, red and jelly-like	Sputum, rust coloured	Throat, sore	Throat, sores and redness	Tonsils, swollen & red	Weight loss

Any extra information about the disease and/or its symptoms:_____

_____

_____

Extra information:

Does anybody in this household smoke? _____

Are there any other uses for the plant? _____

_____

Where do you get your knowledge from about this plant? _____

Why do you choose to use traditional medicine, rather than going to a clinic or a hospital?

_____

_____

Is this plant used in combination with any other plant(s)? _____

Name the plant(s), and describe how this combination is prepared:_____

_____

_____

_____

Any other information:_____

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APPENDIX B

FORM OF CONSENT FOR USING ETHNOBOTANICAL INFORMATION

Researchers:

Dr. Helene de Wet, Prof. Sandy van Vuuren and Talita York

Institution:

University of Zululand - Department of Botany.

Research Project:

This project aims to document Ethnobotanical knowledge on plants which are used to treat respiratory infections in the Maputaland area. The data will be conducted using structured questionnaires. We will also collect plant material for identification and antimicrobial screening. This project is for academic purposes only and is of no commercial value. Results from this study will be presented at conferences and published in academic journals. The data will also be used towards the completion of a Masters Degree by the above mentioned student.

Please take note of the following:

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

Follow up visit:

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

Signature of interviewee:

_____

APPENDIX C

PREVIOUS REPORTS ON THE TOXICITY OF THE MEDICINAL PLANTS USED FOR THE TREATMENT OF RESPIRATORY INFECTIONS IN RURAL MAPUTALAND

Plant species	Previous reports on toxicity	References
A. glabratum	None found	
A. marlothii	Using *A. marlothii* as snuff may be hazardous, as it was reported to contain carcinogens	Watt and Breyer-Brandwijk, 1962, Grace et al., 2008
	Leaf extracts may cause haemolysis (the abnormal breakdown of red blood cells)	Hutchings et al., 1996
B. discolor	No or very low toxicity scores with murine C2C12 myoblasts, preadipocytes and human chang liver cells	Van de Venter et al., 2008
B. cathartica	None found	
C. limon	None found on *C. limon* leaves. The fruit is edible	Van Wyk and Wink, 2004
C. anisata	Brine shrimp test showed that *C. anisata* has potential for toxicity. (ethanol and dichloromethane extracts)	Moshi et al., 2010
C. brachiata.	Effects of aqueous extracts on male Wistar rats showed that it is not completely safe at certain doses. It can affect liver and kidney function	Afolayan et al., 2009

Plant species	Previous reports on toxicity	References
C. molle	*Combretum molle* methanolic or aqueous leaf extracts displayed low levels of cytotoxicity against K562S human monocyte cell lines	Gansané et al., 2010
C. articulatus.	In brine shrimp microwell cytotoxicity tests, dichloromethane and ethanolic extracts showed strong concentration related toxicity	Desmarchelier et al., 1996
E. capensis	None found	
E. caffra	The seeds of many *Erythrina* species contain toxic alkaloids, and *E. caffra* seeds are likely to contain toxic proteins	Munday, 1988; Van Wyk et al., 2002
E. grandis	All *Eucalyptus* species yield an essential oil that is poisonous	Watt and Breyer-Brandwijk, 1962; Munday, 1988
E. tirucalli	Irritant and tumour promoting compounds have been extracted	Chhabra et al., 1990
	Cases of human deaths due to consumption of *E. tirucalli* have been reported	Neuwinger, 1996
	No mortalities were observed in Wistar rats consuming the biopolymeric fraction from *E. tirucalli*	Bani et al., 2007
H. kraussii	Transformed human kidney epithelial cells, MCF-7 breast adenocarcinoma and SF-268 glioblastoma cells were used to determine toxicity of 1:1 chloroform:methanol extracts. Extracts were found to be non-toxic	Lourens et al., 2011
Hypoxis spp.	Recent tests on rats have suggested that *H. hemerocallidea* cause impairment in kidney function. Other studies have shown that aqueous corm extracts may cause brief hypotention and bradychardia in guinea-pigs and rats	Owira and Ojewole, 2009.
K. mosambicina	None found	
L. javanica	The leaf aqueous extracts have been tested for toxicity in BALB/c mice. It was concluded that it might be detrimental to human health if taken at very high doses	Madzimure et al., 2011

Plant species	Previous reports on toxicity	References
O. obovata	Various plant parts, including the leaves, give dermatitis upon touch. The leaves have been reported to be lethal to humans	Verzár and Petri, 1987
P. capensis subsp. *incohata*	None found	
P. neochilus	None found	
P. guajava	Acute toxicology tests in rats have found *P. guajava* leaf extracts to be non-toxic, while *in vitro* genotoxicity and mutagenicity tests on human peripheral blood lymphocytes found no disturbances in cell division	Gutiérrez et al., 2008
S. hyacinthoides	None found	
S. puniceus	Recorded human deaths due to ingestion of the bulbs	Watt and Breyer-Brandwijk, 1962
	Poisoning from *S. puniceus* bulbs or leaves cause symptoms such as dizziness, visual disturbances or depression and are potentially fatal if taken in large quantities	Veale et al., 1992
S. birrea	*Sclerocarya birrea* stem bark aqueous and methanolic extracts were tested on mice, and LD50 values suggested these extracts to be relatively safe. In a brine shrimp test, no toxic effects were evident for hexane, methanol and aqueous extracts	Ojewole et al., 2010
	Dichloromethane:methanol (1:1) extracts showed *in vitro* toxicity with tests done in Chang liver, 3T3-L1 adipose and C2C12 muscle cells	Van de Venter et al., 2008
S. deltoideus	None found on *S. deltoideus*. *Senecio* alkaloids are highly toxic liver poisons	Van Wyk et al., 2009.
S. serratuloides	None found on *S. serratuloides*	
S. cordatum	None found	

Plant species	Previous reports on toxicity	References
T. sericea	In the brine shrimp assay *T. sericea* acetone extracts were relatively non-toxic	Eloff et al., 2008
T. riparia	None found	
T. emetica	The toxicity of leaf and stem bark extracts were studied on lymphocytes using the lymphoproliferation assay and results showed that the methanolic leaf extracts are very toxic. The dichloromethane extract of the stem bark was found to be highly toxic to monkey kidney cells. Leaf aqueous and diethyl ether extracts did not show cytotoxicity in the brine shrimp assay	Komane et al., 2011