ANTIBACTERIAL ACTIVITIES OF BOTH COMBINED AND INDIVIDUAL MEDICINAL PLANTS EXTRACTS TRADITIONALLY USED TO TREAT PNEUMONIA

By

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DECLARATION

I, Immaculate Mhango, s214305864, I hereby declare that this dissertation is my own work. As required by research rules and conduct, I also declare that I have fully cited and referenced all material and results that are not original of this work. It is being submitted for the fulfilment for the Degree Master of Biomedical Technology at the University of Nelson Mandela Metropolitan University. It has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

Signature: 

Date: 16th March 2017

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ABSTRACT

Pneumonia is one of the five major leading causes of death in children under-fives years and the elderly worldwide. Antibiotics used for its treatment are less potent due to bacteria development of bacteria resistant to antibiotics. This has led to a surge in search of novel drugs. There are already some drugs in clinical use that have natural products and derivatives such as quinine, morphine, vincristine, and taxol among others. The healing value of medicinal plants has been well accepted since Stone Age across the globe. This plant therapy has been prescribed and prepared independently or in combination. The following plants: *Terminalia sericea, Warburgia salutaris, Dodonea angustofolia, Eucalyptus camaldulensis, Ballota africana, Kigelia africana* and *Acorus gramineus*. These plants are most commonly used for treatment of pneumonia and other ailment, were studied to validate their antimicrobial activity based on scientific determination.

The primary aim of this study was to evaluate the efficacy of these plants against bacteria pneumonia pathogens. Seven medicinal plants, independently and in combinations were relatively analysed for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae.* Ground plant material of roots, bark and leafs were prepared with acetone, ethanol and distilled water. Dimethyl sulfoxide (10 &100%) was used as a reconstitution solvent and ciprofloxacin (10 %) as a positive control. The antimicrobial efficacy was determined using agar well diffusion and microtiter plate methods. Interaction between plants was evaluated by calculating fraction inhibitory concentration index (∑FIC). Noteworthy activity for individual studies with all test organisms was observed with *T. sericea.* However, highest ZOI (30 mm) was observed for *B*. *africana* ethanol extract for *S. pyogenes*. Weak microbial activity was noted in *W. salutaris* and *D. angustofolia* extracts with all test organisms.

Good antimicrobial activity was observed in combination studies with all organisms. The potency of different plant combinations varied with highest ZOI observed with *B. africana* and *W. salutaris* ranging from 33-35 mm, conversely ZOI of 35 mm was also noted for *S.* aureus in *B. africana* and *E. camaldulensis* ethanol extract. Noteworthy antimicrobial activity was observed in *T. sericea* and *D. angustofolia* against all test pathogens. weak antimicrobial activity with highest MICs was observed in combinations where *W. salutaris*

was involved. After calculating ∑FICs, strongest synergistic effect was displayed for *W. salutaris* and *D. angustofolia* against all test organisms (lowest ∑FICs 0.0491). Most plant extract combinations, displayed either synergistic, additive or indifferent effect, with few demonstrating antagonistic interactions. Significant antagonism effect was noted for *S. pyogenes* with T. sericea ethanol extract ∑FIC value of 15.51.

Based on results of this study use of plants in combination increase antimicrobial efficacy. The antimicrobial activities; synergistic and additive effects observed adds credibility in the use of plant combination for therapeutic value in treatment of pneumonia. Future studies are recommended to identify and isolate specific active compounds involved in plant combination interactions. The importance of combination studies for possible development of new antimicrobials that can succumb bacterial resistance need to be highlighted.

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LIST OF ABBREVIATIONS

ACIP – Advisory committee in immunization practices
AIDS - Acquired immunodeficiency syndrome
ANOVA- Analysis of Variance
ATCC - American Type Culture Collection
BAL – Broncho alveolar lavage
BOOP – Bronchiolitis obliterans organizing pneumonia
BPM – Beats per minute
C – Carbon
CFU - Colony Forming Units
CNS – Central nervous system
CT – Computed tomography
DMSO - Dimethyl Sulfoxide
DNA- Deoxyribonucleic acid
TMP – Thymidine monophosphate
FIC - Fractional Inhibitory Concentration
H1N1 – Influenzae A virus/ swine flu
Hib - *Haemophilus influenzae* type b
HIV - Human immunodeficiency virus
HPLC High - performance liquid chromatography
HPTLC High - performance thin-layer chromatography
INT - *p*-iodonitrotetrazolium-chloride/violet
KHz – Kilohertz
Mg - Milligram
MH Mueller - Hinton agar
MHB Muelle r- Hinton broth
MIC - Minimum Inhibitory Concentration
Min - Minutes
Ml - Millilitre
mm - Millimetre
mRNA - Messenger ribonucleic acid
MRSA - Methicillin Resistant *Staphylococcus aureus*
NMMU - Nelson Mandela Metropolitan University
PABA - Para-aminobenzoic acid
PBP - Penicillin binding protein
PCV13 – Pneumococcal conjugate vaccine
PGE_{2-} Prostaglandin E2
PH - Power of hydrogen
PPSV23 – Pneumococcal polysaccharide vaccine

RNA - Ribonucleic acid
RSV – Respiratory syncytial virus
Spp – Species
THF - Tetrahydrofolic acid
tRNA - Transfer RNA
TSB – Trypticase soy broth
UNICEF - United Nations Children's Fund
UV - Ultra Violet
WBC - White blood cell
WBC/µL - White blood cell per microliter
WHO - World Health Organisation
ZOI – Zone of inhibition
B – Beta
µL - Microliter
µl- Micro-litre
µm – Micrometre

CHAPTER ONE

INTRODUCTION

1.1 Background

Many prescription drugs, are derived from natural products and their compounds. The use and discovery of medicinal plants have progressed for years with a close analysis on their special properties. Medicinal plants have more than one chemical compound, and advanced research has been conducted on some of them, to extract individual components and identify the unique properties they contain (Fawzi, 2013). More than two thirds of the anticancer drugs approved between the 1940s and 2006 are either natural products, or were produced based on the knowledge obtained from natural products (Newman & Cragg, 2006). Other familiar examples of plant derived medicines include quinine, morphine, codeine, colchicine, atropine, reserpine, digoxin, taxol and vincristine (B E Van Wyk & Wink, 2004). Compounds like alkaloids, poly phenols, glycosides, terpenes, terpenoids, oils and many more have specific roles in living organisms.

Currently antimicrobial agents are globally one of the most valued therapeutic agents used in treatment of infectious diseases. Van Vuuren (2010) in his PhD thesis explained that the treatment of infectious diseases focuses on cure rather than easing the symptoms or only on pharmacological management of the disease. Plants can produce exceedingly hundred thousand molecules; not all of them have bacterial potential, but some have significant activity against pathogens. The magnitude of natural resources is not the same as existing antimicrobials but stills provides some extent of hope (Khurram et al., 2009). This has led to an increase in the search of such antimicrobials in recent years. This increase can be observed in the number of articles published on the antimicrobial activities of medicinal plants during 1966 – 1994 which amount to 115, while the number of articles on the same subject appearing during 1995 – 2004 period are 307; that is more than a two fold increase in just one decade, showing the growth of Interest in the search of antimicrobials of natural origin (Khurram et al., 2009; Ríos & Recio, 2005).

Substantial evidence from recent studies highlight increased resistance to commonly used antibiotics and informs us that our protection against these organisms is running out at an increased rate. In order to overcome this challenge there is a need to develop new antimicrobials, which requires continued research on indigenous plants. Relying on natural products such as plants and natural substances can therefore be good alternatives. If these compounds are not potent as conventional antimicrobials they can be used as adjuncts, enhancing mechanism of their therapeutic effectiveness (Khurram et al., 2009). Herbalist, prescribe the use of singular plant and at times a combination, in attempts to improve potency for treatment of respiratory infection. During the 20th century, attempts were focused on one-target, the one drug which was key to finding a single chemical entity being able to inhibit a distinct molecular target (Keith, Borisy, & Stockwell, 2005). This led to the isolation of some successful single molecular compounds from various plant species for example, Artemisinin from *Artemisia annua L*. and quinine from *Chinchona spp*. (Talika, 2012).

In order to reduce the emergence of antibiotic resistance, strict infection control measurements as well as logical and appropriate use of antibiotics have been established (Tseng, Ke, & Chang, 2014). Many studies have been conducted that emphasize the use of drug combinations to reduce resistance, toxicity and side effects, and at the same time maximize therapeutic advantage with high efficacy and bioavailability. Moreover, many synthetic drugs available in pharmacies, and administered to patients in hospitals possess a variety of chemical compounds that have useful biological functions. The use of combination therapy is based on the knowledge that many diseases have multi-casual aetiology and a complex pathophysiology. The complexity of treating multi- drug resistance infections has led to an enormous search for novel and effective antibiotics, especially structures/compounds originating from natural products (Wagner & Ulrich-merzenich, 2009). Compound and molecule identification through phytochemical analysis and microbial activities has played an important role as lead components for the development of new antimicrobials (Drewes, 2012; Frum & Viljoen, 2006). Studies have shown that the plant extracts in combination of two or more are exhibiting effective

antimicrobial activity against a wide range of microorganisms including drug resistant bacteria (Prakash et al., 2006).

According to the World Health Organisation (WHO) (2003), 80% of the total population in some Asian and African countries presently use herbal medicine for some aspects of primary health care. New strategies are being implemented in botany, information systems, regulations, chemistry, and clinical trials which are present to ensure that traditional medicine is improved globally. Pneumonia is one of the leading causes of death in developing countries especially in children and older people with debilitating conditions. Bacterial infections contribute disproportionately to pneumonia mortality in developing countries. Although bacterial infections account for not more than 50% of cases of pneumonia, they cause nearly 70% of deaths that are caused by pneumonia (WHO & UNICEF, 2009). Resistance of microorganisms to antibiotics that are used to treat pneumonia has been reported to make it difficult to treat pneumonia.

1.2 Pneumonia Prevalence

Globally, lung infections and gastrointestinal infections are responsible for most death in developing countries**.** Respiratory diseases are regarded as a common cause of death in developed nations, accounting for about 14% of deaths in both sex (Cross & Underwood, 2013). Pneumonia is an opportunistic disease for HIV/AIDS patients and individuals with other compromised health conditions. Pneumonia is regarded as a common cause of death overall, and the most common fatal hospital acquired infection. “Pneumonia remains the leading infectious cause of death among children under five, killing 2,500 children a day” (Campbell et al., 2013).

In 2008, pneumonia manifested itself in approximately 156 million children, where 151 were from developing countries and 5 million from developed countries. In 2015, pneumonia accounted for 16 % of deaths of children under five years, killing 920 136 children (WHO, 2016). In developing countries it still remains the leading cause of death with rates being highest in children under five, adults above 75 years and the chronically ill (Kabra, Lodha, & Pandey, 2010). In 2010, pneumonia resulted in 1.3 million deaths worldwide, of which 18% of all deaths were of children under five years,

and 95% of this occurred in developing countries (L. Liu et al., 2012). It is estimated that pneumonia affects 450 million people per year globally, 7% of population, and results in about 4 million deaths (Ruuskanen, Lahti, Jennings, & Murdoch, 2011). Children in developing countries are nearly 18 times more likely to die before the age of five than children in high-income countries due to pneumonia and other acute infections. Pneumonia is the single largest contribution to child mortality, accounting close to 28-34% of all under five globally and responsible for death of more than 2 million children under five annually (Tong, 2013).

Pneumonia affects children and families all over, but is widespread in South Asia and sub-Saharan Africa (WHO, 2016). The larger part of death of children mortality caused by pneumonia affect the poor due to high exposure rates to risk factors associated with developing acute respiratory infections such as overcrowding, poor environmental conditions, malnutrition as well as limited access to curative health sciences. Preventive measures to reduce pneumonia are available, which aim at reducing indoor air pollution, promoting adequate nutrition and increasing immunisation rates with vaccines that prevent children and adults >65yrs from developing infections that cause pneumonia (Ronald, 2005; WHO & UNICEF, 2009).

1.3 Aim and specific objectives of the study

The main aim of the study is to evaluate antimicrobial activity of the following medicinal plants *Kigelia Africana, Ballota Africana, Dudonea Angustofolia, Warbugia Salutaris, Termialia Sericea, Acorus Calamus, and Eucalyptus camldulensis* by testing the activity of individual plant extract preparations against microbial isolates that cause pneumonia.

1.3.1 The specific objectives of this study:

1. To collect and to identify the medicinal plants for the study.
2. To prepare and extract the plants materials separately and in some combined preparations using a variety of solvent, like distilled water, ethanol, dimethyl ether and acetone.
3. To test the antimicrobial sensitivity of the plant extracts and combined preparations by agar well diffusion.

4. To perform interactive plant combination studies to determine the efficacy when plants are used together.
5. To determine the minimum inhibition concentration (MIC) of both combined and individual plant extracts against bacteria isolates.
6. To determine the fractional inhibitory concentration index (∑FIC) of the plant extracts in the combined preparations.

1.4 Significance of the research

Medicinal plants can be used as alternatives for antibiotics and chemotherapeutic agents in certain circumstances. Many infections are difficult to treat because of resistance that emerge in the organisms against the antibiotics. One way of overcoming this problem is discovering new compounds to use that are not based on existing synthetic antimicrobial agents and those that are viable and exhibiting no resistance. Combinations studies of plant extracts have been included in this study to help in discovering new alternatives that can be used to overcome drug resistance development to known antibiotics. For example vaccine for *Streptococcus pneumoniae* and influenza infections have already been discovered and are administered in hospitals (Ashby et al., 2012). Considering that disease-causing agents may develop resistance if a single plant is used for the treatment of specific disease, the use of plant in combinations is preferred, since there is minimal chance of developing resistance and can be used for long time.

Synergistic or polyvalent effect from interactions of natural compounds will be useful in discovering new chemical constituents that can be used for developing novel antimicrobials hence overcoming the surge of resistance. The use of medicinal plants in combinations has an advantage of having their polyphenols that act as antioxidant and free radical scavengers that remove harmful metabolite and toxins from the body hence boosting the immunity. Although people from rural areas, and some traditional healers, believe that medicinal plants are more effective in treating infectious conditions than synthetic antibiotics, it is still very important to evaluate with scientific experiments. Another significance of medicinal plant is that it can cure more than one ailment, as seen in literatures, where one plant has numerous medicinal benefits,

hence advantageous over synthetic drugs. Fresh and dry parts of studied plants can be used for preparation of herbal drugs, herbal processed products and traditional herbal drugs. Availability and cost effectiveness of medicinal plants can benefit less privileged people and those living in remote areas far from health facilities (Mohlakoana, 2010; Suliman, 2011). This study will help scientists to validate the usage of these plants.

CHAPTER TWO
LITERATURE REVIEW

2.1 Pneumonia

Globally, pneumonia is a number one killer disease affecting children under the age of five years. Out of an estimated 9 million child deaths as resorted in 2007, about 20% or 1.8 million deaths were due to pneumonia. WHO (2015) and UNICEF (2009) reported that child mortality due to pneumonia is as a result of malnutrition, poverty and inadequate access to the health care. The burden that pneumonia impose on families and the health system in developing countries aggravate unfairness completely exposing children who are poor, hungry and living in remote areas.

Pneumonia infection has different pathogenic causes which results in unpredictable and varying antibiotic treatment selections. These pathogens have developed resistance to antibiotics such as the B-lactams, macrolides, vancomycin and fluroquilones. Global prevalence of drug resistance streptococcal pneumoniae has increased. This has resulted in the establishment of risk factors in individuals who are on B–lactams treatment within the previous three months. The highly affected individuals include; the alcoholics, those with simultaneous presence of two chronic or immunosuppressive diseases. Resistance displayed by methicillin resistance *Staphylococcus aureus* is also a major concern leading to massive search of medicinal plant for possible antimicrobial effect (Kamanga, 2013).

Pneumonia is a lung infection that is caused by either bacteria, viruses or fungi. Indeed pneumonia infection is mainly characterized by inflammation of the lungs, primarily the alveoli (Cross & Underwood, 2013; Reid, Roberts, & MacDuff, 2011). It arises when from weak immune system, pre-existing illness and when the body fails to filter out microorganisms from the air that we breathe in. Pneumonia refers to pneumonitis (lung inflammation) that is usually due to either infection or non-infectious, that has additional feature of pulmonary consolidation (Ashby et al., 2012). There are a number of ways in which pneumonia has been discussed; Stegman and Branger (2005) describe pneumonia as inflammation of the lung parenchyma characterized by consolidation of the affected part with the alveolar air spaces being

filled with exudates and inflammatory cells fibrin. Underwood (2013) stipulated that pneumonia is usually due to an infection affecting distal airways and alveoli with the formation of an inflammatory exudate.

Depending on how and where it was acquired, pneumonia can be classified as either community acquired-aspirations, headache associated, hospital acquired or ventilator-associated pneumonia. Pneumonia can also be classified based on the affected area of the lung namely; lobar pneumonia, bronchial pneumonia, and acute interstitial pneumonia, clinical circumstances and causative agents (McLukcie, 2009). From Underwood (2013), pneumonia can be classified into four major pathological classes.

2.1.1 Classification of Pneumonia

2.1.1.1 Bronchopneumonia

Bronchopneumonia is commonly manifested at old age, infancy and on patients with debilitating diseases such as cancer, chronic renal failure, cardiac failure, or cerebrovascular accidents (Reid et al., 2011). It also occurs as a manifestation of secondary infection in viral conditions like influenza and measles. Moreover, bronchopneumonia can co-exist with other respiratory tract infections in patients with acute bronchitis, chronic obstructive airways diseases or cystic fibrosis (Underwood, 2013). In other occasions when there is failure in cleaning the respiratory secretions, as in post- operative period, it creates a liability to the development of bronchopneumonia. Bacteria known to cause bronchopneumonia include *Staphylococcus aureus, Streptococci Pyogenes* and *Haemophilus influenza* (Cheesebrough, 2006).

Bronchopneumonia has a distinguishing patchy distribution, centred on inflamed bronchioles and bronchi with succeeding spread to surrounding alveoli (Cross & Underwood, 2013; Kamanga, 2013). Areas of the affected lung tend to be bottom lateral and bilateral, and appear focally grey or red at post-mortem with filled bronchi and histological diagnosis. The affected lung also shows atypical acute inflammation with exudation in the bronchi and adjacent alveolar spaces. There is a chance of an

inflammation resolution when antibiotic and physiotherapy is administered (Cross & Underwood, 2013; Reid et al., 2011).

2.1.1.2 Lobar pneumonia

Lobar pneumonia is relatively unfamiliar in infancy and old ages, but normally pneumococcal pneumonia affects healthy adults in between 20-50 years of age. However, lobar pneumonia caused by *Klebsiella* quintessentially affects the elderly, diabetics or alcoholics (Cross & Underwood, 2009, 2013). 90 % of lobar pneumonia is due to *Streptococcus Pneumoniae* (pneumococci). Pneumococci may spread from the lungs into the pleural cavity or pericardium and cause abscesses (Kamanga, 2013). The infection of the pleural space (known as pleurisy) is very painful with striking changes that occur in the alveoli where pleural exudate is common (O'Grady, Torzillo, Frawley, & Chang, 2014). Lobar pneumonia is characterized by diffuse inflammation that affects an entire lobe or even two lobes of the affected lung (Cross & Underwood, 2013).

2.1.2 Clinical presentation

The patient shows symptoms of high fever that can be over 40 ^{0}C, cough and production of sputum. The sputum appears purulent and sometimes with flecks of blood referred as rusty sputum (Cross & Underwood, 2013). The chest signs range from dullness to percussions with bronchial breathing, as the lung becomes consolidated.

2.1.3 Pathogenesis

The pathology of lobar pneumonia undergoes sequential combined phases in which the alveoli in the lobe are evenly affected (Reid et al., 2011). The pathological phases of lobar pneumonia can be summarized as follows:

Phase 1: Congestion: This can last for 24 hours and represents the outpouring of protein rich exudate into alveolar spaces, with venous congestion. Gross appearance of the lung is heavy, oedematous and red in colour.

Phase 2: Red hepatisation: This usually lasts for 2 to 5 days. At this phase, there is massive accumulation of polymorphs, lymphocytes and macrophages in the alveolar

spaces. The red cells are also extraverted from the distended capillaries. The overlying pleura bear a fibrinous exudate where the lung is red, solid and airless with consistency resembling liver.

Phase 3: Grey hepatisation: This phase last a few days (4 to 8 days) and it constitutes further accumulation of fibrin with destruction of white and red blood cells. The lung is now grey brown and solid.

Phase 4: Resolution: This takes place at about 8 to 10 days in untreated cases and represents the resorption of exudate and enzymatic digestion on inflammatory debris, with preservation of underlying alveolus wall architecture (Cross & Underwood, 2013; Reid et al., 2011).

2.1.4 Atypical pneumonia

Other organisms rather than well-known organisms cause atypical pneumonia. It is sub-classified according to the host, thus either immunocompromised, suppressed or non-immunosuppressed hosts (Denis & Et-al, 2014; Reid et al., 2011).

In non-immunosuppressed host, pneumonia may be due to viruses like influenza, RSV, adenovirus and mycoplasma (Augenbraun, 2014; Cross & Underwood, 2013). The clinical cause of viral pneumonia depends on the severity and extent of the diseases. In fatal cases, the lungs appear heavy, red and consolidated in adult respiratory syndrome. Mycoplasma pneumonia is caused by *Mycoplasma pneumoniae* and often affects individuals less than 40 years of age. It usually causes low grade pneumonia with interstitial inflammation and less exudation (Cross & Underwood, 2013; Kamangar & Harrington, 2015).

In non-immunosuppressed host, pneumonia can be caused by bacillus, *Legionella pneumophilia* that result in legionnaires diseases. It mostly appears in middle-aged and older adults, smokers and those with chronic illness and weak immune system. The condition worsens within the first 4 to 6 days and reach resolution next 4 to 5 days, but it takes time for symptoms to completely go away (Denis & Et-al, 2014). Patient infected by legionnaires diseases, may be previously well, although a proportion have underlying chronic illness like heart failure or carcinoma. Symptoms

include cough, dyspnoea and chest pains with systematic features like headache, confusion, nausea, vomiting and diarrhoea (Cross & Underwood, 2013).

Immunosuppressed host may be correlative, as it occurs in patients at the extreme age, diabetics, malnourished, those on high dose steroid therapy, undergoing chemotherapy for malignancy, immunosuppression for transplantation and those with HIV/AIDS infection (Cross & Underwood, 2013; Denis & Et-al, 2014; Kamangar & Harrington, 2015). Patients with immunosuppression are prone to opportunistic non-pathogenic infections and they present clinical features such as onset fever, shortness of breath, cough and pulmonary infiltrates (Augenbraun, 2014). Common opportunistic agents include *pneumocystis jirovecii*, fungi (*candida* and *aspergillus*); viruses (cytomegaloviruses, herpes simplex and varicella zoster) and HIV lung disease (Cross & Underwood, 2013; Reid et al., 2011).

2.1.5 Non- infective pneumonias

Non-infective pneumonias are described as occurrence of non-contagious pneumonia. Below is brief information on some causes and clinical features of non-infective pneumonias:

a) Cryptogenic organizing pneumonia is the idiopathic form of organizing pneumonia (formerly called bronchiolitis obliterans organizing pneumonia (BOOP)). This is a type of diffuse interstitial lung disease that affects the distal bronchioles, respiratory bronchioles, alveolar ducts, and alveolar walls (Cross & Underwood, 2013; Kamangar & Harrington, 2015). The primary area of injury is within the alveolar wall. This clinical syndrome is characterized by mild systemic upset with possible cough, low-grade fever and breathlessness. Radiology diagnostic shows evidence of focal lung consolidation (Cross & Underwood, 2009, 2013).
b) Aspiration pneumonia also known as anaerobic pneumonia is an inflammation of the lung and bronchial tubes that occur when food, vomitus or fluid is aspirated into the lung, resulting in secondary inflammation and consolidation (Dock, Boskey, & Brian Wu, 2015).

c) Lipid pneumonia is a lung inflammation that develops when vacuoles of lipid are ingested by foreign-body giant cells that might result in interstitial fibrosis. Lipid pneumonia may be endogenous, associated with airway obstruction causing distal collections of a foamy macrophages and giant cells, alternatively lipid pneumonia may be exogenous as pertaining to aspiration of materials containing lipid content like paraffin or oily node drops (Cross & Underwood, 2013).

d) Eosinophilic pneumonia which is also referred as Loffler's syndrome is characterised by numerous eosinophils in the interstitium and alveoli (Cross & Underwood, 2009). Acute eosinophilic pneumonia is usually idiopathic and is associated with blood eosinophilia. The lung shows extensive infiltration with eosinophils and presence of organizing exudates which may go on to give rise to finding fibrosis (Cross & Underwood, 2013; Reid et al., 2011).

2.1.6 Pneumonia diagnosis

Different tests and procedures are performed to come up with the correct diagnosis so that proper management and treatment is provided. The following methods and tests may not be useful for diagnostic purposes but are useful for classifying illness severity and site-of-care or admission decisions: laboratory tests, trans-tracheal aspiration, chest radiotherapy, chest CT scanning, chest ultrasonography, bronchoscopy with BAL thoracentesis and other specific test (Kamangar & Harrington, 2015)

2.1.6.1 Laboratory analysis

In cases where culturing blood is considered, blood samples should be obtained before the administration of antibiotics. These cultures require a minimum of 24 hours to incubate and grow. When blood cultures are positive, they correlate well with the microbiologic agent causing the pneumonia. Unfortunately, blood cultures show poor sensitivity in pneumonia where findings are positive in approximately 40% of reported cases (Kamangar & Harrington, 2015). Even in pneumococcal pneumonia, the results are often negative. Their yield may be higher in patients with severe pneumonia infection. These findings probably have minimal clinical effect in treating bacterial pneumonia since the use of blood cultures only rarely dictates a change in empiric

antibiotics. Cell blood count analysis may show leukopenia <4000 white blood cell count per microliter or leucocytosis >12000 wbc/ul, which may be an inauspicious clinical sign of impeding sepsis (Kamanga, 2013). The white blood cell (WBC) count should be more than 25 per low-power field in non-immunosuppressed patients, and there is presence of neutrophils in patient's sputum smear (Reid et al., 2011). Other laboratory test includes biochemistries such as C-reactive protein, urea, electrolytes, liver function test and pulse oximetry (Kamanga, 2013; Kamangar & Harrington, 2015). Capsular serotyping is used by doing Quelling reaction in detecting streptococcus pneumoniae (Cheesbrough, 2006).

2.1.7 General Signs and Symptoms of Pneumonia

The following are the signs and symptoms exhibited by patients suffering from pneumonia:

- Cough up mucus from the lungs, rusty or tinged with blood
- Fever typically >38.8 ^{0}C
- Difficulty in breathing
- Shaking and teeth chattering chills
- Severe chest pains that worsens when coughing or breathe in
- Fast heart beat
- Cough which result in tachypnea (>18 respiration/min) and tachycardia >100 bpm or bradycardia <60 bpm.
- Tiredness and weakness
- Central cyanosis
- Nausea, vomiting and diarrhoea
- Children may experience headache, loss of appetite and may develop wheezing.
- Young infants may suffer convulsions, unconsciousness, hypothermia < 35°C, lethargy and feeding problems.
- In adult 70 year or older they have altered mental status with no recognized cause (Cross & Underwood, 2013; Kamangar & Harrington, 2015; Reid et al., 2011; WHO, 2010).

Symptoms caused by bacteria and viruses are often the same, but viral symptoms are gradual and not severe.

In general, the organisms causing pneumonia include:

- Bacteria: *Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pyogenes, Neisseria meningitidis, Moraxella catarrhis, Legionella pneumophilia, Mycoplasma pneumoniae* and *Chlamydophilia pneumoniae*.
- Viruses: Respiratory syncytial virus*, rhinovirus,* herpes simplex *and* severe acute respiratory syndrome.
- Fungi*: Pneumocystis jirovecii*
- And various chemicals (Cheesebrough, 2006; Cross & Underwood, 2013):

Physical findings in bacterial pneumonia may include the following:

- Adventitious breath sounds, such as rales/crackles, rhonchi, or wheezes
- Decreased intensity of breath sounds
- Egophony
- Whispering pectoriloquy
- Dullness to percussion
- Tracheal deviation
- Lymphadenopathy
- Pleural friction rub (Kamangar & Harrington, 2015).

Examination findings that may indicate a specific aetiology for consideration are as follows:

- ✓ Bradycardia may indicate a *Legionella* aetiology.
- ✓ Periodontal disease may suggest an anaerobic and/or polymicrobial infection.
- ✓ Bullous myringitis may indicate *Mycoplasma pneumoniae* infection.
- ✓ Physical evidence of risk for aspiration may include a decreased gag reflex.

- ✓ Cutaneous nodules, especially in the setting of central nervous system (CNS) findings may suggestion *Nocardia* infection (Kamangar & et al, 2015).

2.1.8 Treatment, prevention and management of bacterial pneumonia

The following personal health practices help in preventing the spread of bacterial pneumonia:

- Health habits, good hygiene practice like frequent hand cleaning and isolation of patients with multiple resistant respiratory tract pathogens helps prevent pneumonia (Tong, 2013).
- Hand washing in-between patient contacts, sneezing or sneezing into an elbow or sleeve instead of hands, avoid interacting with the sick, getting proper nutrition and having enough rest (WHO & UNICEF, 2009).
- Refrain from smoking and other pollutants, at the same time reducing indoor and outdoor indoor air pollution, and become familiarizes about warning signs to identify infection, specifically a cough, fast breathing or difficult breathing (Chang, Ooi, Perera, & Grimwood, 2013; WHO & UNICEF, 2009).
- For breastfeeding mothers, during the first six months breastfeeding is advised to critically prevent pneumonia, since breast milk contains a nourishing supply of nutrients, antioxidants, hormones and antibodies a child needs for growth and development (Marie B.Coyle, 2005).

Vaccination is one of the key method that is preferred as a preventive measure (Marie B.Coyle, 2005). Many vaccines can prevent infection by bacteria or viruses that may cause pneumonia. Some of these vaccines are namely; pneumococcal conjugate, Haemophilus influenza type-b (Hib), pertussis (whooping cough), varicella (chickenpox), measles, seasonal and 2009 H1N1 influenza (flu) vaccines (Kamangar & Harrington, 2015; WHO & UNICEF, 2009). The advisory committee in immunization practices (ACIP) in 2015 gave a recommendation on the pneumococcal polysaccharide vaccine (PPSV23) and the pneumococcal conjugate vaccine (PCV13). Immunocompetent adult aged 65yrs and order, who have not previously received

pneumococcal vaccine: A dose of PPSV23 should be given 1yr or more following a dose of PCV13, these two vaccines should not be co-administered (Kamangar & Harrington, 2015; WHO & UNICEF, 2009).

In treatment, the ACIP currently recommend that a dose of PCV13 be followed by a dose of PPSV23 in persons aged 2 years or older who are at high risk for pneumococcal diseases because of underlying medical conditions (Kamangar & Harrington, 2015). Children with immunocompromising conditions, functional or anatomical asplenia should receive a second dose of PPSV23 5years after the first PPSV23 dose (Kamangar & Harrington, 2015). The ACIP on October 12, 2012, published the updated recommendations for pneumococcal vaccination on high risk adults; which propose use of Prevnar 13 in addition to the previously Pneumovax 23 for adults aged 19 years and older with immunocompromising conditions (Chang et al., 2013; Kamangar & Harrington, 2015; WHO & UNICEF, 2009). The purposes of bacterial pneumonia pharmacotherapy are to eliminate the infection, reduce morbidity, and prevent complications. Treatment of pneumonia depends largely on the practical use of antibiotic regimes directed against potential pathogens as determined by the setting in which the infection took place and the potential for exposure to multidrug-resistant organisms (Kamangar & Harrington, 2015). Table 1 presents first and second-line antibiotic choices for specific organisms that cause bacterial pneumonia.

Table 2: Shows different choices of pathogen-driven antibiotics as obtained from (Kamangar &Harrington, 2015)

Streptococcus pneumoniae			
	Penicillin susceptible (MIC < 2 mcg/mL)	Penicillin G, amoxicillin	Macrolide, cephalosporin (oral or parenteral), clindamycin, doxycycline, respiratory fluoroquinolone
	Penicillin resistant (MIC ≥2 mcg/mL)	Agents chosen on the basis of sensitivity	Vancomycin, linezolid, high-dose amoxicillin (3 g/d with MIC ≤4 mcg/mL
Staphylococcus aureus			
	Methicillin susceptible	Anti-staphylococcal penicillin	Cefazolin, clindamycin
	Methicillin resistant	Vancomycin, linezolid	Trimethoprim-sulfamethoxazole
Haemophilus influenza			
	Non–beta-lactamase producing	Amoxicillin	Fluoroquinolone, doxycycline, azithromycin, clarithromycin
	Beta-lactamase producing	Second- or third-generation cephalosporin, amoxicillin/clavulanate	Fluoroquinolone, doxycycline, azithromycin, clarithromycin
Mycoplasma pneumoniae		Macrolide, tetracycline	Fluoroquinolone
Chlamydophila pneumoniae		Macrolide, tetracycline	Fluoroquinolone

Legionella species	Fluoroquinolone, azithromycin	Doxycycline
Chlamydophila psittaci	Tetracycline	Macrolide
Coxiella burnetii	Tetracycline	Macrolide
Francisella tularensis	Doxycycline	Gentamicin, streptomycin
Yersinia pestis	Streptomycin, gentamicin	Doxycycline, fluoroquinolone
Bacillus anthracis(inhalational)	Ciprofloxacin, levofloxacin, doxycycline	Other fluoroquinolones, beta-lactam (if susceptible), rifampin, clindamycin, chloramphenicol
Enterobacteriaceae	Third-generation cephalosporin, carbapenem	Beta-lactam/beta-lactamase inhibitor, fluoroquinolone
Pseudomonas aeruginosa	Antipseudomonal beta-lactam plus ciprofloxacin, levofloxacin, or aminoglycoside	Aminoglycoside plus ciprofloxacin or levofloxacin
Bordetella pertussis	Macrolide	Trimethoprim-sulfamethoxazole
Anaerobe (aspiration)	Beta-lactam/beta-lactamase inhibitor, clindamycin	Carbapenem
MIC = Minimal inhibitory concentration.		

2.2 General Review of Traditional Medicine

Approximately 80 % of the world's population rely on traditional medicine. Traditional medicine is regarded as traditional herbal medicine when the material being used for treatment originates from plant material. According to the WHO, traditional medicine

relates to health practices, approaches, knowledge, and beliefs incorporating plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to treat, distinguish, control illness and maintain wellness (WHO, 2003). As seen from literature, studies on herbal medicines they are described as plant medicine, phytomedicine, pharmacognosy and natural products. “Natural products” usually refer to by-product processed or derived from living organisms, including plants, animals, insects, microorganisms and marine organisms. This explains why traditional herbal medicine is only a small part of a more cohesive and holistic health care (W. J. H. Liu, 2011; WHO, 2003).

2.2.1 Traditional Herbal Medicine

Plant-based system progresses to play an important role in health care and their use by different cultures has been extensively documented. A survey of pure plant-derived compounds used as drugs in countries having WHO-traditional medicine centres show that of the 122 compounds identified, 80 % were used for related ethnomedical purposes and derived from 94 species. For example, “khellin from *Ammi visnaga (L) Lamk* led to the development of chromolyn (in the form of sodium chromoglycate) as a bronchodilator and galegine from *Galega officinalis L.* which was the model for the synthesis of metformin and other bisguanidine-type antidiabetic drugs. Also, papaverine from *Papaver somniferum* which formed the basis for verapamil is used for the treatment of hypertension. The latter plant is better known for being the source of painkillers such as morphine and codeine. The best example of ethnomedicine's role in guiding drug discovery and development is that of the antimalarial drugs, particularly quinine and artemisinin” (Cragg & Newman, 2013).

African traditional medicine is the oldest medicine regime culturally referred as the cradle of mankind. Although ethnobotanical literature is well documented, little is known of its scientific information like efficacy, phytochemistry, on indigenous medicinally used plants. From (1997-2008) a number of discovery has surface of the chemistry and biological activity of plants used in traditional healing. South Africa owns a distinctive and varied botanical heritage with over 30, 000 plant species of which approximately 3000 species are therapeutically used. Despite South African unique botanical heritage, it also has a cultural diversity with traditional healing being integral

to each ethnic group. It is known to be rich in flora endemic and diversity. Although a number of publications has focused on the isolation and identification of bioactive compounds it's important to consider complexity of plants. regarding that single compound may not be responsible for the observed activity, but preferably a combination of compounds either major or minor working together in an additive or synergistic manner (SF van Vuuren, 2008, 2010).

2.2.2 Medicinal plants

Currently, medicinal plants are widely used for primary healthcare in the developing countries, and is regarded as a possible source of important bioactive compounds. The conduction of ethnobacterial studies are required to unveil locally vital medicinal plant species and document popular knowledge, which is under threat of being lost. Some of the ethnobotanical studies, it resulted in discovery of digoxin which was extracted from *Digitalis Pupurea*, a plant used by European populations for its positive cardiovascular effects and recogutum of anticancer etoposide and teniposide extracted from *Pidophyllum peltatum* (Benarba et al., 2015).

Medicinal plants carry substances that can play a role in treatment purposes or which can be used as precursors for the synthesis of important drugs. Studies on plant use as the source of drugs and dietary supplements have accelerated in recent years. Out of this studies plants have been found to contain in-vitro antimicrobial property because of the presence of wide variety of secondary metabolites. With reference to the past events, pharmacological screening of compounds of natural or synthetic origin has been the source of countless therapeutic agent. Erratic evaluation instrument in discovering new biological active molecules, has been most productive in the area of antibiotics (Dhanalakshmi, Dhivya, & Manimegalai, 2013).

Globally the scientists are inspecting the possibilities of deploying or uncovering pharmacologically active compounds from medicinal plants. For instance, screening of medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial activities is the paramount review for finding out successful phytochemically active theory. Many investigations of this nature were concerned with the study of aqueous or solvent extracts of plant fraction and testing them individually for particular

pharmacological activities like, antibacterial, hepatoprotective, hypoglycaemic and hypolipidemic activities. Recent studies has shown that the plant extracts in combinations of two or more are revealing effective antimicrobial activity against many organisms including drug resistance bacteria's (Natchimuthu Karmegam, Jayakumar, & Karuppusamy, 2012).

2.2.3 Medicinal plants secondary metabolites

Medicinal herbs continue to contribute significantly to modern prescription drugs by providing lead compounds upon which the synthesis of novel drugs can be developed. "The American Society of Pharmacognosy defines pharmacognosy as ''the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources'' (Balunas & Kinghorn, 2005). Approximately 60 % of the anticancer drugs and 75% of the anti-infectious disease drugs approved from 1981-2002, could be traced to natural origins. Moreover, 61% of all new chemical existence introduced worldwide as drugs during the same period were inspired by natural products. The use and search for drugs, and dietary supplements derived from plants have accelerated up to date. Pharmacologists, microbiologists, biochemist, botanists, and natural-products chemists globally are currently investigating medicinal herbs for phytochemicals and lead compounds that could be developed for treatment of different diseases (Bashar & Omar, 2011). Plants synthesize a wide range of organic compounds that are traditionally categorized as primary, secondary metabolites, despite the fact that the exact boundaries between the two groups can in some case to a certain extent not be clear. Primary metabolites are compounds that have essential roles to do with photosynthesis, respiration, growth and development like phytosterols, acyllipids, nucleotides, amino acids and organic acids. Secondary metabolites are other phytochemicals, many of which accumulate in surprisingly high concentration in some species, these are structurally diverse and many are distributed among a very limited number of species (Crozier, Clifford, & Ashihara, 2006).

Despite secondary metabolites being disregarded, the function in plants is now attracting attention as some appear to have a key role protecting plants from herbivores and microbial infection. They may act as attractants for pollinations and seed-

dispersing animals, as ellolopathic agents, UV protectants and signal molecules in the formation of nitrogen-fixing root nodules in legumes. Secondary metabolites are also of interest, with the fact that they are used as dyes, fibres, waxes, glues, oils, flavouring agents, drugs and perfumes. They are viewed as possible sources of novel drugs, antibiotics and herbicides. Based on their biosynthesis origin they are divided into three major groups i) flavonoids and allied phenolic and polyphenolic compounds, ii) terpenoids and iii) nitrogen- containing alkaloids and Sulphur containing compounds (Crozier et al., 2006). Below are some of the secondary herbal metabolites which are going to be discussed briefly as follows:

2.2.3.1 Flavonoids

Flavonoids are polyphenolic compounds comprising of 15 carbons, with two aromatic rings by a three carbon bridge. They are the most prevalent out of the many phenolic, and are present through-out the plant domain. Principle subclasses of flavonoid are flavones, flavan-3-ols, isoflavones, flavoners and anthocynanidins. The flavonoids groups in relation to minor components of the diet are dihydrofravonols, flavan-3, 4 diols, coumarins, chacones, dihydrochalconers and aurones. Solubility of flavonoids depend on their existing forms (Crozier et al., 2006).

Table 2: Specific example of each of the major subclasses of flavonoids, botanical sources and some of their pharmacological properties

Medical Properties	**Botanical Sources**	**Examples**	**Flavonoids**
Anti- inflammatory analgesic	Fruits of various citrus trees	Naringenin, hesperetin	Flavanones
Anti-tumour activity	Generally, in herbaceous families,	Apigenin, luteolin	Flavones

	e.g. Labiate, Umbelliferae		
Antioxidant and microbial activities Enzyme inhibitors	Generally, in woody angiosperms, anions and green tea leaves	Myricetin kaempferol, quercetin	Flavonols
Powerful antioxidants	Found in tea leaves	Catechins, gallocatechins	Flavanols
Anti-hepatotoxic, anti-lipolytic, vasodilatory effects	Fruit and vegetables	Catechins, gallocatechins	Anthocyanidins
Powerful anticancer and heart disease properties	Cereals and legumes	Daidzein, genistein, glycitein	Isoflavonoids
Antioxidant, anti-cancer, anti HIV activities	Abundant in grapes, wine and coffee pulp	Procyanidin, prodelphinidins	Condensed tannins (proanthocyanidin)

Taken from; (Kashani, Hoseini, Nikzad, & Aarabi, 2012)

Flavonoids are termed as biological response modifiers because of their intrinsic ability to modify the body reaction to allergens, viruses, and carcinogens. Flavonoids are known for their antioxidant activities and also for their functions in producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. They display several medicinal properties such as, anti-allergic, anti-inflammatory, antimicrobial, anti-cancer activity, and anti-platelet (Anon et al, 2008). Flavonoids are everywhere in photosynthesing cells, and are usually found in fruits, vegetables, nuts, seeds, stem, flowers, tea, wine, propolis and honey (Kamanga, 2013). Flavonoids have

the capacity to strongly inhibit the topoisomerase enzyme that are responsible for DNA cleavage in replication, which result to mutations of DNA that often leads to natural acute leukaemia. Their antimicrobial activity of flavonoids is based on their ability to bind in a covalent complex with bacterial cell wall and interrupt the cellular activity, hence making them target for antimicrobial effect (Nkosi, 2013). Their antiviral function has been revealed with HIV as well as herpes simplex virus (W. J. H. Liu, 2011).

2.2.3.2 Coumarins

Coumarins are a group of 1-benzopyron derivatives that are normally present in higher plants. They serve as growth inhibitors (anti-auxins) and defence compounds in plants. Coumarins are present in nearly every plant family, but found in large volume in *Legumioseae* (bean family), *Rutecae* (citrus family) and *umbelliferae* families (also known *Apiaceae*, parsley-fenenl family) (W. J. H. Liu, 2011). Coumarins were found to have many-biological activities including anti-HIV, antitumor, antihypertension, anti-arrhythmia, anti-inflammation, anti-osteoporosis, pain relief, and prevention of asthma and antisepsis. Coumarin derivatives are extensively used as anticoagulants for the treatment of excessive or undesirable blood clotting because of their competitive bonding to vitamin K reductase and vitamin K epoxide reductase, which are vital to blood clotting. 7-hydroxyl coumarins are used to absorb ultraviolet (UV) rays in sunscreen cosmetics and for synthesis of anticancer drugs (Kamanga, 2013; W. J. H. Liu, 2011).

2.2.3.3 Lignans

Lignans are found in flax seeds, pumpkin seeds, rye, soya beans, broccoli, some barriers, and in herbs like *Magnolia officinalis*, *schizardia*, and *chinensis*, and *Podophyllum peltunum*. Lignans are widely studied for their possible anti-cancer properties, and their effect on cancer preventions was approved by a number of initial studies in humans and animals. They are one of the major classes of oestrogens like chemicals called photoestrogeners. They are efficient on binding to oestrogen receptors and interfering with the cancer promoting effects of oestrogen on breast tissue, hence inhibiting breast growth, prostate and colon cancer. Etoposide is a podophyllotoxin derivatives now used to treat lung cancer, testicular cancer, and acute lymphocytic leukaemia. Lignans are also well-known as good antioxidant, potent

antioxidant scavenge free radicals that can damage tissue and are thought to play a role in the treatment of many diseases like inflammation (W. J. H. Liu, 2011).

2.2.3.4 Quinones

Quinones occur as pigment in bacteria, fungi and some higher plants. Their derivatives have been isolated from plants and animals like Juglone in unripe walnuts, Spinulosin from *Penecillium spinuhsum*, arnebinone and arnebifuranone from *Aenebia euchroma*, tanchinone derivatives from *Salvia miltiorrhiz*, and sennoside A-D from palmatum. Quinones have various biological activities such as antimicrobacterial, antitumor, inhibition of PGE_2, biosynthesis, and anti-cardiovascular diseases (W. J. H. Liu, 2011). Quinones are known for their ability to target cell wall polypeptides, surface exposed adhesions and membrane bound enzymes. Coenzymes Q10 is benzoquinone derivatives used for cardiovascular diseases, hypertension, and cancer in clinics. Vitamin K compound like K_1 and K_2 belong to naphthoquinones. Capable of improving blood coagulation, thereby used for the treatment of natal bleeding(W. J. H. Liu, 2011; Nkosi, 2013).

2.2.3.5 Terpenoids

Terpenoids, also known as isoprenoids constitute the largest group of herbal secondary metabolites, they play a role in defence, wound scaling and thermotolerance of plants as well as in the pollination of seed crops. They are also accountable for the flavour of fruits, the fragrance of the flowers and the quality of agricultural products (Kashani et al., 2012)**.** Most terpenoids are optically active, hydrophobic and readily dissolves in liposoluble solvents. Terpenoids are further classified into many classes namely, hemiterenoids (C 5), monoterpenoids (C 10), sesquiterpenoids (C 15), diterpenoids (C 20), sesterterpenoids (C 25), triterpenoids (C 30), tetraterpenoids (C 40) carotenoids, and polyterpenoids (C 5n) based on the number of carbon atoms in the same manner as in isoprene. Both triterpenoids and steroids are normally present in plants in the form of saponins. Sesquiterpernoids exhibit a wide range of biological activities and a number of them have displayed remarkable antimicrobial activity (Kamanga, 2013; W. J. H. Liu, 2011).

Table 3: Distinct examples of each of the major subclasses of terpenes, their botanical source (s) as well as some of their pharmacological properties

Medical Properties	**Botanical Sources**	**Examples**	**Terpenes**
Analgesic and anti-inflammatory activities	Essential oils of some Pinus Spp and coniferous woods	Camphor, limonen	Monoterpenes (C10)
Antibacterial, antifungal, antimalarial, mulluscicidal	Essential oils of many plant species	Bisabolol, Ngaione Hymenoxin, Santonin	Sesquiterpenes(C15**)**
Anti-hypertensive Anti-cancer activities	Gymnosperm woods (Larix spp) Taxus (brevifolia)	Forskolin, Phorbo esters,Taxol (Paclitaxel)	Diterpenes (C20)
Ant-inflammatory Hemolytic properties	Bark of the birch Betulaalba, Larix, Picea, Pinus, Fagus, Quercus spp	Betulin (Pentacyclic triterpene) Phytosterols β-Sitosterol and campesterol	β-Carotene
Antioxidant activity	Vegetables such as carrots and pumpkin	β-Carotene	Tetraterpenes (C40) Carotenoids

Taken from**:** (Kashani et al., 2012)

2.2.3.6 Cardiac Glycosides

Glycosides are active compounds made up of two components, a carbohydrate component identified as the glycone and a non-carbohydrate component known as the

aglycone. Medicinally important glycosides are made up of anthraquinone glycosides, coumarin glycosides and steroidal (cardiac) glycosides (Kashani et al., 2012). Cardiac glycosides also known as cardenolides are named after the effect of their group compounds on the heart. Mostly cardiac glycosides are toxic and have many pharmacological activities such as oleandrin (the toxic component from the leaves the leaves of Oleander), and digitoxin one the component of the heart drug digitalis. These natural compounds the cardiac glycosides are used for the treatment of cardiac failure in clinic for congestive heart failure and cardiac arrhythmia. Some cardiac glycosides are used as arrowhead poisons in hunting (Anon et al, 2008; W. J. H. Liu, 2011).

2.2.3.7 Alkaloids

Alkaloids are well known for their varied pharmacological activities. A lot of synthetic drugs are developed from naturally occurring alkaloids or their synthetic analogues. They are classified further based on major classes these are; pyrrolidine (e.g., occur in betonicine from white horehound); pyridine (e.g., gentianine from gentian); piperidine (e.g., occur in plants such as pelletierine); pyrrolizidine common in *Senecio*; and many more (Kamanga, 2013).

Alkaloids have following biological activities such as, (e.g., berberine), anti-malaria (e.g., quinine), analgesia (e.g., morphine), anaesthesia (e.g., cocaine), anticancer (e.g., vincristine), cardiant (e.g., dl-demethylcoclaurine), antihypertension (e.g., reserpine), cholinomimeric action (e.g., galatamine), relieving cough (e.g., codeine), spasmolysis (e.g., atropine), vasodilatation (e.g., vincamine), anti-arrhythmia (e.g., quinidine), and anti-asthma (e.g., ephedrine). For instance the morphine alkaloids are powerful pain relievers and narcotics, and vincristine, isolated from vinsa rosea (*Catharansus roseus*) is one of the viable anti-leukemic in use today (Kamanga, 2013; W. J. H. Liu, 2011).

Alkaloids are a huge class of nitrogen, containing secondary metabolites of plants, microbes and mammals. From 1806 when morphine was discovered from the opium poppy, *Papaver somniferum*, more than 10,000 alkaloids have been purified and identified from natural resources (W. J. H. Liu, 2011). Alkaloids have different effect on prokaryotic DNA, and have ability to interact within prokaryotic DNA, hence making

them potential candidate for antimicrobial study in discovery of new antibiotics (Nkosi, 2013).

Table 4: Botanical source(s), and pharmacological properties of some well-known alkaloids

Alkaloids	**Examples**	**Botanical Sources**	**Medical Properties**
Opium alkaloids	Morphine, heroin	*Papaver Somniferum* (Opium poppy)	Analgesics (pain relievers and narcotics
Belladonna alkaloids	Cocaine, atropine, scopolamine, hyoscyamine	*Datura species*, *Atropa belladonna, Hyoscyamus nige*r (henbane).	Anti-cholinergic (local anaesthetics) and stimulants
Cinchona alkaloids	Quinine, quinidine	*Cinchona species*	Antimalarial, antiarrhythmic Activities
Cantharansus alkaloids (Vinca)	Vinblastine, Vincristine	*Catharanthus roseus* (Madagascar rosy periwinkle)	Anti-cancer (antileukemic) Activity
Rauwolfia alkaloids	Reserpine	*Rauwolfia Species*	Anti-hypertensive activity

Taken from: (Kashani et al., 2012)

2.2.3.8 Saponins

Saponins are found in different plant parts; leaves, stems, roots bulbs, flowers and fruits. Saponis are characterized by their bitter taste and their ability to haemolyse red blood cells. Medically they are used as expectorant, epilepsy, emetic and as a treatment for excessive salivation, chlorosis and migraines'. In Ayurvedic medicine saponins are used as treatment of eczema, psoriasis and for removing freckles. In

human diet they are known to be vital in controlling cholesterol, while as for digitalis-type saponins they strengthen the heart muscle causing the heart to pump more efficiently. Saponins are known as plants immune system activity, they act as an antibiotic to protect the plant against microbes and fungus. In animas saponins inhibit cancer tumour growth, especially in lung and blood cancers without affecting normal cells (Anon et al, 2008).

2.2.3.9 Tannins

Tannins occur as two major types – the hydrolysable tannins and the non-hydrolysable (condensed) tannins. Hydrolysable tannins are compounds which are made up of one or more sugars bonded to phenolic acid molecules. The phenolic acids are either gallic acid or ellagic acids. However, condensed tannins or proanthocyanidins are made up of two or more flavonoid units which decompose into anthocyanidins when treated with acids at high temperature. Cola tannins are examples of proanthocyanidins. Tannins have antiseptic effects which are usually used pharmaceutically to cure intestinal disorders such as diarrhoea and dysentery (Anon et al, 2008; Kamanga, 2013).

2.2.4 Methods for extraction of medicinal plants

All the substances in the universe, including plants, are composed of chemical compounds. In order to study herbal medicine, the major bioactive chemical components should first be known. Biochemical, biological, or pharmacological studies can be performed scientifically only after the biological compounds in herbs are correctly extracted, isolated, and identified. Chemical studies of herbal medicines provide fundamental substances for further studies of biological and pharmacological activity. During the earlier decades of the 1800s, chemical studies in plants could only be performed on active compounds that were highly concentrated and isolated into a relatively pure form by techniques such as distillation or extraction with water, acid, base, or alcohol. Their structures were mainly determined by chemical degradation and proven by synthesis in an unambiguous manner. Scientists were unable to determine the stereochemistry of compounds (W. J. H. Liu, 2011). Some of the methods include:

2.2.4.1 Maceration

In this process, the whole coarsely powered crude drug is placed in a stoppered container with solvent, it is left at room temperature for 3 days or weeks depending on

material and solvent, with continuous agitation till the point the soluble matter dissolves. The mixtures are strained, the marc (the damp solid material) is pressed, and the mixture of liquids are classified by filtration after standing. The maceration method is easy but time consuming and less efficient. It is suitable for thermolabile compound (Kashani et al., 2012; W. J. H. Liu, 2011; Swami Handa, Singh Khanuja, Longo, & Dutt Rakesh, 2008).

2.2.4.2 Sonification assisted solvent extraction

Sonification assisted solvent extraction is a modified maceration whereby ultrasound is utilized to improve the extraction efficiency. It is same as maceration only that the container is placed in ultrasound bath, whereby ultrasound transfers the mechanical power onto the plant cells, resulting in breakdown of cell walls and increase of solubilisation of metabolites in the solvents. The length, frequency and temperature of sonification are the main factors affecting the extraction yields. This technique is easy and efficient commonly used in the lab. Ultrasound frequency ranging from 20 KHz to 2000 KHz is normally used, it increases the permeability of cell walls and produces cavitation. Known disadvantages of this procedure though minimal, is deleterious effect of ultrasound energy on active constituents of medicinal plants through formation of free radicals therefore resulting in undesirable changes in drug molecule (W. J. H. Liu, 2011; Swami Handa et al., 2008).

2.2.4.3 Percolation

The percolator is a cylindrical or conical container made from glass or metal with a tap at the bottom. Ground plant material is added first until the whole plant material has submerged. The percolator is selected with the cover and allowed to soak for 24hrs, after this period the liquid is slowly flow out of the bottom of the percolator with a certain flow by adjusting the switch. Fresh solvent is continuously added on top of plant material, the process continues until recovered residues of extraction reduces (W. J. H. Liu, 2011; Swami Handa et al., 2008). Factors influencing the extraction yields are, percolation late, temperature of solvent and solvent used. In this process hot solvent is refluxed through herbal material. Usually, it has an advantage of providing high yields, it is quick and uses less solvent, but one disadvantage is that decomposition may occur due to heat (Kashani et al., 2012).

2.2.4.4 Soxhlet extraction

In this procedure finely pulverized powder is placed in a porous bag or thimble of strong filter pores placed in one chamber of the soxhlet apparatus. Then extracting solvent in a flask is heated, and its vapours condense in a condenser. The condensed extract drips into the thimble containing the crude drug, and extracts it by contact, the process continues and is carried out until a drop of solvent from siphon tube doesn't leave residue when evaporated. It has an advantage over other extraction methods, since large amounts of crude drug can be extracted with much smaller solvent. The commercially available soxhlet instrument is composed of an extraction chamber with reflux condenser and a collecting flask (W. J. H. Liu, 2011; Swami Handa et al., 2008).

2.2.4.5 Steam distillation

There is special apparatus for distilling volatile oil which is immiscible with water. In this process a vapour mixture of essential oil and water is produced by heating the plant material immersed in water. Vapour mixture is condensed and distillate is separated into two or three immiscible layers. Stem distillation is an effective method in preparation of fragrance and flavouring water insoluble natural products (W. J. H. Liu, 2011; Swami Handa et al., 2008).

2.2.4.6 Infusion

Fresh infusion are prepared by macerating the crude drug for a short period of time with cold or boiling water and filtered through a filter. These are dilute solutions of the readily soluble constituents of the raw drugs, suitable for aromatic drugs to prevent volatile oil to evaporate at other temperatures (Swami Handa et al., 2008).

2.2.4.7 Digestion

Digestion is a form of maceration with slight warming during the extraction process. It is accompanied by moderately increased temperature in order to avoid altering active ingredients in the use of menstruum. The common used temperatures are between 35-40 ^{0}C which might rise to 50 ^{0}C. Usually this process is used in tougher plants parts or those that contain poorly soluble substances (Swami Handa et al., 2008)

2.2.4.8 Decoction

This involves boiling of crude drugs in a specific volumes and definite times depending on the plant or the active ingredient to extract. It is then cooled and strained. The

decoction process is actually used for active ingredients that do not change with temperature. The decoction are prepared for immediate use and should not be stored for more than 24 hours (Swami Handa et al., 2008).

2.2.5 Combination Studies

Synergy has been defined as an outcome of the combination action of constituents or parts of plants being greater than expected from a collection of individual plants. From reported views and reviews, it was deduced that synergy play part in the medicinal effects of plant extracts and is considered to be one of the greatest assets of phytotherapy. Based on knowledge obtained from herbalist, it is always assumed that extract from whole plant have higher effect over single isolated constituents and it is even acclaimed that combined herbs have synergistic effects (Barnes, 1999).

Combination therapy is popular in antimicrobial chemotherapy and is usually used commercially available drugs. An example of commercial drug is Augmentin which is used to treat infections such as sinusitis, pneumonia, ear infections, bronchitis, urinary tract and skin infections. Herbalists often use combinations of plants to treat or cure diseases. An example from ethnobotanical literature in the combined administration of Salvia species and *Leonidas leonurus* to treat different kinds of infections (Kamatou, Viljoen, van Vuuren, & van Zyl, 2006).

Barnes (1999), described synergy as a kind of moderation of activity between ingredients that is either a potentiation of beneficial effects or an attenuation of undesirable effects. So often the term synergy is frequently used not accurately and intends to include all kinds of interactions between constituents and a single plant extract and a mixture of multiple components of herbal plants. It is defining that interaction between ingredients will take place. However, it is not easy to decide whether the effect is truly synergic or solely addictive which question precise evidence. Antagonism is as vital as synergy since one of the basic principles of herbalism is that the toxicity of plant extracts is minimal than in single constituents (Barnes, 1999; Williamson, 2001).

Herbalists, traditionally use multi-herb preparations which makes the evaluation of additive or synergistic effects even more difficult. However, it is still possible to test the effect of an individual herbal extract or in combination that gives an indication of synergy or antagonism with no real evidence as to which constituents are interacting. In synergy, polyvalent action is sometimes used to outline the effect of various active constituents acting in combination, in harmony and possibly innate and to describe the total effect as synergistic (Barnes, 1999).

The application of combination therapy has gained a wider acceptance, mostly in the infectious disease treatment. For example, WHO has advised pharmaceutical companies to stop encouraging the use of Artemisia derivatives in monotherapy against malarial infection. Alternately, artemisim combination therapy should be promoted not only because of its 95 % against malarial parasite (specifically *Plasmodium falciparum*) but also due to its restrain to resistance. A non-interactive or indifferent effect represents an expected linear response when two agents are combined and show either an additive nor antagonistic effect. Antagonism explains the scenario where two or more agents in combination have an overall activity which is less than the sum of their individual effects. For clarification, words such as synergism, additives, indifferent and antagonism will be used in this paper to describe a variety of interactions as adapted from (SF Van Vuuren et al., 2011).

2.2.6 Medicinal plants selected for this study

In this study, indigenous medicinal plants were selected and tested for their antimicrobial activity against some of the bacteria that cause pneumonia. The criterion for selection was based on traditional knowledge, previous studies, as well as history of folk-use in the treatment of infectious diseases (B.E. Van Wyk & Gericke, 2000; B. Wyk, Outtshoom, & Gericke, 1997).

The following indigenous medicinal plants from South Africa and Malawi were selected for study: *Kigelia africana, Ballota africana, Dudonea angustofolia, Warbugia salutaris, Terminalia sericea, Acorus calamus, Eucalyptus camldulensis.*

2.2.6.1 *Salutaris warburgia (Cannelaceae)*

This is portrayed as a universal remedy of Africa and a member of cinnamon family (cannelaceae). It is known by the following vernacular names, amazwecehlayo fever tree (E), pepper-bark (E), pepper- bark tree (E), pepper-leaf tree (E), pepper-root tree (E), koorsboom (A), peperbasboom (A), peperblaarboom (A), sterkbos (A) ZULU amazwecehlabayo, isibaha, isibhaha and mlombwa. It's a forest tree which grows southwards as far as Kwazulu-Natal, eastern and northern Gauteng and across Swaziland and in Malawi. Its growth habitat is forest and kloofs. *W. salutaris* has been used in curing fever, respiratory disorders, toothaches, dermatological disorders, sinusitis, stomach ailments, pains, malaria, venereal disease and cancer. The pepper like, bitter stems and root bark are used to cure many ailments. Smoked or expectorant *salutaris* are used diversely as a remedy for common colds (Grace, Prendergast, Jager, & van Staden, 2003; Leonard & Viljoen, 2015; Maroyi, 2013; Sprague, n.d.; B.E. Van Wyk & Gericke, 2000).

Figure 1: An image of *Warburgia salutaris*

Taken from (SANBI, 2016).

The powdered bark is most often used for various herbal formulations. Sometimes, the leaves, roots and stalk are also used. Dried and grounded *Warbugia salutaris* is most often prepared as a decoction or infusion and is to be taken orally, inhalation, snuff (to clear the sinuses), or consumed as tea. Usually, monotherapy treatments are administered but polyherbal treatments have also been explained in literature. For example, *Warbugia salutaris*, mixed with fat and at times with leaves of *Hibiscus surattensis L* stalks are applied topically to treat genital sores and inflammation. The bark of *W. salutaris* is used together with *Artemisia afra* leaves and *Acorus calamus* loot as an infective agent. When mixed with approximately 5 ml water, *W. salutaris* bark powder is taken for a dry cough, or is mixed with leaves *of Cannabis sativa L*. and smoked as herbal remedy for cough (Grace et al., 2003; Leonard & Viljoen, 2015; Maroyi, 2014; B.E. Van Wyk & Gericke, 2000).

W. salutaris has a number of major chemical constituents which include; polygodial, isopolygodialor, isoditadeonal, warbuganal, muzigadial, 3-Hydroxymuzigadial, sesquoterpenes and flavonoids. From previous studies, *W. salutaris* have been tested for antifungal properties of various secondary metabolites and activity was noted against most yeast and filamentous fungi. Rabe and van Staden (1997) also reported minimal activity against *S. aureus* (MIC 0.5 mg/ml) *S*. *epidermidis* (2.0 mg/dl) and *bacillus subtilis* (0.5 md/dl) as well as *E. coli*. Antibacterial activities were also reported in by Sprague (n.d.) on organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Candida utilis, Bacillus subtilis* (Grace et al., 2003; Leonard & Viljoen, 2015; Rabe & Van Staden, 1997; Sprague, n.d.).

2.2.6.2 *Terminalia sericea (Combritaceae)*

Terminalia Sericea also known as silver cluster leaf is endemic to Tanzania, Angola, Namibia, Zimbabwe, Botswana, democratic republic of Congo and South Africa. It grows well in open mixed woodland on sandy oils.

Figure 2: An image of leaves and fruits of *Terminalia sericea* Taken from (Lembede, 2014)

In traditional medicine, both the roots and leaves are boiled in water and the infusion is taken. This has been used as a remedy for stomach ailments and for treating bilharzia, diarrhoea and pneumonia (B. Wyk et al., 1997).

The bark is used against diabetes and to dress wounds. In case of bleeding, a paste is made by cooking the leaves in water and placing them on wounds. The bitter root decoction is used as a remedy to treat infected eye, diarrhoea and colic, to treat infertility and venereal diseases (syphilis and gonorrhoea). Parts of plants are also used to treat dysentery, wounds and menorrhagia, but in Tanzania, it is used in the treatment of fever, hypertension and bacterial infections (Talika, 2012).

T. sericea contain phytochemical constituents namely; tannins, triterpenoid saponins and flavonoids are found in the leaves, bark and roots. The triterpenoid saponin consist

of sericoside, arjunglucoside and sericic acid. Anolignan B, termilignan B and arjunic acid are some of the phytochemicals that have been recently isolated in *T. Sericea,* some of these phytochemicals are identified to have antimicrobial activity (Lembede, 2014). From previous studies *Terminalia sericea* have demonstrated antimicrobial activity against Proteus vulgaris and Proteus mirabilis (Cock & van Vuuren, 2010). From a study done by Nkwanyana (2013), *T. sericea* aqueous extract displayed activity on *B. cereus, E. faecalis, P. vulgaris, S. typhimurium S. flexneri* and *S*. *aureus*. Antifungal properties had also been confirmed in some investigation done.

2.2.6.3. *Ballota africana (Lamiaceae)*

Ballota Africana is found in South Africa and extends from the southern part of Namibia down to the west coast and cape peninsula, throughout the little Karoo and further along to the Eastern Cape and the Free State. A recipe for a cough is recommended in which boiled brown sugar, cloves, lemon juice and water are mixed with a few springs of *B. africana* (Roberts, 1990). Traditionally, use of *Ballota Africana* for treatment of haemorrhoids by making brandy tincture is still famous today.

The Khoi often used the leaves in combination with fresh Salvia and dry *B. africana* is said to be good for colds, influenza, asthma, bronchitis, colic, typhoid fever, hysteria and over-excitement, snakebite, hoarseness, heart trouble, insomnia headaches, liver problems, piles and as a foot-bath for arthritis (Ben Erik Van Wyk, Van Oudtshoorn, & Gericke, 1997). Microchemical tests done by some researchers indicated the presence of saponins and tannins and have reported insufficient information in the scientific literature concerning the secondary chemistry of this species (“Ballota africana herba,” n.d.). In a study done by Cock and van Vuuren (2014) *Ballota africana* showed potency on *Klebsiella pneumoniae*.

Figure 3: A picture showing a description of *Ballota africana* leaves (Photo: I. Mhango)

2.2.6.4 *Kigelia africana (Bignoniaceae)*

Kigelia africana (syn. Kigelia pinnata, Kigelia aethiopica) is commonly referred to as sausage or cucumber tree because of its huge sausage or cucumber-like fruit and belongs to the *Bignoniaceae family.* Due to its extended existence, it has vernacular names in many languages*:* Rawuya (Hausa, Nigeria); Uturubein (Igbo, Nigeria); Pandoro, Iyan (Yoruba, Nigeria); Balmkheera Hindi (India); Bechi (Nupe, Nigeria); Mwegea (Swahili: Kenya, Tanzania); Mvunguti (Malawi); Umfongothi (Zulu, South Africa) and Ebie (Igala, Nigeria) (Atawodi & Olowoniyi, 2015).

Figure 4: An image of *Kigelia africana* taken from Kumbula Indegenous nursery (KumbulaNursery, 2016).

K. africana is widely used throughout Africa for local medicine and more recently in commercial applications to treat skin ailments. It occurs throughout tropical Africa, especially in dry regions, in South Africa (Northern Province and KwaZulu Natal), Tanzania, Malawi and Swaziland. Each and every tree part is used diversely. For instance, in Malawi, roasted fruits are used to flavour beer and aid fermentation and dried powder or fresh fruit is boiled in water to boost immunity to various infections. Traditional healers use it to treat a wide range of skin ailments such as fungal infections, boils, psoriasis and eczema, dysentery, ringworm, tape-worm, post-partum haemorrhage, malaria, venereal diseases, diabetes, pneumonia and toothache (Gill, 1992; Owolabi, Omogbai, & Obasuyi, 2007).

In Botswana and Zimbabwe, dugout canoes are made from the tree. Bark and root infusion stepped in either cold or hot water are used for treatment of pneumonia.

Kigelia africana contains naphthoquinones and iridols dihydroiscocon marines in extracts of the bark phenylopropanoids caffeic acid, P-coumaric acid and ferulic acid, sterols and flavonoids (Joffe, 2003). The ethanolic extract of *K. africana* exhibit antibacterial and antifungal activity against *S. aureus* and *C. albicans* but not against the strains of *E. coli* and *P. aeruginosa*. However, the aqueous extract on the same study demonstrated no activity indicating that ethanol is a better extracting solvent (Owolabi et al., 2007).

K. africana extracts have showed significant growth inhibitory activity towards *K. pneumoniae* (Cock & van Vuuren, 2014). Moreover, *K. africana* showed the presence of glycosides, phenolic compounds (hydrolysable tannins), alkaloids, flavonoid and reducing sugar in all extracts while cardiac glycoside was noted only in chloroform extract (Abdulkadir, Adedokun, & John, 2015). It has been reported that the extracts from many species of bignoniaceae contain secondary metabolites such as saponins, tannins, flavonoids, quinones, alkaloids, anthralene derivatives, reducing sugars, glycosides, carbohydrates, querletin, kaempferol, α-sitosterol, terpenes, steroids and coumarins and their derivatives (Azu, 2013).

2.2.6.5 *Dodonaea angustofolia (Sapindaceae)*

D. angustofolia grows in variety of habitats ranging from arid, semi-arid to high rainfall regions and in frost-hardly regions. It is found in a wide strip along coast from Namaqualand through the Western Cape, Eastern Cape to KwaZulu Natal as well as further north in Mozambique and Zambia. It also occurs naturally in Saudi Arabia, Australia and New Zealand. *D. Angustofolia* is widely used for colds, influenza, stomach complaints and measles. Other early uses of the plant included treatment of pneumonia, tuberculosis and skin rashes and it can be combined with viscum capense (B E Wyk, Endtsshoon, & Gericke, 2009).

Figure 5: The fruits of *Dodonaea angustofolia.* Taken from (Plantbook, 2016).

For a sore throat and oral thrush, it is used as a gargle. The khoi-khoi used a concoction of the root for the colds and influenza whilst in Namaqualand the green leave were boiled slowly, then left to steep, strained, and the extract was used for influenza, colds, and induce sweating (Harris, 2015). It is used to relieve coughs and the congested feeling typical of influenza, croup and diphtheria. The leave extracts of *D. angustofolia* is considered to alleviate stomach ailments and fever, as a mild purgative for rheumatism, sore throat and haemorrhoids (Shuizen et al., 2011).

In a study powdered plant material of *Dodonaea Angustofolia* demonstrated the presence of many secondary metabolites, such as tannins, alkaloids, phytosteriods, saponnins and polyphenols(Al-baker, Ahmed, Hanash, & Al-hazmi, 2014) . From an investigation on antimicrobial activity of selected Eastern Cape medicinal plants *D. Angustofolia* displayed antimicrobial activity against *B. cereus* (Mohlakoana, 2010).

2.2.6.6 *Eucalyptus camaldulensis (Myrtaceae)*

This plant range in Australia in all mainland states where it has widespread natural distribution, but it is a plantation species in many parts of the world. *Eucalyptu*s have many uses; leave decoction are used for gastrointestinal disorders, sore throat, urinary tract infection, colds, asthma, coughs, diarrhoea and dysentery, haemorrhage, laryngalgia, laryngitis, spasm, trachagia and vermifugent (Abubakar, 2010; Jouda, 2013a).

Figure 6: An image showing Eucalyptus camaldulensis. As taken from (Lucidcentral, 2016).

Poultice of leaves applied over catarrh is used for nasal congestion. The plant is aromatic, astringe tonic herb that strives to the teeth and turns the saliva red. Essential oils and volatile oils are used for treatment of lung disease and tuberculosis, and as expectorant and cough stimulant respectively. Apiarists use the flower for honey production, providing bees with good quality pollens and heavy yields of nectar. Chemical compositions of the *E. camaldulensis* include essential oils, cineol, cuminal, phellandrene, aromadendren, Valery aldehyde, geraniol, cymene, tannins, kitiotanic acid, glycoside catechol, flavonoids and sterols terpenes and isoprenoids, phenolics, cardiac glycosides, sterols, saponins and flavonoids terpenes and isoprenoids,

phenolics, cardiac glycosides, sterols, saponins and flavonoids (Abubakar, 2010; Grbovc et al., 2010). *E. camaldulensis* aqueous and acetone extract when tested against *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* Proteus *mirabilis* and *Klebsiella pneumoniae* they demonstrated antibacterial activity, and highest was observed in acetone extract (Abubakar, 2010). It was noted that ethanolic leaf extract of *E. camaldulensis* had marked fungicidal effect against clinical dermatophytic fungal isolates; *Microsporium gypseum* and *Trichophyton mentagrophytes* (Jouda, 2013a).

2.2.6.7 *Acorus gramineus (Acoraceae)*

Acorus gramineus is indigenous to Asia, but has been introduced across Europe, Australia, New Guinea, South Africa, Reunion and North America. The dry rhizome of *A. gramineus*, called *Acori graminei* rhizome has been used largely in Korean traditional medicine (Lee, Yun, & Hwang, Byung KookLee, 2004).

Figure 7: An image of *Acorus gramineus*. Taken from: (grasses on Pinterest)

It has common names like grass-leaved sweet rush, Japanese sweet flag, dwarf sweet flag (R. Balakumbahan, 2011). The plants root has medicinal uses like: antifungal, antibacterial, antiperiodic, antirheumatic, antispasmodic, aromatic, cardiac, carminative, diaphoretic, emmenagogue, febrifuge, sedative, stimulant, stomachic, tonic and vermifuge It is also powdered and applied to bleeding gums, digestive problems (gastralgia and diarrhoea), cough, bronchial asthma, neurasthenia, depression, epilepsy, body parasites, dermatosis and haemorrhoids.

The root contains an essential oil consisting of asarone and asaryl aldehyde, and bitter glucoside acorin. Asarone increases the hypnotic effect of barbiturates and ethanol lowers blood pressure and has antibacterial activity against Staphylococcus aureus, streptococci and mycobacterium. The whole plant is anodyne, antiperiodic, antispasmodic, digestive, diaphoretic, diuretic, expectorant, sedative, stimulant, stomachic, sudorific, tonic, vermifuge. The dried root repels insects likely as an insecticidal and is used in Vietnam to kill lice, bugs and fleas. Antifungal activity of beta-asarone from rhizomes of Acorus gramineus was recently demonstrated in a study whereby a number of fungal under study were inhibited (Lee et al., 2004).

2.3 Classification of antimicrobial therapy

In an effort to battle the various forms of diseases that have continued to invade humans from ancient times up to present, variety of antimicrobials have been developed against pathogens for these diseases. Antimicrobials are substances that kill or inhibit the growth of microorganisms, which refers to all agents that act against microbial organisms; bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal) (Cheesbrough, 2006; Michigan State University (institution), 2011). This is not synonymous with antibiotics, “antibiotic” refers to substances produced by microorganisms that act against another microorganism. Some vital antibiotics include culture extracts and filtrates of fungi such as penicillium, amphotericin B, griseofulvin and cephalosporium, and bacteria such as aminoglycosides (Streptomyces, kanamycin, amikacin, gentamicin and tobramycin) tetracycline’s, polymyxins, rifampicin, fucidin, chloramphenicol, erythromycin, clindamycin, vancomycin and Bacillus species (bacitracin

by *B. licheniformis* or *B. subtilis*, polymyxin by *B. polymyxa* and gramicidin by *B. brevis*). Therefore, antibiotics does not include antimicrobial substances that originate from synthetic derivatives (sulphonamides and quinolones), or semisynthetic (methicillin and amoxicillin), or those which come from plants (quercetin and alkaloids) or animals (lysozyme). Some broadly antifungal agents include nystatin, flucytosine and imidazole agents (Cheesbrough, 2006; Michigan State University (institution), 2011).

Because of the re-occurring resistance of pathogenic microorganisms to antibiotics, and their side effects has resulted to active investigation of other sources of antimicrobials such as medicinal plants for their potential antimicrobial activity. Plants produce secondary metabolites (phytochemicals), which have potential as antibacterial when used individually and in combination or potentiators of other antibacterial agents. Since phytochemicals usually act through different mechanisms rather than the conventional antibiotics, they could therefore be of use in treatment of resistant bacteria (Chinyama, 2009; Frum & Viljoen, 2006).

2.3.1 Mechanisms of Antimicrobials

In order to understand the mechanism of bacterial resistance, bacterial physiology, pharmacology and antimicrobial drugs and molecular biology of infectious agents need to be understood. In this study, some of these processes will be briefly discussed to gain an insight of resistance concept. Broad spectrum is related to antimicrobials with activity against a wide range of gram positive and negative organisms like tetracycline's, aminoglycosides and chloramphenicol. Sometimes, antimicrobials are used in combination to treat mixed infections, prevent drug resistance from developing. They can also be used to treat infection when organism is not known, or to obtain greater antimicrobial effect from two bactericidal drugs activity together (synergistic effect) (Cheesbrough, 2006; Michigan State University (institution), 2011).

Antimicrobials are mostly categorized according to their primary functions. However, they are sometimes classified according to their function, thus to say agents that kill microbes at usual dosages are called bactericidal, and those that inhibit their growth are bacteriostatic (Cheesebrough, 2006). Different ways on how antimicrobials act on

bacteria based on structure and degree of affinity to certain target sites within bacteria cells are as follows:

a) **By inhibiting cell wall formation**: This mechanism leads to cell wall lysis. Examples include; penicillin's, cephalosporin's, carbapemarins and vancomycin's. Since human and animal cells have no cell walls, drugs that target cell walls selectively kill or inhibit bacterial organisms since this structure is critical for the life and survival of bacterial species. Bacteria drugs in this group disrupts normal balance between cell wall and its degradation, hence exposing cell wall to destruction by losing out inclusion or allowing water to flow into the cell causing it to burst (Cheesebrough, 2006; Kamanga, 2013; Michigan State University (institution), 2011; Nkosi, 2013).

   Antimicrobial agents that interfere with cell wall synthesis are often bactericidal. Activity of bet-lactam antimicrobials on gram-negative bacteria is through passage of this drugs into the cell through porin channels in the outer membrane. In susceptible cells, beta-lactam molecules bind to penicillin binding proteins (PBPs) enzyme involved in cell wall production. The attachment of the beta-lactam molecules to the PBPs, located on the surface of the cytoplasmic membrane, obstruct the process, resulting in weakened or defective cell walls that leads to cell lysis and death. Since gram-positive bacteria do not possess an outer membrane, beta lactam antimicrobials diffuse through cell wall and the following steps are similar to those in gram negative (Marie B.Coyle, 2005).

b) **By inhibition of cytoplasmic membrane function** or damaging bacterial cell membrane, leading to loss of cell contains and cell death. Cell membranes in a cell they act as a vital barrier that isolate and control the intra and extra cellular flow of substances into and outside living cell. Any possible damage to cell membrane could result in leakage of important cell contains (solutes) responsible for the cells survival. The action of this this class of antibiotic are frequently poorly selective and can often be toxic for systematic use in mammalian host, as some of this structures can be found in both eukaryotic

and prokaryotic cells (Cheesebrough, 2006; Marie B.Coyle, 2005; Michigan State University (institution), 2011). As a result, most clinical usage is limited to relevant applications. Example of drugs that fall under this category are, polymixins, amphotericin B and colistin (Cheesebrough, 2006; Michigan State University (institution), 2011). Drug like gramicidin is known for disrupting the bacterial cytoplasmic membrane by forming a channel through the membrane that result in damaging its integrity, antifungal drugs polyeners they are known to have similar mechanism (Kamanga, 2013; Nkosi, 2013).

c) **By interfering protein synthesis** as a result arresting bacterial growth and also cause further damage leading to cell death by binding to the machinery that builds proteins. Enzymes and cellular structures are essentially made of proteins, and their synthesis is a basic process necessary for the multiplication and survival of all bacterial cells. Several type of antibacterial agents' target bacteria protein synthesis by binding to either 30s 0r 50s sub units of the intracellular ribosomes. This process result in the disruption of the normal cellular metabolism. Tetracycline's (tetracycline, minocycline and doxycycline) bind to the 30S subunit of the ribosome and block the attachment of transfer RNA (tRNA). Therefore, new amino acids cannot be added to the growing protein chain, as synthesis of protein is inhibited. The action of tetracycline's is bacteriostatic (Cheesebrough, 2006; Kamanga, 2013; Marie B.Coyle, 2005; Michigan State University (institution), 2011).

   Aminoglycosides (e.g. gentamicin, tobramycin, amikacin, and streptomycin) also bind to the 30S ribosomal subunit and they block protein synthesis in two possible ways. First they bind to 30S subunit of the ribosome and prevent it from attaching to messenger RNA (mRNA), secondly, the presence of the aminoglycoside on the ribosome may cause misreading of the mRNA. "This leads to the insertion of the wrong amino acid into the protein or interference with the ability of amino acids to connect with one another. These activities often occur simultaneously and the overall effect is bactericidal" (Marie B.Coyle, 2005). Inhibition of protein synthesis by binding to the 50S ribosomal

subunit happens when macrolides (erythromycin, azithromycin and clarithromycin) and lincosamides (clindamycin) attach to the 50S ribosomal subunit triggering termination of the growing protein chain and inhibition of protein production. Chloramphenicol binds to the 50S subunit of the ribosome and interferes with binding of amino acids to the growing protein. Antimicrobial agents that interferes protein synthesis in this way are bacteriostatic (Marie B.Coyle, 2005).

d) **By inhibiting the production of nucleic acids,** hence preventing bacteria from reproducing. Nucleic acids production is important for the synthesis of DNA, some antibiotic they work by binding to vital components for DNA and RNA synthesis, causing interference in normal cellular process, eventually compromising bacterial multiplication and survival. In order for nucleic acid to be produced it requires tetrahydrofolic acid, which is normally reduced to tetrahydrofolic acid by enzymes dihydrofolate reductase (DHFR). THR is primarily important in thymidine monophosphate (dTMP) synthesis, which are substrates for DNA production. Anti-folate is known to inhibit nucleic acid by inhibiting DHFR enzymes. The antimicrobial action happens when antifolates which are DHFR analogy competitively and irreversibly bind with DHFR at a greater affinity than DHF. Leading in inhibition of the supply of THF and obstruction of DNA synthesis, as a result disrupting cellular growth. Trimethoprim works by inhibiting dihydrofolate reductase interfering with nucleic acid production by inhibiting purine production (Cheesebrough, 2006; Kamanga, 2013; Michigan State University (institution), 2011; Nkosi, 2013).

Fluoroquinolones (e.g. nalidixic acid, ciprofloxacin, levofloxacin and gemifloxacin) interfere with DNA synthesis by blocking the enzyme DNA gyrase, which helps to wind and unwind DNA during DNA replication. The enzyme binds to DNA and initiate double stranded breaks that allow the DNA to unwind. Fluoroquinolones bind to the DNA gyrase-DNA complex and permit the broken DNA strands to be released into the cell, which leads apoptosis.

Rifampicin binds to DNA-dependent RNA polymerase, which blocks the production of RNA and result in apoptosis (Marie B.Coyle, 2005)**.**

e) **By inhibition of other metabolic pathways or processes** some antibiotics they act on selected cellular processes necessary for the survival of bacterial pathogens. For example, sulphonamides and trimethoprim disrupt the folic acid pathway which is imperative for bacteria to produce precursors important for DNA synthesis. For many organisms' para-amino-benzoic acid (PABA) is an essential metabolite and is involved in the synthesis of folic acid, important precursor for nucleic acids synthesis. Sulphonamides are structural analogs of PABA and compete with PABA for the enzyme dihydropteroate synthetase. Trimethoprim acts on the folic acid synthesis pathway at a point after the sulphonamides. It inhibits the enzyme dihyrofolate reductase. Trimethoprim and sulphonamides can be used separately or together. When used together they produce a sequential blocking of the folic acid synthesis pathway and have a synergistic effect. Both trimethoprim and the sulphonamides are bacteriostatic (Cheesbrough, 2006; Kamanga, 2013; Nkwanyana, 2013).

2.3.2 Action mode of antimicrobial resistance

Modification of active site of the target results in the inefficiency on binding of the drugs. Bacteria they change the patterns and other essential compartment of bacterial cell, which are used as binding site for antimicrobials. One target for interposition, at the active site for instance penicillin-binding protein (PBP). Beta-lactam antibiotics they alter penicillin-binding proteins in order to hinder antibiotics access to the enlarging peptidoglycan. For example, the mode of action for the high level resistance of staph A to PBP2 to a variant that is designated PBP2a. As another option, may be loss of the binding proteins that have a greater affinity for the antibiotics (Winn, Allen, Janda, & Koneman, 2006). With literature revelation, a lot of natural products do exist, which are specialised to overcome resistance microorganisms for example changes of genetic origin are associated with resistance to aminoglycosides, lincomycin and erythromycin (Cheesebrough, 2006; Wagner & Ulrich-merzenich, 2009). Changes in the affinity of receptors for other antibiotics such as DNA gyrase and quinolones or

ribosomes and aminoglycosides are also important. Despite modification of active site the second most important mechanism is the change in receptors for antibiotic attachment to critical structures whereby there's alteration of normal binding proteins on the cell membrane of gram positive bacteria to binding proteins with reduced affinity for antibiotics (Winn et al., 2006).

Efflux of antibiotic from the cell by hindering antibiotics from penetrating into the bacteria cell or after penetration to eject the accumulated dry out of bacteria cell (Wagner & Ulrich-merzenich, 2009). Some bacteria they actively remove antimicrobial agent from the bacterial cell so that intracellular concentration of antimicrobial agents won't reach suitable amount to exert their effective antimicrobial activity. The energy-dependent efflux mechanism is a prime defence for bacteria against tetracycline's and macrolides, these groups interfere with protein synthesis at the level of ribosome. Relatively as with staphylococci against the quinolones, removal of antibiotics is resistance mechanism which actually interfere with DNA gyrase (Marie B.Coyle, 2005). The resistance of gram negative bacilli to many antibiotics was originally attributed to poor entry of the antibiotic into the bacterial cell because of size, hydrophobicity and charge. The efflux of these antibiotics from bacterial cells is vital, since the pumps can excrete chemical compounds, including antimicrobial agents, into extracellular medium directly, whereby their re-entry is prohibited (Cheesbrough, 2006; Michigan State University (institution), 2011; Winn et al., 2006).

Direct destruction or modification of the antibiotics by enzymes produced by the microorganisms, thus enzymes destroy or inactivate antimicrobials. Genes that code for the production of resistance enzymes are carried by plasmids and they can be moved from one species to another. An individual plasmid possesses genes that code resistance to several antimicrobial drugs like streptomycin, chloramphenicol, tetracycline's and sulphonamides. Plasmids of the nature are called resistance (R) factors, but other plasmids they carry genes that code for resistance to penicillin. Examples of enzymes that causes resistance are Beta lactamases which destroy the beta lactam ring that forms part of the structure of penicillin and cephalosporin. These organisms, *staphylococcus aureus*, *haemophilus influenzae* and *Neisseria gonorrhoea* are associated with Beta-lactamase production. Certain gram negative

bacteria equally produce acetylating, adenylating, and phosphorylating enzymes that have ability of inactivating aminoglycosides, like chloramphenicol is inactivated by the enzymes acetyltransferase. Some enzymes in gram negative bacteria that inactivate streptomycin and kanamycin they are widely distributed making these antibiotics vulnerable to common clinical use. A minor resistance for tetracycline is enzymatic inactivation. Resistance to macrolides and lincosamides in gram positive bacteria may be yielded due to several methods; alone or in combination-enzymatic inactivation, alteration of ribosomal target or active efflux (Cheesbrough, 2006; Marie B.Coyle, 2005; Wagner & Ulrich-merzenich, 2009; Winn et al., 2006).

Bypass of a metabolic block imposed by antimicrobial agent like trimethoprim-sulfamethoxazole and enterococci also by bacteria altering the permeability of their cell membrane, making impossible or difficult for antimicrobial to enter bacteria cell. This kind of resistance have been observed in bacteria resistance to polymyxins and tetracycline's, but the cell wall of streptococci form a natural barrier to aminoglycosides (Cheesebrough, 2006; Wagner & Ulrich-merzenich, 2009). Auxotrophic mutants that use thymine for growth may bypass the activity of trimethoprim and sulphonamides in-vitro making use of available substrate and alternative pathway. Important clinical example of bypass process is the ability of naturally occurring strains of enterococcus species to use compounds like folic acid growth in vivo in the presence of trimethoprim – sulfamexazole. Strains of enterococci may appear susceptible in-vitro but they don't respond to chemotherapy in-vivo. In order to check the in-vitro result of resistant, there's a need to add folic acid to the testing media. For this reason hereof enterococci should not be used for susceptible testing against trimethoprim (Marie B.Coyle, 2005; Michigan State University (institution), 2011; Wagner & Ulrich-merzenich, 2009; Winn et al., 2006).

2.4 Microorganisms

Organisms selected to for this study were clinical strain of; *Klebsiella pneumoniae, Streptococcus pyogenes,* and *ATCC 43300* strain of *Staphylococcus aureus.*

2.4.1 Staphylococcus aureus

Staphylococcus aureus is one of the important human pathogen among the staphylococci species. *S. aureus* is gram positive coccus, uniform in size, can occur in groups, singly and also in pairs, tetrads and short chains. Predominantly it appears in grape like structures and is non- motile, non-capsulated, non-spore-forming. Commonly found in the external environment like, on the intertriginous skin folds, mucous membranes, perineum, the axillae, vagina, and the intestine of humans and other animals. Common specimens from which *S. aureus i*s isolated are; sputum, blood, urine, faeces, vomit, pus and anterior nasal perineal swabs which are analysed by microscopy and culture (Cheesebrough, 2006; Kamanga, 2013; Winn et al., 2006).

S. aureus grow well aerobically and in carbon dioxide enriched atmosphere (facultative anaerobes), expect for *S. aureus* sub-species *anaerobius S. saprophyticus*. Desirable temperature range for growth is 10-42^{0}c with an optimum of 35-37^{0}c. On blood agar and chocolate, it produces yellow to cream or occasionally white 1-2 mm in diameter colonies. When grown aerobically they are beta- haemolytic, on MacConkey smaller colonies are seen after overnight incubation. Biochemically *S. aureus* are non -lactose fermenter, coagulase positive, DNases positive and catalase positive (Cheesbrough, 2006; Winn et al., 2006).

S. aureus is known to cause a number of infection processes ranging from benign infections to life threatening systemic illness. It causes conjunctivitis in new-born, septicaemia, endocarditis, osteomyelitis, mastitis, food poison, toxic shock syndrome, scalded skin syndrome, and antibiotic associated enteritis (Cheesebrough, 2006). Skin infection include simple folliculitis and impetigo, furuncles and carbuncles which involves subcutaneous tissues and causing systemic symptoms, such as fever. In postsurgical infections it suffices as a breeding ground for the development of systematic infections. Pneumonia and empyema is also experienced, which is categorized into community acquired, bronchopneumonia which is manifested in elderly individuals and is associated with viral pneumonia as a predisposing factor, and also nosocomial pneumonia due to

S. aureus which happens in hospital because of cross infection of obstructive pulmonary diseases, intubation, and aspiration (Cheesbrough, 2006; Winn et al., 2006).

S. aureus produce enzyme and toxins which are termed virulence factors which believed to contribute to their ability to cause infection. Initially, penicillin was the preferred drug for *S. aureus* treatment, but due to acquisition of plasmid borne genetic elements coding for Beta lactamases production has led to emergence of resistant to penicillin. For the mean time over 80% of *S. aureus* isolates are resistant to penicillin, as a result semisynthetic penicillinase-resistant penicillin's have become the drug of choice for treatment of infections. The latest mode of resistant is acquired from the presence of an altered penicillin-binding protein (PBP 2a) that come up from acquisition of a chromosomal gene called *mecA.* Other drugs that have developed resistance to *S. aureus* are glycopeptide agent vancomycin (Kenneth & Ray, 2004; Winn et al., 2006).

2.4.2 *Streptococcus pyogenes*

Streptococcus pyogenes consist of Lancefield group A, Beta haemolytic streptococci, which is among the most prevalent of human bacterial infections and their common reservoirs in nature are the skin and mucus membranes. Just like *Staphylococci aureus, streptococcal* group A also produce several other products that contribute to virulence. *S. pyogenes* is a gram positive cocci and non-motile, they can grow both aerobically and anaerobically. Specimens normally found are pus, throat swab, or blood depending on site of infection, and blood samples are also used for antistreptolysin O antibody (Kenneth & Ray, 2004).

*S. pyogenes g*row well on blood agar whose colonies look grey white or colourless, dry or shinny and usually irregular in arteria. They are beta-haemolysis which is markedly when incubated anaerobically. *S. pyogenes* does not grow on MacConkey (Cheesbrough, 2006). Group A streptococci cause's streptococcal pharyngitis, impetigo, erysipelas, cellulitis, puerperal sepsis, acute rheumatic fever, acute glomerulonephritis and ear infections. Oral penicillin V or intramuscular benzathine penicillin G, is currently recommended for streptococcal pharyngitis and other

infection however, erythromycin and cotrimoxazole is an alternative to those who are allergic to penicillin. Resistance to macrolides has been reported globally and less than 5% in U.S have demonstrated to be resistant to erythromycin (Winn et al., 2006).

2.4.3 Klebsiella pneumoniae

Klebsiella pneumoniae are gram negative, non-motile, encapsulated, non-sporulating, facultative, aerobic shaped rod bacterium that is normally found in the human gastro-intestinal tract (oropharynx). This colonization may prove to be the origin of lung infections that generally manifested in patients with chronic conditions such as alcoholism, diabetes mellitus, and chronic obstructive pulmonary diseases. The pneumonia is prone to destruction with extensive necrosis and haemorrhage, leading to production of sputum which is thick, mucoid and brick red, or thin and currant jelly-like appearances. When cultured on blood agar or MacConkey they produce large and usually mucoid colonies, also when cultured on broth a stingy type of growth is produced. Specimens collected depends on site of infection and includes urine, sputum, pus, and infected tissue. Most strains are lactose fermenters and urease test is positive after 18-24hrs (Cheesbrough, 2006; Kenneth & Ray, 2004; Ryan & Ray, 2004; Winn et al., 2006).

An adhesion to a mucosal surface is often the first step in the development of an infection. A survey of the presence of *Klebsiella* in urban residents, hospital personnel, and newly admitted patients showed that 30-37% of individuals carried *Klebsiella*, including a 29-35% faecal carriage and a 3-4% throat carriage. Strains of *K. pneumoniae* and *K. oxytoca* which have not acquired any resistance are determined as naturally resistant to ampicillin and carboxypenicillin but susceptible to other beta-lactam antibiotics. This is due to the production of a chromosomal penicillinase which is inhibited by clavulanic acid. *K. pneumoniae* also causes enteritis and meningitis, urinary tract infection, wound infections and peronitis, and septicaemia (Cheesbrough, 2006; Kenneth & Ray, 2004; Winn et al., 2006).

CHAPTER THREE

ANTIMICROBIAL EVALUATION FOR PLANT USED TO TREAT BACTERIAL PNEUMONIA

3.1 Introduction

Medicinal plants are used worldwide for primary health care which is usually administered by herbalist or a local inhabitant with herbal knowledge. As such in developing countries and South Africa inclusive, up to present medicinal plants, forms a cornerstone of health care. Traditional medicine use has been known as a source of biologically important active compound or as an element in complex mixture of comprehensive constituents (Suliman, 2011).

currently, due to an increase of bacterial resistance there has been a dramatic increase in the demand for medicinal plants. This resulted in an urgent need to develop new antibiotics and immune modulating compounds with a broad range chemical formulation and different mechanism of action (Mohlakoana, 2010).

This chapter discusses the materials and methods that were used in this study to meet objectives. As observed by Chinyama (2009) revitalized interest in traditional *pharmacopeias'* has encouraged researchers not to focus only on determining scientific logic but also to concentrate on discovering new compounds with pharmaceutical value. The plants being investigated in this study were selected based on their reported ethnobotanical use and ethnopharmacological studies. In this study the antimicrobial evaluation of medicinal plants was done by testing them against a number of pathogens associated with pneumonia infections.

3.2 Materials and methods

3.2.1 Plant sample selection and collection

Seven plants that are used traditionally to treat pneumonia and related conditions were selected based on indigenous ethnopharmacological reports from the available literature and herbal knowledge usage (B.E. Van Wyk & Gericke, 2000; B E Van Wyk & Wink, 2004; B. Wyk et al., 1997). The plant materials used in this study consisted of Kigelia africana (bark), Ballota africana (leaves), Dudonea angustofolia (leaves), Warbugia salutaris (bark), Terminalia sericea (roots), Acorus gramineus (roots), and Eucalyptus camldulensis (leaves). Three plants namely K. africana, T. sericea and W. salutaris were collected were collected from Malawi to be precise central region in Lilongwe rural area, Msalu village which was later identified at Chitedze Research Station. The remaining four plants were collected from different places in Port Elizabeth Eastern Cape Province of South Africa.

3.2.2 Plant preparation

Fresh collected plant material of roots, leaves and bark were washed with tap water a number of times to remove dirt. The roots and bark were further cut into small pieces to enhance drying; the plant materials were oven dried at 40 °C until absolutely dry. There after the dried plant material were pulverized into fine and course powder depending on its property with mortar and pestle, and the powder was kept in zip lock bags and airtight containers until required for further processing (Mohlakoana, 2010; Nkwanyana, 2013).

3.2.3 Plant Extraction Methods

Aqueous solvent, (preferably hot distilled water) and two organic solvents namely (ethanol and acetone) were used for extraction for each plant under study investigation. 2 g of powdered plant material was weighed and subjected to extraction, with 45 ml of extracting solvent in in polyester centrifuge tubes. The mixtures were vigorously shaken with a vortex for 5 minutes and left overnight while being heated to 30^0c for the thorough dissolving of the plant compounds. Tubes were centrifuged at 3500 ×g for 5 min and the supernatant was filtered using Wattman No.1 filter paper before being transferred into pre-weighed glass containers. The procedure was repeated twice using the same amount of a solvent in each tube.

The beakers with aqueous extract supernatants were then placed into 40 °C water-bath to allow evaporation of the extractant so that the plant extract/residues remain. The organic solvent was removed by evaporation under a stream of air in a fume hood at room temperature to quantify extraction effectiveness and comparison of different plant species. After evaporation, the beakers containing the residues were weighed and were re-dissolved in 10% and 100% dimethyl sulfoxide (DMSO). The amount of DMSO added to reconstitute dry extract was dependent on an individual extract. All the plants were able to dissolve in 10% DMSO except *B. africana, D. angustofolia, A. gramineus extracted* with acetone and ethanol, which were dissolved with 100% DMSO. Concentrations were then standardized between the different plants and then it was kept in refrigerator at 4 °C till further use for the antimicrobial screening assay. The method of extraction was adopted from (Eloff, 2000; Eloff JN, 2004; Kamanga, 2013)

3.3 Antimicrobial assays

3.3.1 Panel of bacterial strains

Strains of organisms tested during the present study are some of the predominant causes of pneumonia. Bacteria strains used in this study were provided by the Faculty of Health Science, Medical Laboratory Sciences department, microbiology laboratory at Nelson Mandela Metropolitan University. Three organisms were used for the study, the strains included, *streptococcus pyogenes, Klebsiella pneumoniae* and American Type Culture Collection (ATCC) of *Staphylococcus aureus* 43300*.*

3.3.2 Media and culture preparation

Nutrient agar and broth were prepared by adding 1 L of distilled water to 30 g of TSB powder. The mixture was dissolved and autoclaved for 15 minutes at 121 °C. Muller Hinton agars were prepared by dissolving 38 g powdered of agar in 1 litre of distilled water. The mixture was boiled on Bunsen burner to dissolve and thereafter it was autoclaved for 15 minutes at 121 °C. Shortly before solidifying, the liquid agar was poured into 90 mm petri dishes to a depth of 4mm, the plate was allowed to dry and stored in fridge at 2.2°C. Mueller-Hinton agar plate was preferred for use in sensitivity because it gives good result in batch-to-batch reproducibility, lack in sulphonamide, trimethoprim and tetracycline inhibitor, and the growth of many bacteria pathogen gives

satisfactory result. The agar should have a PH of 7.2-7.4 at room temperature and the agar surface should be left to dry without droplet of moisture (Cheesbrough, 2006; Motlhatlego, 2014; Saad, Sadia, Asmani, & Yusuf, 2014).

Bacterial strains were sub cultured in laboratory to get colonies/new strains of these bacteria, and nutrient agar and Muller Hinton was used for the initial sub-culturing for all organisms. The microorganisms were incubated at 37°C for 24 hrs and 32 hrs for *streptococcus pyogenes*. The bacterial strains were preserved and maintained on nutrient agar plates and Muller Hinton agar plates at 2°C - 8°C for short-term storage (Cheesebrough, 2006). Single colonies of each bacterium were also respectively transferred into McCartney bottles each with 10 ml Oxoid Mueller-Hinton Broth (MHB). These bottles were then incubated in a shaking incubator overnight at 37°C, but for the viability maintenance of the bacterial strains, every 30 days the organisms were sub-cultured (Motlhatlego, 2014).

3.3.2 Antimicrobial assessment using Agar well diffusion method

Antibacterial activity of the plants under investigation were tested using Agar well diffusion method. Stock cultures were maintained at 4 °C on Nutrient and Muller-Hinton. In order to come up with active cultures for experiments, organisms were prepared by transferring a loopful of culture to 5 ml of nutrient broth and incubated at 37 °C for 24 hours. Few isolated colonies (3-5) were selected and suspended in 5 ml sterile physiological saline using sterile wire loop or disposable swab. (Jouda, 2013b; Kamanga, 2013).

The turbidity of the bacterial suspension was adjusted and visually compared to a 0.5 McFarland turbidity standard. Turbidity was also verified using spectrophotometric comparison with a 0.5 McFarland standard to ensure that the suspension contained approximately 1 to 2 x 108 CFU/ml (Hariprasath, 2010; Kamanga, 2013).

An inoculum suspension was swabbed uniformly to solidified 25 ml Mueller-Hinton agar (MH) for bacteria, and the inoculum was allowed to dry for 5 minutes. Holes of 6 mm in diameter were made in the seeded agar using glass Pasteur pipettes. Aliquots of 100 µl from each plant crude extract (10.5 mg/ml) was added into each well on the seeded

medium and allowed to stand on the bench for 15-20 minutes for proper diffusion and thereafter incubated at 37 °C aerobically for 18 hours. Control wells were maintained by introducing extract solvents (10% & 100% DMSO) as negative controls into the well. Antimicrobial paper discs of streptomycin (S10), chloramphenicol (C30), Penicillin (G10), Tetracycline (30), Ciprofloxacin (CIP5), Amoxicillin (A10) were used as positive control. After 18 hours of incubation, the plates were monitored making sure that the growth was consistent. After incubation the growth inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm, inhibition > 8 mm were considered to be sensitive. The interpretation of inhibition zone results, were reported as susceptible, intermediate, or resistant to the organisms tested. The experiments were done in triplicates to make sure the results are reproducible, reliable and valid. the mean clear zones was calculated and values were compiled (Jouda, 2013a; Wizemann, Olsen, & Choffnes, 2013).

3.3.3 Minimum inhibition concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that completely inhibits the growth of an organism under specific condition. After initial screening by agar diffusion assay which aided in identification of an extract with antibiotic activity**,** the micro-dilution bioassay for MIC was performed. This method is based on the principle of exposing test organism to a series of dilutions of test antimicrobial/substance (Suliman, 2011). There are a number of ways in which MIC can be determined like agar dilution, the broth dilution method, and microplate or microdilution method. The microplate is regarded as a “sensitive” and a “quick” method specifically developed to obtain MIC values for plant extracts against micro-organisms. This method was developed by Eloff (1998).

The well microtiter plate method is preferred since:

- It is not expensive, and approximately 30 times more sensitive than any other method found in literature.
- it requires small quantities of test substance.
- It has ability to screen large number of samples.

- Different sample of micro-organisms, controls can be run on the same plate at the same time with samples.
- reasonable time is spent which makes it a quick and efficient method (Eloff, 1998).

100 µl of Muller Hinton broth was added from second row of the 96-well microtiter plate. 200 µl of each dissolved plant extract (10.5mg/ml) was then added on the first rows and diluted with Muller Hinton broth in the other wells. These were serially diluted row by row and 100 µl of the mixture was discarded from the last row, thus leaving each diluted well with a volume of 100 µl. The same procedure was carried out for ciprofloxacin (positive control), during which 100 µl of 2.5 mg/ml of dissolved ciprofloxacin was serially added in triplicate. 100 µL of each bacterial suspension in suitable growth medium (nutrient broth) was then added to all the wells except the last column, which served as the sterile control (containing 200 µL of nutrient broth). A few organic extracts could not be re-dissolved adequately in 10% DMSO, and were therefore dissolved in 100% (DMSO). Wells containing bacterial suspensions and growth medium, as well as wells containing 10% and 100% DMSO, bacteria suspensions and growth medium, were used as negative control. The final volume in each well was 200 µl. The microtiter plates were incubated at 37°C overnight at moist conditions, to prevent evaporation and drying of volatile extracts (Nkwanyana, 2013; Suliman, 2011).

Following incubation of microtiter plates, microbial MIC was detected by using a 0.04% solution of *p*-iodonitrotetrazolium (INT). INT chloride was prepared by weighing 0.08 g of INT of and added to 200 ml of distilled water to make up the required stock solution. INT (50 µl) of 0.2 mg/ml of (INT) violet (Sigma) was to all microtiter plates wells to detect microbial growth. The INT was used as the bacterial growth inhibition indicator where by INT changes to pink purple or red colour in the presence of actively metabolizing organisms or colourless appearance representing inhibition of bacteria growth. Each microtiter plate was investigated for microbial growth as soon as efficient colour change took place for each test organism's corresponding culture control. To achieve efficient colour change, MIC plates were incubated at to 37°C for 30 minutes or more 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 (Nkwanyana, 2013; Talika, 2012).

The MIC value was reported as the lowest concentration of extract having no indication of microbial growth. The concentration of the extract in each well ranged from 10.5 mg/mL in the top row, to 0.048 mg/mL in the last row of wells. In cases where there was no detection of growth for a specific plant sample (i.e. MIC value ≤ 0.048), the extract was then further diluted before repeating the MIC assay. Each extract was tested in triplicate against each microorganism to test for reproducibility of results.

3.3.4 Combination studies

The healing value of synergistic interaction has been known for long period of time, and many traditional medicine treatments are based on the fact that combinations treatments might be able to produce the desired results (Williamson, 2001). Many herbalists rely on a routine where single plant is used for treatment but often, various plants parts or species are combined to achieve favourable results (Shealy, 1998). The conceptualization of antimicrobial synergy is based on the belief that when drugs are used in combination; efficacy increases, toxicity is minimized, side effects decreases, bioavailability increase, and allow minimum dosage use at the same time it reduces the progress of antimicrobial resistance (Van Vuuren & Viljoen, 2011).

Three methods are usually used in plant combination studies to determine presence of synergy, antagonism, indifference or additive interactions. These methods are chequerboard method, time-kill curves and disk diffusion methods. Qualitative evaluation of plant interactions the results are obtained by with killing curves and disk diffusion studies, whereas quantitative assessment is expressed by microtiter-plate method using FIC and isolobogram assays. Microtiter plate was preferred method for this current study (Suliman, 2011).

In this study all seven medicinal plants were involved in combination studies. These plants were combined in 1.1 combination to come up with 21 different plant combinations. All these 21 plant combinations were extracted with ethanol, acetone and sterile distilled water. Despite evaluation of single plant conducted, interactive antimicrobial plant combination studies were done for all 21 combinations. Just like

with individual studies, bacterial cultures were grown in nutrient broth at 37º C for 24 hours. The prepared suspension was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, holes were made with Pasteur-pipette and 50 µl (10.5mg/ml) of each plant extract in 1.1 combination was placed into the wells. The plates were incubated at 37º C for 24 h. after incubation diameters of clearing zones was measured. The table below displays the list of plant combinations involved in this study.

Table 5: Tested plant combinations

Plant samples used in this study	**Plant combinations tested extracted with aqueous and organic extract**
T. sericea	T. sericea + w. salutaris, T. sericea + D. angustofolia, T. sericea + E. camaldulensis, T. sericea + B. africana, T. sericea + K. africana, T. sericea + A. gramineus
W. salutaris	W. salutaris + D. angustofolia, W. salutaris + E. camaldulensis, W. salutaris + B. africana, W. salutaris + K. africana, W. salutaris + A. gramineus
D. angustofolia	D. angustofolia + E. camaldulensis, D. angustofolia + B. africana, D. angustofolia + K. africana, D. angustofolia + A. gramineus
E. camaldulensis	E. camaldulensis + B. africana, E. camaldulensis + K. africana, E. camaldulensis + A. gramineus
B. africana	B. africana + K. africana B. africana + K. africana
K. africana	B. africana + A. gramineus
A. gramineus	N/A

This evaluation was done according to Agar well diffusion method on the three bacterial test cultures. The methodology followed was same as for individual studies. The MICs for combination studies are discussed in FICs determination.

3.3.5 Fractional inhibitory concentration (FIC) determination

Microtiter plates were aseptically prepared by adding 100 µl sterile Muller-Hinton broth into each well of a 96 microtiter plate. A 1:1 combination was prepared from plant stock solutions (10.5 mg/ml for extracts) adding up to 100 µl in each well. First row it was100 µl + 100 µl from each plant extract, whereby 100 µl was transferred to second row where serial dilutions were done. MIC values were determined for these combinations to establish any interaction. Serial dilutions were performed as with MIC assays (Section 3.3.3.). Tests were performed in duplicates and triplicates. The fractional inhibitory concentration (FIC) was then calculated according to the following equation (SF Van Vuuren et al., 2011);

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

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

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

$$FIC\ index\ =\ FIC\ (a)\ +\ FIC\ (b)$$

The following calculations were used by
Berenbaum (1978) in order to determine the FIC index:

$$\Sigma FIC\ =\ FICA\ +\ FICB$$

$$FICA\ = \frac{MICA \text{ combined with } MICB}{\text{MICA independently}}$$

$$FICB\ = \frac{MICB\ combined\ with\ MICA}{MICB\ independently}$$

As projected by previous researchers, in order to determine plant interaction, if a combination is synergistic, additive, non-interactive or antagonistic, the methods 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 (Table 5). The interpretation by Van Vuuren and Viljoen (2011) is a more conservative analysis of interpreting synergism.

During the current study, these calculations has been used, bringing into account the plant extracts forming part of each combination, but not their actual amount in the interaction. The FIC index Schelz (2006), was used to determine the correlation between the two plants and may be classified as either synergistic (≤ 0.5), additive (> 0.5-1.0), indifferent (> 1.0-4.0) or antagonistic (> 4.0). Conventional antimicrobials were included in all repetitions as controls and tests were undertaken in triplicate. In order to measure the activities of combinations, FIC indices were calculated for the 1:1 combination since isobologram studies were not done. The ΣFIC was calculated from MIC values obtained from plants investigated independently (Chapter 3, Section 3.3.3) and for each combination. The ΣFIC 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 MIC results for the combined plant extracts (where applicable) were recorded in triplicate as put in chapter 4, against each of the three test organisms. The average MIC result for each combination was tabulated, along with their sum fractional inhibitory concentration (ΣFIC). A difference of no more than one dilution factor was accepted within these repetitions.

CHAPTER FOUR
RESULTS

4.1 Introduction

In this section the results obtained in the study will be discussed. These include: Extraction, antimicrobial screening, MIC and FIC determination. Data obtained in this study was subjected to statistical analysis using ANOVA followed by post-hoc Tukey HSD to calculate results. Values were considered statistically significant when p value is less than 0.05 ($P<0.05$).

4.2 Extraction results

Bark, root and leave samples of seven medicinal plants under study were subjected to extraction. The following solvents; ethanol, acetone and distilled water were used as extractant on this study to determine antimicrobial activity of the seven medicinal plants investigated. Aqueous preparation was made in such a way to mimic traditional preparation. Water extracts were dried in incubator while acetone and ethanol extracts were dried in fume wood. The dried plant material was reconstituted with DMSO. *T. sericea, W. salutaris, K. Africa and E. camaldulensis* were dissolved in 10% DMSO. Water extracts of *A. gramineus, D. angustofolia* and *B. africana* were able to dissolve in 10% DMSO whilst their organic extracts (acetone and ethanol) were dissolved in 100% DMSO. A final concentration of 10.5 mg/ml was prepared for all plants samples as a working solution.

4.2.1 One-way ANOVA with post-hoc Tukey HSD Test Calculator

Table 6 : Input data on kk=7 independent antimicrobial activity

Treatment →	A	B	C	D	E	F	G
Input Data →	16.3 15.8 15.6 17.45 18.3 14.0 18.5	13.4 14.25 0.0 12.0 16.5 12.0 0.0	22.6 24.0 0.0 0.0 30.0 0.0 0.0	26.3 25.75 26.65 23.3 25.2 23.5 22.0	20.7 20.0 24.0 22.0 19.6 21.5 19.3	13.5 14.0 11.0 13.0 15.6 0.0 12.2	24.0 20.6 13.5 14.0 22.0 14.0 15.0

Treatment →	A	B	C	D	E	F	G
	13.05 13.0	9.0 0.0	12.0 0.0	23.9 23.0	18.7 18.75	13.0 0.0	18.35 13.0

Table 7: Descriptive Statistics of kk=7 independent antimicrobial activity

Treatment →	A	B	C	D	E	F	G	Pooled Total
observations N	9	9	9	9	9	9	9	63
sum $\sum x_i$ $\sum xi$	142.0000	77.1500	88.6000	219.6000	184.5500	92.3000	154.4500	958.6500
mean $\bar{x}$ $\bar{x}$	15.7778	8.5722	9.8444	24.4000	20.5056	10.2556	17.1611	15.2167
sum of squares $\sum x_{2i} \sum xi2$	2,275.6350	1,023.8725	2,130.7600	5,379.3650	3,808.6425	1,229.4500	2,789.3325	18,637.0575
sample variance s^2 s2	4.3988	45.3157	157.3178	2.6406	3.0428	35.3578	17.3499	65.3161
sample std. dev. s s	2.0973	6.7317	12.5426	1.6250	1.7444	5.9462	4.1653	8.0818
std. dev. of mean $SE_{\bar{x}}$ $SE\bar{x}$	0.6991	2.2439	4.1809	0.5417	0.5815	1.9821	1.3884	1.0182

Table 7: One-way ANOVA of your kk=7 independent treatments

Source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
Treatment	1,926.2133	6	321.0356	8.4667	1.4973e-06
Error	2,123.3867	56	37.9176		
Total	4,049.6000	62			

4.2.2 Conclusion from Anova

The p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that there is one or more antimicrobial activity that is significantly different. The Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison tests follow. These post-hoc tests would likely identify which of the pairs of treatments are significantly different from each other.

4.2.3 Tukey HSD Test

The p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.01 which strongly suggests that one or more pairs of treatments are significantly different. With kk=7 antimicrobial activity, for which Tukey's HSD test is applied to each of the 21 pairs to pinpoint which of them exhibits statistically significant difference.

4.2.4 Post-hoc Tukey HSD Test Calculator results

The degrees of freedom for the error term v=56v=56 and the critical values of the Studentized Range QQ statistic: Qα=0.01, k=7, v=56 critical Q critical α=0.01, k=7, v=56 = 5.1516. Qα=0.05, k=7, v=56 critical Q critical α=0.05, k=7, v=56 = 4.3245. Presented below are color coded results where red is for insignificant, and green for significant in order to evaluate whether Qi,j>QcriticalQi,j>Qcritical for all relevant pairs of antimicrobial activity. In addition, also presented is the significance (p-value) of the observed QQ-statistic Qi,jQi,j. The algorithm used here to calculate the critical values of the studentized range distribution, as well as p-values corresponding to an observed

value of Qi,jQi,j, is that of Gleason (1999). This is an improvement over the Copenhaver-Holland (1988) algorithm deployed in the R statistical package.

Table 8: Tukey HSD results

Antimicrobial Activity	**Tukey HSD**	**Tukey HSD**	**Tukey HSD**
Pair	Q statistic	p-value	inferfence
A vs B	3.5105	0.1854115	insignificant
A vs C	2.8907	0.4015151	Insignificant
A vs D	4.2007	0.0623513	Insignificant
A vs E	2.3033	0.6443468	Insignificant
A vs F	2.6904	0.4877991	Insignificant
A vs G	0.6739	0.8999947	Insignificant
B vs C	0.6198	0.8999947	Insignificant
B vs D	7.7112	0.0010053	** p<0.01
B vs E	5.8138	0.0023648	** p<0.01
B vs F	0.8201	0.8999947	Insignificant
B vs G	4.1844	0.0641498	Insignificant
C vs D	7.0914	0.0010053	** p<0.01
C vs E	5.194	0.0091505	** p<0.01
C vs F	0.2003	0.8999947	Insignificant
C vs G	3.5646	0.1719589	Insignificant
D vs E	1.8973	0.8080553	Insignificant
D vs F	6.8911	0.0010053	** p<0.01
D vs G	3.5267	0.1811743	Insignificant
E vs F	4.9937	0.0138495	* p<0.05
E vs G	1.6294	0.8999947	Insignificant
F vs G	3.3643	0.2268453	Insignificant

KEY: A= K.africana , B=W.salutaris, C=B.africana, D=T.sericea, E=E.camaldulensis, F=D.angustofolia, G=A.gramineus,

4.3 Antimicrobial screening

Warburgia salutaris, Eucalyptus camaldulensis, Ballota africana, Kigelia africana, Terminalia sericea, Acorus gramineus, and *Dodonea angustofolia* were tested against three organisms. Plant extracts were screened against strains of *Klebsiella pneumoniae and Staphylococcus aureus ATCC 43300* and results obtained displayed significant antimicrobial activity. Test strains used in this study are known to cause bacterial pneumonia, and were tested against these plants extracts to find their scientific antibiotic effectiveness in pneumonia treatment as speculated.

4.3.1 *Staphylococcus aureus*

Plant extracts tested against *Staphylococcus aureus* demonstrated different ranges of results based on extraction solvent used as shown in T able 5. *K. Africana* displayed sensitivity results with little variation in zone of inhibition when tested with all extraction solvents: 16.3 mm, 15.8 mm, 15.6 mm, water ethanol and acetone respectively.

W. salutaris and *B. africana* demonstrated sensitivity with *S. aureus* on water and ethanol extracts as follows; 13.4 mm on water and 14.25 mm on ethanol and the latter 22.6 mm on water and 24 mm on ethanol respectively with no zone of inhibition (ZOI) on acetone extracts of both plants.

S. aureus was highly susceptible to all three of the *Terminalia sericea* extracts as it showed an inhibition zone of 25.75 mm on ethanol, 26.65 mm on acetone, and 26.3 mm on aqueous extract.

Aqueous, acetone and ethanolic extracts of *E. camaldulensis* were tested for their antibacterial activity, by showing various degrees of activity (24 mm Acetone, 20 mm Ethanol and 20.7 mm with water extracts water).

Table 9: Screening results mean values of antimicrobial activity of plant extracts at a concentration of 10.5 mg/ml against strains of test organisms using the well diffusion method

Plant extracts	***S. aureus ATCC 43300***			***S. pyogenes***			***K. pneumoniae***		
	H_2O	E	A	H_2O	E	A	H_2O	E	A
K. Africana	16.3±1.9	15.8±1.5	15.6±1.8	17.5±2.1	18.3±2.5	14±2.1	18.5±1.45	13.05±3.1	13±3.5
W. salutaris	13.4±1.5	14.25±1.3	-	12±1.0	16.5±0.5	12±0.2	-	9±0.8	-
B. Africana	22.6±4.1	24±4.3	-	-	30±1.0	-	-	12±2.0	-
T. sericea	26.3±2.0	25.7±0.9	26.6±1.8	23.3±2.0	25.2±1.2	23.5±1.0	22±1.9	23.9±1.0	-
E.camaldulensis	20.7±1.7	20±1.5	24±2.1	22±2.0	19.6±1.6	21.5±1.4	19.3±2.6	18.7±3.4	18.7±2.9
D. angustofolia	13.5±2.3	14±1.3	11±0.6	13±1.7	15.6±1.1	-	12.2±1.8	13±1.5	-
A. gramineus	24±4.0	20.6±3.4	13.5±1.5	14±2.5	22±3.3	14±3.4	15±1.3	18.4±3.7	13±1.0
CIP5	28±0.9			10±0.98			35±0.26		
C30	25±1.4			-			14±0.75		
T30	22±0.97			-			-		
PG10	9±0.72			21±0.66			-		
A10	-			25±1.02			12±0.75		
S10	11±0.58			-			19±0.30		
DMSO 0%	-			-			-		
DMSO 100%	-			-			-		

Key: E = Ethanol extracts; **A**= Acetone extract; **H_2O** = water/aqueous extract; **(-)** = No growth inhibition; **Numerical figures** = representing bacteria zones of inhibition measured in millimeter. Ciprofloxacin (CIP5), chloramphenicol (C30), Tetracycline (30), Amoxicillin (A10), Penicillin (G10), Streptomycin (S10), DMSO = Dimethyl-sulfoxide; (-) = No clearing

D. angustofolia plant extracts showed weak antimicrobial activity against *S. aureus* with exhibition of the following results of ZOI; 13.5 mm on water extract, 14 mm ethanol and 11 mm on acetone extract.

A. gramineus displayed antimicrobial activity with the following clear zone of inhibition 24 mm aqueous, 20.6 mm ethanol and 13.5 mm acetone.

4.3.2 *Streptococcus pyogenes*

K. africana demonstrated an antimicrobial effect, measured by ZOI's produced on the following plant extract, water 17.45 mm, ethanol 18.3 mm and acetone 14 mm.

Ethanolic extracts of *W. salutaris* showed 16.5 mm ZOI and both acetone whereas water extract had 12 mm on *S. pyogenes*.

Ethanol extract of *B. africana* had significant antimicrobial activity against S. pyogenes thereby demonstrating the highest zone of inhibition measuring 30 mm, but no zone of inhibition was noted on both acetone and water extract.

All *Terminalia sericea* plant extracts showed noteworthy antimicrobial activity against *Streptococcus pyogenes* with following results aqueous showed 23.3 mm, ethanol and acetone had 25.2 mm and 23.5 mm ZOI's respectively.

E. camaldulensis extracts inhibited *S. pyogenes* by displaying the following ZOI's: water 22 mm, ethanol 19.6 mm and acetone 21.5 mm.

Aqueous and ethanol extracts of *D. angustofolia* inhibited growth of *S. Pyogenes* by displaying zones of inhibition, 13 mm and 15.6 mm respectively, with no zones of inhibition noted on acetone extract.

S. pyogenes was also inhibited by *A. gramineus* with 14 mm ZOI's on both water and acetone extracts. 22.2 mm ZOI was observed on ethanol extract.

4.3.3 Klebsiella pneumoniae

Kigelia africana and *Acorus gramineus* demonstrated considerable antibacterial activity against *K. pneumoniae.* Results on *A. gramineu*s, showed ZOI of 15 mm with water extract, 18.35 mm on ethanol and 13 mm with acetone extracts.

K. africana showed various degrees of activity, and highest observed on water extract 18.5, 13.05 mm with ethanol and 13 mm on acetone extract.

Both aqueous and acetone extract of *W. salutaris* and *B. africana* were not effective against *K. pneumoniae* except ethanol extract which displayed ZOI of 9 mm with *W. salutaris* and 12 mm with *B. africana*

Like the other two organisms tested *K. pneumoniae* was highly susceptible to *T. sericea* with average diameter zone of inhibitions of 23.9 mm on ethanolic extract, 23 mm acetone extract and 22 mm on aqueous extract.

E. camaldulensis also displayed significant anti-*Klebsiella* activity with zones of inhibition of 19.3 mm on water, 18.7 mm ethanol and 18.75 mm on acetone extract.

D. angustofolia displayed minimal antimicrobial activity against *K. pneumoniae* with ZOI of 12.2 mm on water extract and 13 mm ethanol and no inhibition was seen with acetone extract.

Table 10: positive and negative controls of agar diffusion method

Test organism	CIP5	C30	T30	PG10	A10	S10	DMSO 10%	DMSO 100%
S. aureus	28	25	22	9	-	11	-	-
S. pyogenes	10	-	-	21	-25	-	-	-
K. pneumoniae	35	14	-	-	12	19	-	-

Key: Ciprofloxacin (CIP5), chloramphenicol (C30), Tetracycline (30), Amoxicillin (A10), Penicillin (G10), Streptomycin (S10), DMSO = Dimethyl- sulfoxide; (-) = No clearing

The paper diffusion disc produced the following results (table 3) in diffusion assay. No zones on inhibition was observed around wells where DMSO (10 & 100%) was placed

4.4 Evaluation of plant extracts bioactivity in combination studies

The crude extracts of seven medicinal plants were combined in 1.1 ratios and screened for their antimicrobial activity against three bacterial pathogens. Ethanol, acetone and aqueous extracts of all plants studied exhibited broad spectrum of antimicrobial activity. Plant combinations studies revealed different degrees of antimicrobial activity with some

combinations not showing any activity at all. This was especially the case for those plant extracts dissolved in 100% DMSO

The maximum zone of inhibition was 35 mm which was observed for *S. aureus* with ethanol extracts of *B. africana* + *E. camaldulensis*, and *B. africana* + *W. salutaris*

Combination of the ethanol extracts of all plants showed significant antimicrobial activity in combination study against *S. aureus*, and *S. pyogenes.* Ethanol extract of all plants also demonstrated impressive results except the *D. angustofolia* and *K. africana* combination which did not exhibit any activity.

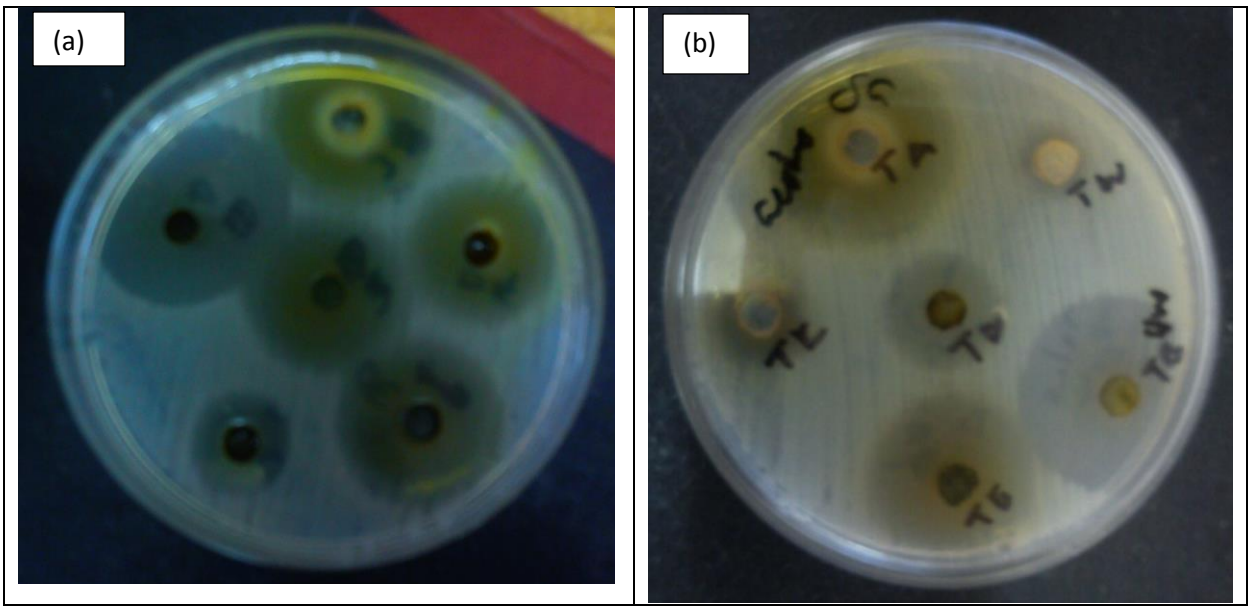
Good antimicrobial activity was also observed against *K. pneumoniae* with the ethanol extract, though there was no zone clearing in some combinations like *D. angustofolia* + *B. africana*, *D. angustofolia* + *K. africana*, *B. africana* + *W. salutaris*, and *A. gramineus* + *T. sericea.*

Table 11: Antibacterial activity of plant extracts tested in combination against bacterial strains using agar well diffusion method (mm)

Combined plant extracts	***S. Aureus ATCC 43300***			***Streptococcus pyogenes***			***Klebsiella pneumoniae***		
	H_2O	**E**	**A**	**H_2O**	**E**	**A**	**H_2O**	**E**	**A**
K. africana + T. sericea	25	25.4	27	24.5	26.25	26	25.5	26	23
D. angustofolia + B. Africana	15	30	32	-	29	28	-	-	-
K. africana + E. camaldulensis	22.3	21	24	22	21	25.3	24	19.25	23.75
D. angustofolia + K. Africana	13	20	17	18.75	-	16.5	21	-	-
K. africana + W. salutaris	23	13.6	12.5	16.3	19.3	13	18.5	13	12
B. africana + W. salutaris	-	35	33	-	33	33	-	-	-
W. salutaris + T. sericea	26.5	23.3	29	25	26	-	26.6	26.6	28
D. angustofolia + A. gramineus	12	22	20	18	22.5	13.3	-	25	20
E. camaldulensis + A. gramineus	19.8	26	27	25	24.4	30	23	24.3	25

K. africana + A. gramineus	17	14.8	16	17	15.25	13	21	13.6	12
W. salutaris + E. camaldulensis	26	22.6	22	23.4	22.25	22	21.6	28	19
T. sericea + A. sericea	24	26.75	27.6	25.5	27	26	25.5	-	27.75
T. sericea + E. camaldulensis	28.7	28	30	28.37	26.75	30	28.4	28.75	29
B. africana + K. Africana	16	26	30	21	22	-	24	20	20
D. angustofolia + W. salutaris	-	23	17	13	23.5	18.5	-	13	-
W. salutaris + A. gramineus	10	20	12	17	18	14.5	15	22	16
B. africana + A. gramineus	-	23	34	22.3	27.6	30	25	25.6	20
D. angustofolia + E. camaldulensis	25.25	22.3	21	24.3	23.3	23.6	25.3	23.5	22.6
T. sericea + B. Africana	25.5	28.75	30	26	27	30.5	23.8	29.25	22
B. africana+ E. camaldulensis	18	35	29	22	25	28.5	25	15	20
T. sericea + D. angustofolia	23.5	25.5	24.3	21	27	27	25	28	25.25

Key: E = Ethanol extracts; **A** = Acetone extract; **H_2O**= water/aqueous extract; **(-)** = No growth inhibition; **(+)** = in combination with; **Numerical figures** are representing bacteria zones of inhibition measured in millimeter.



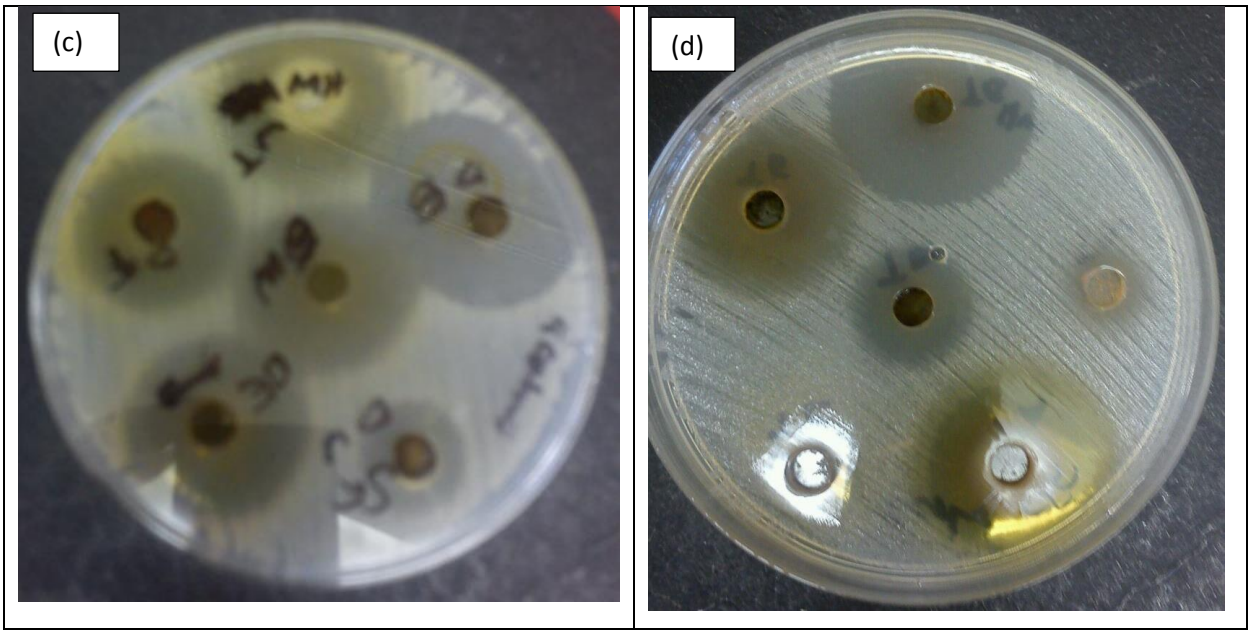


Figure 8 = (a) ZOIs of ethanol extracts combination against *S. pyogenes* plate; (b) = ZOIs of acetone extracts combinations against *S. aureus*; (c) = ZOIs of acetone extracts combinations against *K. pneumoniae* (d) = ZOIs of acetone extracts combinations against *S. pyogenes.*

A susceptibility evaluation of *S. aureus* with all combination of plant extract fractions displayed notable interactions particularly with ethanol and acetone plant extracts, since sensitivity was observed in all 21 combinations. However, not all aqueous extract combination exhibits antimicrobial activity against the bacteria tested. As indicated in Table 11, *B. africana* + *A. gramineus*, *D. angustofolia* + *W. salutaris*, and *B. africana* and W. salutaris did not inhibit bacterial growth.

All plant extract combination displayed significant activity against *S. pyogenes.* The results from activity evaluations of ethanolic extract showed inhibitory potential in all combinations with the exception of *D. angustofolia and K. africana*. The highest activity was observed with ethanol and acetone extracts of *B. africana* and *W. salutaris* with ZOI of 33 mm, followed by *T. Sericea and E. camaldulensis* and also E. c*amaldulensis* and *A. gramineus* acetone extracts with Z0I of 30mm; lowest activity was observed with water extracts of *W. salutaris* and *D. angustofolia* with ZI of 13 mm.

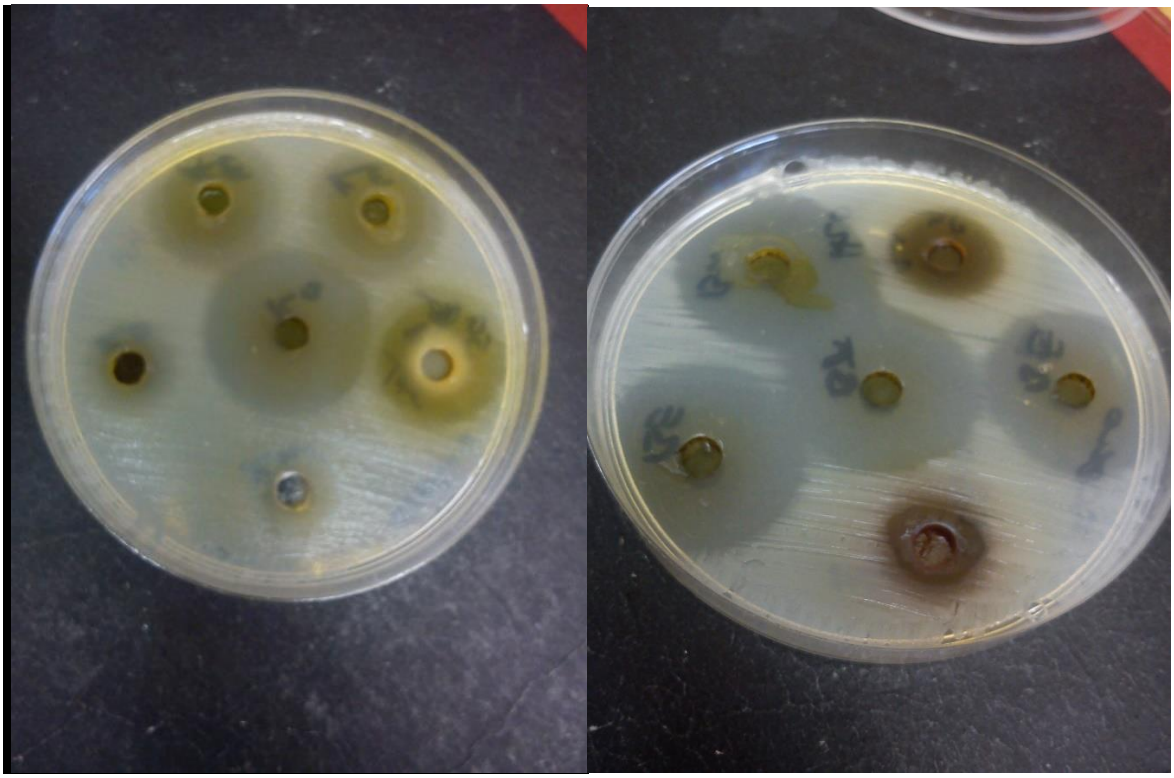


Figure 9: (a) = ZOIs of aqueous extracts combination against *K. pneumoniae* (b) = ZOIs of ethanol extracts combinations against *S. aureus*.

Klebsiella pneumoniae also exhibited pronounced antimicrobial activity with all plant extract combinations, with a few of them as shown in the Table 11. *D. angustofolia* + *B. africana*, *B. africana* + *W. salutaris* did not show any antimicrobial activity with any crude plant extract. The *D. Angustofolia and K. africana combination*, demonstrated antimicrobial activity with only water extract showing a ZOI of 21 mm. Conversely, the *A. gramineus* and *D. angustofolia* combination exhibited antimicrobial activity in ethanol and acetone crude extract, unlike water extract which did not inhibit growth of *K*. *pneumoniae*.

The *A. gramineus* and *T. sericea* combination had a ZOI of 25.5 mm and 27.75 mm on aqueous and acetone respectively against *K. pneumoniae*, with no activity on ethanol extract. The combination of *D. angustofolia* + *W. salutaris* showed antimicrobial activity with only ethanol extract for *K. pneumoniae*.

4.5 Evaluation of Minimum Inhibitory Concentration (MIC) Using Microdilution Method

The MIC results of all plant extract showed antibacterial activity against *S. aureus*, *S. pyogenes* and *K. pneumoniae* with MIC values from 0.082 – 10.5 mg/dl. Broth microdilution method was used to determine the MIC for the plant extract, and the highest dilution of the plant extract that retained its inhibiting effect resulting in no growth (absence of pink color after adding INT) of microorganism was reported as the MIC value of the extract. MIC of all seven medicinal plants namely *Terminalia sericea, Warbugia salutaris, Kigelia africana, Dodonea angustofolia, Ballota africana, Acorus gramineus,* and *Eucalyptus camaldulensis* were extracted with the following extracts, ethanol, water and acetone. Plant extract displayed varied activity from maximum to minimum with each test strain organism.

The MIC interpretation was adapted from Suliman, (2011) and Van Vuuren, (2008), whereby MIC values < 1.0mg/ml were considered noteworthy, and <8.0 mg/l were considered to have some antimicrobial activity. False positive MIC values were excluded by including solvent controls. Positive control results with ciprofloxacin were as follows S. pyogenes had MIC value of 0.78 µg/ml, S. aureus MIC OF 0.31 µg/ml and K. pneumoniae MIC value of 0.625 µg/ml. To check purity of culture, organisms were cultured and observed for any sign of contamination.

Out of 7 plants tested *Terminalia sericea* was found to have noteworthy activity against all test organisms with MIC value ranges of 0.0144 – 0.607 mg/ml. The other plants investigated displayed noteworthy activity against one or more test organism with aqueous or the organic extract.

Table 12: MIC's of individual medicinal plants against three test organism (mg/ml)

Plant Extract	***S. aureus***			***S. pyogenes***			***K. pneumoniae***		
	W	E	A	W	E	A	W	E	A
K. Africana	1.81	1.94	6.83	**0.985**	1.31	3.94	**0.984**	3.94	6.56
D. angustofolia	5.25	1.75	3.28	10.5	1.09	1.31	**0.656**	1.64	1.42

E. camaldulensis	1.31	2.63	1.31	1.31	**0.656**	**0.328**	**0.739**	1.31	1.37
B. Africana	3.94	**0.76**	1.31	1.48	**0.164**	**0.492**	2.30	2.63	1.31
W. salutaris	5.25	2.66	4.38	7.88	3.5	5.25	1.72	7.22	3.06
A. gramineus	5.25	**0.739**	1.31	2.63	1.31	1.97	1.64	2.63	2.63
T. Sericea	**0.607**	**0.328**	**0.492**	**0.191**	**0.0144**	**0.267**	**0.369**	**0.547**	**0.191**

Key: E= Ethanol extracts; **A**= Acetone extract; **W** = water/aqueous extract; **Numerical Figures** are representing concentration of plant extracts at which it was able to inhibit bacterial growth in mg/ml; Noteworthy activity values are indicated in bold.

Minimum antimicrobial activity was observed with *Dodonea angustofolia* and *Warburgia salutaris* with the highest MIC values of 10.5 and 7.8 mg/ml respectively with water extract on *S. pyogenes*. In contrast, *Dodonea angustofolia* water extract on *K. pneumoniae* demonstrated noteworthy activity with MIC values of 0.656mg/ml, as well as water extract of *Warbugia salutaris* which displayed a good MIC of 1.72mg/ml. The highest antimicrobial activity observed against *S. aureus* with an ethanol extract of *Dodonea angustofolia* was MIC value of 1.75mg/ml and 2.66mg/ml obtained with *Warbugia salutaris*.

The *Ballota africana* Ethanol extract had the overall strongest antibacterial activity, having MIC values of 0.164 as shown in Table 12 against *S. pyogenes*. The activity was observed against *S. aureus* with the water extract having a MIC of 3.94 mg/ml. The aqueous and organic *extracts of Ballota africana* also had microbial activity against *K. pneumoniae* with MIC ranges 1.31 – 2.63 mg/ml.

The aqueous extract of *Kigelia africana* showed noteworthy MIC values of 0.985 and 0.984 mg/ml against *S. pyogenes* and *K. pneumoniae* respectively. The same organisms exhibited weakest activity against the acetone extract of *Kigelia africana* as displayed in Table 12 with MIC values ranging from 6.56 – 6.85 mg/ml.

The organic extracts of *Eucalyptus camaldulensis* showed noteworthy activity with organic extract against *S. pyogenes*, specifically the acetone extract which had a MIC value of 0.328 mg/ml and ethanol had a MIC of 0.656 mg/dl. With the aqueous extract of

Eucalyptus camaldulensis, noteworthy activity was observed with MIC value of 0.739mg/ml for *K. pneumoniae*. Antibacterial activity was also observed against *S. aureus* with all plant extracts with MIC values ranging from 1.31 – 2.63 mg/ml.

The ethanol extract of *Acorus gramineus* ethanol extract also had noteworthy activity against *S. aureus* with a MIC value of 0.739 mg/ml. All *A. gramineus* plant extracts exhibited antibacterial activity against all test organisms with MIC values ranging from a minimum of 0.739mg/ml and maximum of 5.25 mg/ml. It is important to take note that each plant extract in this study was tested in triplicate against test organisms, and mean value was calculated to come up with final MIC value, moreover, within these tests a rerun difference of no more than one dilution factor was accepted.

Controls used in the MIC assays; antimicrobial control ciprofloxacin was used at initial concentration of 2.5 mg/ml. This conventional antimicrobial drug was used to serve as a positive control, where it was added to each MIC assay to validate antimicrobial susceptibility of the test organisms.

Some organic extracts like *B. africana*, *D. angustofolia* and *A. gramineus* could not be re-dissolved adequately with 10 % DMSO as a result they were dissolved in 100% DMSO. Since all plant extracts were dissolved in 10 and 100% DMSO, these solvents were added to each MIC assay as a solvent control in order to verify that inhibitory activity observed were from plant extract. In order to check the viability of tested organisms streak plate was prepared to determine purity of culture by identifying single colonies.

4.5.1 MIC values of combination studies

As before, MIC assays were performed independently to determine antimicrobial activity of plants being investigated. The MIC values of the combined plant extracts MIC were determined by broth dilution, and the highest dilution of the plant extract that retained its inhibitory effect with no growth of microorganism was recorded as the MIC value. The antibacterial activities of extract in combination of the plants displayed different degrees of MIC result as shown in Table 13. Results displayed a variety of pharmacological activity, from synergistic, additive, indifferent to antagonistic against the bacterial strains under investigation. Ciprofloxacin was used as the positive control in this study and 10%

DMSO as a negative. 10% DMSO failed to dissolve some plant extracts, it was replaced with 100% as the negative control

The purpose of the combination studies was to determine if there were an increase ineffectiveness of plant extracts when used in combination. Some of these plants were reported to be used in combination in previous papers against bacterial pneumonia and also generally in respiratory ailment (Suliman, 2011). Some plants in this study were used in combination with other plants not included in this paper by other researchers and traditional healers ("Ballota africana herba," n.d.; Mabona, Viljoen, Shikanga, Marston, & Van, 2013; Talika, 2012). For A. gramineus and D. angustofolia plants used in this study there is no literature supporting their use in combination to treat pneumonia or other ailments, it mostly used independently.

Table 13: MIC mean values of plant extracts in combination against test organisms (mg/ml)

Plants extracts	***Staphylococcus aureus***			***Streptococcus pyogenes***			***Klebsiella pneumoniae***		
	W	**E**	**A**	**W**	**E**	**A**	**W**	**E**	**A**
T. sericea + A. gramineus	1.313	1.313	1.313	1.3.13	**0.656**	1.313	2.625	**0.164**	1.313
T. sericea + W. salutaris	1.313	**0.656**	**0.875**	1.313	**0.328**	**0.656**	1.313	**0.656**	**0.875**
T. sericea + E. camaldulensis	1.313	**0.656**	**0.656**	**0.656**	**0.437**	**0.437**	1.313	**0.437**	**0.875**
T. sericea + K. Africana	1.313	1.750	**0.656**	1.313	**0.656**	**0.656**	2.625	1.312	**0.845**
T. sericea + B. Africana	2.625	**0.875**	**0.656**	1.313	**0.219**	**0.437**	1.313	**0.437**	1.313
T. sericea + D. angustofolia	1.313	**0.656**	**0.656**	**0.656**	**0.437**	**0.656**	1.313	**0.219**	**0.875**
B. africana + A. gramineus	5.25	1.313	1.313	5.25	**0.875**	1.313	5.25	1.313	1.313

B. africana + D. angustofolia	5.25	**0.656**	2.625	5.25	**0.875**	1.313	1.313	**0.656**	1.313
B. africana + E. camaldulensis	1.313	1.313	2.625	**0.875**	1.751	1.313	1.313	2.625	**0.609**
B. africana + K. Africana	2.625	2.625	1.313	1.313	2.206	**0.875**	2.625	2.625	2.625
B. africana + W. salutaris	5.25	2.625	1.313	5.25	1.313	2.625	2.625	2.625	1.75
K. africana + E. camaldulensis	2.625	2.625	**0.328**	**0.875**	2.625	**0.656**	1.313	2.625	5.25
K. africana + W. salutaris	5.25	7.0	5.25	2.625	5.2	10.5	6.563	7.0	10.5
K. africana + A. gramineus	2.625	5.25	2.625	2.625	1.313	2.625	5.25	5.25	5.25
K. africana + D. angustofolia	2.625	1.313	1.313	2.625	2.625	**0.328**	2.625	2.625	**0.875**
D. angustofolia + A. gramineus	10.5	1.313	1.313	10.5	1.313	1.313	**0.656**	**0.656**	**0.656**
D. angustofolia + E. camaldulensis	2.625	**0.41**	2.625	1.313	1.313	1.313	1.313	**0.825**	**0.492**
W. salutaris +D. angustofolia	1.313	1.750	**0.875**	5.25	**0.875**	1.313	5.25	**0.656**	1.313
W. salutaris + A. gramineus	5.25	2.625	2.625	5.25	2.625	5.25	5.25	1.313	2.625
W. salutaris +E. camaldulensis	2.625	2.625	2.625	2.625	1.750	2.625	1.313	2.625	3.5
A. gramineus + E. camaldulensis	2.625	1.751	1.313	5.25	1.313	2.625	**0.656**	1.313	**0.656**

Key: E= Ethanol extracts; **A**= Acetone extract; **W** = water/aqueous extract; **Numerical figures** are representing concentration of combined plant extracts at which it was able to inhibit bacterial growth in mg/ml; noteworthy activity values are indicated in bold.

The organic extracts in the combination studies had the highest MIC values in comparison to aqueous extract as shown with values in Table 9. The noteworthy MIC values were observed against all microorganisms under study with plant combination of *T. sericea* + *E. camaldulensis* and *T. sericea* + *D. angustofolia* and *T. sericea* + *A. gramineus* with ranges from 0.164 – 0.875 mg/ml.

Strong to weak antibacterial activity was observed with mixtures of the following plants; *K. africana* + *W. salutaris*, *B. africana* + *W. salutaris*, *K. africana* + *A. gramineus,* and also *A. gramineus* + *W. salutaris*. Their MIC values ranged from 1.313 – 10.5 mg/ml. Aqueous extracts of a number of combinations demonstrated very weak antimicrobial activity. For instance, the water extract of *D. angustofolia* + *A. gramineus* against *S. aureus* and *S. pyogenes* had MIC value of 10.5 mg/dl, the same MIC value was also observed on *K. africana* + *W. salutaris* acetone extract against *S. pyogenes*. Generally organic extracts exhibited good antibacterial activity compared to aqueous extract with all test organisms investigated.

4.6 Fractional inhibitory concentration in 1:1 combination

The ΣFIC values of seven plants which were extracted by distilled water, acetone and ethanol and tested against three pneumonia pathogens which were in 1:1 combination are given in Table 14.

Table 14: FIC values of all plant extracts in various combinations against three strains of organisms

Plant	***Staphylococcus Aureus***			***Streptococcus Pyogenes***			***Klebsiella Pneumoniae***		
	H_2o	Eth	Ace	H_2o	Eth	Ace	H_2O	Eth	Ace
T+A	1.207	2.86	1.835	3.687	23.05	2.792	4.3505	**0.181**	3.585
T+W	1.207	1.1235	**0.973**	3.521	11.4	1.291	2.161	**0.649**	2.434
T+E	1.582	1.125	**0.917**	1.968	15.51	1.485	2.668	**0.567**	2.61
T+K	1.444	3.119	**0.715**	4.104	23	1.312	4.491	1.366	2.967
T+B	2.501	1.911	**0.917**	3.881	8.273	1.263	2.065	**0.483**	3.937
T+D	1.207	1.188	**0.767**	1.749	15.38	1.478	2.031	**0.774**	2.599
B+A	1.167	1.753	1	2.774	3.002	1.668	2.742	**0.5**	**0.75**
B+D	1.167	**0.619**	1.4	2.024	3.069	1.835	1.286	**0.328**	**0.963**
B+E	**0.667**	1.114	2.004	**0.63**	6.675	3.335	1.174	1.5	**0.445**
B+K	1.058	2.404	**0.596**	1.11	7.567	1	1.905	**0.832**	1.2
B+W	1.166	2.221	**0.65**	2.107	4.193	2.92	3.337	1.816	5.723
K+E	1.725	1.176	**0.149**	**0.778**	3	1.084	1.556	1.333	2.316
K+W	1.951	3.12	**0.984**	1.5	2.728	2.333	5.243	1.374	2.442
K+A	**0.975**	4.905	2	1.833	1	1	4.268	1.665	2.852
K+D	1.95	**0.714**	**0.296**	1.458	2.205	**0.167**	3.334	1.134	**0.375**
D+A	2	1.264	**0.7**	2.5	1.103	**0.834**	**0.52**	**0.325**	**0.356**
D+E	1.25	**0.195**	1.4	**0.563**	1.603	2.5	1.889	**0.567**	**0.359**
W+D	**0.25**	**0.829**	**0.467**	**0.583**	**0.527**	**0.625**	11.05	**0.0491**	**0.677**
W+A	1	2.276	1.3	1.333	1.375	1.833	3.127	**0.341**	**0.929**
W+E	1.25	**0.993**	1.3	1.167	1.584	4.25	1.27	1.182	1.852
E+A	1.25	1.518	1	3.01	1.5	4.666	**0.644**	**0.751**	**0.364**

Key: Eth = Ethanol extracts; **Ace** = Acetone extract; **H_2O** = water/aqueous extract; **B** = *Ballota africana*; **A** = *Acorus gramineus*; **W** = *Warburgia salutaris*, **E** = *Eucalyptus camaldulensis*; **D** = *Dodonea angustofolia*; **K** = *Kigelia africana*; **T** = *Terminalia sericea*; **Numerical Figures** are representing the calculated FIC's from individual and combined MIC's; synergistic and additive activity values are indicated in bold.

Out of the 21 combinations of plants tested in this study against the pathogens, varied results were obtained with different combinations and with different plant extracts. Noteworthy antimicrobial activity with synergy was noted with some combinations, while

some combinations were showing additive interactions, as well as indifferent and antagonistic interactions.

The combination of the ethanol extract of *T. sericea* and *A. gramineus* showed noteworthy activity which proved to be synergistic against *K. pneumoniae* with ΣFIC values = 0.181. The remaining results with this combination showed indifferent results with all extracts in all three organisms.

When combined, *T. sericea* + *W. salutaris* showed broad spectrum antimicrobial activity, though additive interactions were only portrayed with ethanol extract against *K. pneumoniae* and acetone extract against *S. aureus.* The rest of organic extracts of this combination displayed indifferent interactions to all three organisms except *S. aureus* with ethanol extract which demonstrated antagonistic interactions.

Noteworthy antimicrobial activity was reported with the ethanol combination of *T. sericea* and *E. camaldulensis* for *K. pneumoniae* with ΣFIC value of .567, *and* acetone extract for *S. aureus* with calculated ΣFIC of 0.917, both depicting additive interactions. The rest of the results displayed indifferent values in all extracts against all organisms, excluding ethanol extract on S*. pyogenes* which had antagonistic interactions (ΣFIC value = 15.51).

0ut of *T. sericea* and *K. africana* plant combination extracts, additive interactions were observed with acetone extracts for *S. aureus* with ΣFIV value of 0.715, but no synergy was observed on this combination. The remaining extracts of this combination had non-interactive antimicrobial activity with ΣFIC values ranging from 1.312 – 3.119, but water and ethanol extract demonstrated antagonistic reactions with ΣFIC range value = 4.104-23.

The combined effect of *T. sericea* and *B. africana* ethanol extract showed synergistic effect on *K. pneumoniae*. Acetone extract on *S. aureus* demonstrated additive interactions. All aqueous and acetone extracts of this combination when tested for *K. pneumoniae* and *S. pyogenes* demonstrated indifferent activity, same as ethanol extract on *S. aureus* and *S. pyogenes*.

Ethanol extract of *T. sericea* and *D. angustofolia* combination for *K. pneumoniae* and *S. aureus* acetone extract showed additive reactions with ΣFIC value = 0.774 and 0.767

respectively, but the rest of plant extracts displayed indifferent antimicrobial interactions expect ethanol extract on *S. pyogenes* which had antagonistic interactions with ΣFIC value =15.38.

Both plant combinations of *B. africana* + *A. gramineus and B. africana* + *K. africana* displayed additive interactions with ΣFIV value ranging 0.596 – 1.0 with ethanol and acetone extracts on *S. aureus*, *S. pyogenes* as well as *K. pneumoniae.* Moreover, *K. pneumoniae* demonstrated synergistic interactions with *A. gramineus* and *B. africana* ethanol extract with ΣFIC value of 0.5. Both combination also showed indifferent interactions against all test organisms, with the rest of plant extracts.

When testing *B. africana* and *D. angustofolia* against all bacteria test strains, ethanol extract on *K. pneumoniae* proved to be synergistic with ΣFIC value of 0.328 (Table 14). Additive interaction was observed with ethanol extract combination for *S. aureus* and acetone extract for *K*. *pneumoniae* respectively.

Worth noting antibacterial activity was reported with *Ballota africana* and *Eucalyptus camaldulensis* combination acetone extract on *K. pneumoniae*. Aqueous extract of the same combinations displayed additive interactions against *S. aureus* and *S. pyogenes*. Ethanol extracts showed antagonistic interactions with ΣFIC value = 6.675 against *S. pyogenes* but the rest of extract combinations had indifferent interactions with test pathogens.

These combinations; *Ballota africana* and *Warburgia salutaris*, *Kigelia africana* and *Warbugia salutaris,* portrayed additive interactions with acetone extract on *S. aureus*. The rest of the results were indifferent plant with all three organisms (Table 14).

The combination of *Kigelia* and *Eucalyptus* showed noteworthy antimicrobial activity with synergistic interaction with acetone extract on *S. aureus* and ΣFIC value was reported, while water extract of this combination demonstrated additive interaction on S. Pyogenes, but the rest of results showed indifferent interactions with all organisms.

On combining the water extracts of *K. africana* and *A. gramineus,* good antimicrobial activity was observed which proved to be additive against both *S. aureus* and *S. pyogenes* with ΣFIC values of 0.975 and 1.0 respectively. Ethanol extract combinations against *S.*

pyogenes also demonstrated additive interactions but the rest of extracts demonstrated indifferent interactions.

Both organic extracts of the *Kigelia africana* and *Dodonea angustofolia* combination displayed strong antibacterial activity against *S. aureus* with ΣFIC values of 0.296 (synergistic) and 0.714 (additive) for acetone extract and ethanol extract respectively. Synergistic interactions were also observed with acetone extract against *S. pyogenes* with ΣFIC value of 0.167 and 0.375 with *K. pneumoniae*, the rest of extracts in all tested pathogens had indifferent interactions.

K. pneumoniae was strongly inhibited by all extract of the *Dodonea angustofolia* and *Acorus gramineus* combination. The water extract demonstrated an additive interaction (ΣFIC value = 0.52). Acetone and ethanol plant extracts had synergistic interactions with ΣFIC values of 0.325 and 0.356 respectively. *S. aureus* and *S. pyogenes* both displayed additive interactions with acetone extracts but the rest showed indifferent interactions with these organisms.

The Combination of *D. angustofolia* and *E. camaldulensis* showed noteworthy antibacterial activity against pneumonia pathogens in this study. Synergistic interactions were observed with ethanol and acetone extract of this combination. Against *S. aureus* and *K. pneumoniae* with ΣFIC values of 0.195 and 0.359 respectively. Additive interactions were observed for *S. pyogenes* and *K. pneumoniae* with ethanol extract of the combination of D*. angustofolia* and *E. camaldulensis* but the rest of plant extracts demonstrated indifferent interactions with all three organisms (Table 14).

The most interesting synergistic interactions at the ΣFIC value of 0.0491 with *Warburgia salutaris* and *Dodonea angustofolia* was detected against *K. pneumoniae*. Synergistic interaction was also noted with water and acetone extract against *S. aureus* with ΣFIC values of 0.25 and 0.467 respectively. Additive interactions were observed for *Warburgia salutaris* and *Dodonea angustofolia* combination with ethanol extract against *S. aureus* and *S. pyogenes*, water extract for *S. pyogenes* and acetone extract against *K. pneumoniae*. Antagonistic interactions were observed with water extract of this combination against *S. aureus*.

A combination of *Warburgia salutaris* and *Acorus gramineus* showed major antibacterial activity. For the ethanol extract when screened against *K. pneumoniae,* synergistic interaction was observed with ΣFIC value of 0.341. An additive interaction was noted for *S. aureus* with water extract and *K. pneumoniae* with acetone extract. However, the rest of the extracts displayed indifferent interactions.

The Combination of *Warburgia salutaris* and *Eucalyptus camaldulensis* exhibited antibacterial activity and a ΣFIC value of 0.993 was found with ethanol extract against *S. aureus.* But the remaining plant extracts displayed indifferent interactions with all pneumonia test pathogens.

When testing the acetone extracts of *Eucalyptus camaldulensis* and *Acorus gramineus* against *K. pneumoniae*, synergistic interaction was depicted with ΣFIC value = 0.364. However, ethanol and water extracts displayed additive interactions against *K. pneumoniae*. Additive interaction was also observed with acetone extract against *S. aureus*. All the plant extract combinations against the three test organism displayed results which all fall within the non-interactive range.

CHAPTER FIVE
DISCUSSIONS

5.1 Introduction

Seven medicinal plants namely *Terminalia sericea, Warburgia salutaris, Ballota africana, Kigelia africana, Acorus gramineus, Dodonea angustofolia*, and *Eucalyptus camaldulensis* were used in this study. As discussed in chapter three, these plants were extracted with aqueous and organic solvents, which were eventually re-dissolved in 10% and 100% DMSO. Thereafter they were analysed for their antimicrobial activity against pneumonia pathogens precisely *staphylococcus aureus, Streptococcus pyogenes*, and *Klebsiella pneumonia*. Information related to extract reaction on tested organism for antimicrobial activity which was reported in chapter 4 will be further discussed in this chapter in relation to previous studies where these plants or similar ones were used. Some of the plants used in this study have interesting records that are locally vital and widely used but little or nothing has been recorded about them in scientific literature. As expressed in chapter 3, the following methods: agar well diffusion, microtiter dilution method were used for the current study to come up with plant extract antimicrobial efficacy.

The purpose of the study was to determine the prospective antimicrobial activity of the plants under study, which are traditionally used to treat pneumonia. With the widespread problem of bacterial resistance development to antibiotics, there is an urge to look for new potential antimicrobials(Van Vuuren, 2010). Results obtained in this study showed that organic plant extracts relatively had higher inhibitory antimicrobial activity when compared with aqueous extract. As related to previous antimicrobial studies, Bamuamba et al, in agreement with van Vuuren and Naidoo, Kerinki and Nyiroge, Ncube et al., (as cited in Talika,2012) suggested that in scenarios where water was used for extraction it showed poor or no inhibitory activity, since it is too polar to efficiently dissolve organic elements derived from plant components. In chapter two each plant was discussed based on their chemical constituency, medicinal use, and originality value of the incident and possible new uses that haven't been provided in harmony with list of critical literature references.

Agar well diffusion assay which was preferred method in this study for testing antimicrobial activity for plant extract showed interesting zones of inhibition and poor inhibitions with some of the plant extracts.

5.2 Susceptibility testing of individual plants

The aqueous and organic extracts of *Terminalia sericea* showed very good antimicrobial activity against all test organism with zones of inhibition ranging from 22-27.75 mm. In all three test organisms, organic extracts had a slightly large inhibition zone compared to aqueous extracts. *S. aureus* demonstrated very good susceptibility activity among the three organisms with *Terminalia sericea*. MIC values obtained for this plant also demonstrated noteworthy antimicrobial activity with all test bacteria strains, eventually *S. pyogenes* reporting high MIC values. *Terminalia sericea* was the strongest /or potent inhibitor of all seven plants under study with each and every extract against all tested organisms. MIC values ranging from 0.0144-0.607 mg/ml were reported after calculating mean values from triplicates results at a concentration of extracts from 0.0821 to 10.5 mg/ml.

Eyhrqust et al., Mushi and Mbwambo, Eldeen *et al*: a, Eldeen *et al*: b (as cited in Lembede, 2014) reported that both aqueous and organic decoction extracts of *T. sericea* displayed antimicrobial activity against *S. aureus, S. epidermidis, S. pyogenes*, *E. aerogenes, B. subtlis, M. luteus, E. coli, K. pneumonia* and *Sacini specia.* Apart from the organisms mentioned above its antifungal activity against *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigates, Cryptococcus neorformans, Microsporium canis*, and *Sporothrix schenckii* (Eldeen *et al* & Fyhrqustet *et al* as cited in Lambede, 2014) described the potentiation of *T. sericea* antimicrobial activity to be due to presence of triperpenesaponins, tannins, flavonoids and aligananon B.

In previous study, Cock and Vuuren (2014) about African plants with anti-Klebsiella activity for the treatment and prevention of ion Ankylosing Spondylitis, *Terminalia sericea* methanol and water extracts were potent inhibitors of *K. pneumoniae* with ZOIs of 13±0 and 12.0±1.0 mm respectively. Unlike other plants that were used in that study *T*. *sericea* were amongst the most potent inhibitors of *K. pneumoniae* growth. *Terminalia sericea* has been extensively studied by many researchers especially for conditions associated

with diarrhea and respiratory ailments (Suliman 2010). Mabira et al, (2013) reported that *T. sericea* display mostly worthy noting broad spectrum antimicrobial efficacy against skin conditions related organisms used in the study, supporting its folkloric use for dermatological related ailments.

As discussed by Cock and Vuuren (2014), anti-*Proteus* activity of some medicinal plants, such as *Terminalia sericea* were evaluated, and displayed outstanding antibacterial activity with inhibition zones >10 mm. It was also reported that T. *sericea* and *W. salutaris* have sesuiterponoids (including polygidal and tannins) which are known to have antibacterial activity. Kwanyama (2013) also concurred with the results for the current study, since noteworthy activity results to six pathogens with MIC range 0.04-1.00 mg/ml were reported. Talika (2010) also reported the antimicrobial activity of *Terminalia sericea* exhibited in bark extracts against *S. aureus*. Therefore, based on results obtained from previous and current study we can easily stipulate that, almost all plant parts of *Terminalia sericea* have some antibacterial activity against Pneumonia causing pathogens.

Eucalyptus camaldulensis, showed good antimicrobial activity having highest inhibition zone of 24mm with acetone extracts but overall all three organism displayed good antibacterial activity with ZOI ranging from18.7 – 24mm. A worthy noting activity with *E*. *camaldulensis,* MIC value of 0.325 mg/dl against *S. Pyogenes* with acetone extract was observed. Based on results obtained for the present study it indicated that *E. camaldulensis* can work better in some organisms at higher dosages as an antimicrobial agent in treatment of bacterial pneumonia, because extracts displayed different antibacterial activity with different organisms.

Behbahan and Yadzi (2013,) found *E. camaldulensis* ethanol and aqueous extract to exhibit good antimicrobial activity against *S. aureus* with ZOIs average diameter of 20.2 mm at (20 mg/ml) 22 mm at (40 mg/ml) 25.1 mm (60 mg/ml) 26.7 mm at (80 mg/ml), whilst 15 mm, 19 mm, 21 mm, and 24.5 mm were also observed at the same working concentration as above. When tested against *S. aureus* for MIC determination ethanol extract demonstrated half down equal MIC value, and least MIC values were reported on remaining organisms involved in the study. In a previous study Ayepola and Adeniyi (2008) revealed that the methanol extracts, dichloromethane fraction and the methanol

residue showed varied degrees of bacteria inhibition on *S. aureus, B. subtlis, P. aeruginosa*, *K. pneumoniae* and *Y. enterocolitica*. The MIC of methanol extract and dichloromethane fraction which ranged from between 0.04-0.079 mg/ml correlated to current study which also demonstrated good antimicrobial activity with some extracts.

Jouda (2013), observed antibacterial activity of *E. camaldulensis* ethanol extract against *S. aureus* with ZOI of 13 mm, which was corresponding to current study which reported 20 mm with same organism. As to aqueous extract results were discordant with current study, Jouda (2013) observed ZOI of 8 mm and 20.7 mm was reported in present study. Lowest MIC with ethanol extract was from 6.25 mg/ml according to Jouda (2013), 2.63 mg/ml with the same extract against *S. aureus*. This conformity of result can be due to varied concentration used, growing conditions, extraction methods and so many parameters.

Kigelia africana extracts had antibacterial activity against all three organisms involved in this study with ZOIs ranging from 13 – 18.5 mm and interesting MIC values, which showed noteworthy antimicrobial activity of 0.985 mg/ml and 0.984 for *S. pyogenes* and *K. pneumonia* with aqueous extract: supporting its use traditionally for the treatment of different ailment. In a study Abdulkadir (2015), discovered that *K. africana* chloroform, methane and aqueous extracts displayed pronounced activity against *S. aureus*, compared to *K. pneumoniae* and other organisms involved in the study. Based on that research K. africana was found to be rich in flavonoids and phenolic compounds which are assumed to be responsible for their antimicrobial activity and antioxidant potential.

In another study Owalabi and Omogbai (2007), reported that *K*. *africana* at 20mg/ml showed inhibitory zone of 15.0 ± 0.95 mm with ethanolic extract, which is in alignment with present study which had 15.6 – 16.3 mm with all extracts against *S. aureus*. In that same study antifungal activity was observed for *Candida albicans* with 20.25 ± 4.60 mm ZOI, but the rest of organisms like *E. coli* and *P. aeruginosa* did not show any susceptible activity with *K. africana*. MIC values of 6.25mg/ml and 7.92 mg/ml were observed on *S. aureus* and *Candida albicans* respectively, while in the current study MIC values ranging from 0.984-6.83 mg/ml were reported with all three extracts against all test pathogens.

Naidoo and van Vuuren (2013), examined antimicrobial activity of *K. africana* in bacterial and fungal pathogens, and found broad spectrum activity with MIC value ranging from 0.25 – 16 mg/ml with organic and aqueous extract.

It has been noted that aqueous and organic of (methanol and acetone) *K. africana* demonstrated good antimicrobial activity in the following organisms, *E. coli, S. aureus*, *MRSA*, *S. flexneri* and activity was observed with *S. typhi*. MIC values ranging from 0.139 – 5.54 mg/ml with *Kigelia africana,* which corresponds to the present study whereby MIC values of 0.984 – 6.56 mg/ml were reported, hence concurring that the use of *Kigelia africana* for potential antimicrobial effect (Nkosi, 2013).

Acorus gramineus in this study demonstrated good antimicrobial activity against all three organisms with both water and organic extracts. Ethanol extract displayed good results compared to aqueous extracts, and noteworthy activity was observed with ethanol extract against *S. aureus* whilst the rest of MIC results indicate good antibacterial activity. Lee et al., (as cited in Asha and Ganjewala (2009), reported *A. graminieus* rhizome (methanol extract) to have antimicrobial and antifungal properties particularly against phytopathogenic fungi, due to the presence of α-asarone and asaronaldehyde compounds. Arm (n.d) also reported *A. gramineus* to have antibacterial activity against *S. aureus*, *Streptococci* and *Mycobacterium* which is similar to the present study whereby *S. aureus* and *S. pyogenes* growth were inhibited. Based on information encountered in search of literature, few scientific studies have been reported by researchers about A. gramineus, compared to *Acorus calamus* which has been studied intensively. Initially *Acorus calamus* was planned to be used for this current study, but due to difficulty in accessing it in Eastern Cape and near provinces the hybrid species *Acorus gramineus* was used instead.

According to results obtained in this study acetone and aqueous extracts of *D. angustofolia* showed antimicrobial activity with ZOI ranging from 11 – 15.6 mm, while ethanol extract of *Dodonea angustofolia* did not inhibit *K. pneumoniae* and *S. pyogenes*. This observation was probably due to polarity of plant extracts since it was dissolved in 100% DMSO and the particles were unable to diffuse in Muller Hinton agar, as compared with MIC assay where all extracts showed antibacterial activity with all organisms. Worth

noting antimicrobial activity was observed with water extract against *K. pneumoniae*, and yet same aqueous extract had weak antimicrobial activity for *S. aureus* and *S. pyogenes* with MIC value 5.25 and 10.5 mg/ml respectively. Organic extracts of *D. angustofolia* showed some good antimicrobial activity with all bacterial pathogens.

According to Mohlakoana (2010), *D. angustofolia* exhibit some antimicrobial activity against *B. cereus* but other organisms like *S. aureus*, *K. pneumoniae* which were also involved in the study do not show any activity which is in contrary to the current study where antimicrobial activity was reported to these organisms. Gemeda et al (2011), reported that *D. angustofolia* had bioactive compounds responsible for their antibacterial activity. Water solvents were reported to have slight antibacterial prospective, followed by hexane extractant, yet dichloromethane, acetone and methanol extractant they portion out resemblance in bioactive compounds on bio-autographs, and extracted the highest number of antibacterial compounds with variety of polarities.

Ballota africana displayed good antibacterial activity with all three organisms and noteworthy activity was observed with organic extracts against *S. pyogenes* with ZOI 30 mm, but water and acetone extracts of *B. africana* did not show any antibacterial activity with *S. pyogenes* and *K. pneumoniae*. *S. aureus* were inhibited by both water and ethanol extract of the plant. Surprisingly MIC values showed noteworthy antimicrobial activity of 0.164mg/ml and 0.492 mg/ml with ethanol and acetone extract for *S. pyogenes*. Good antibacterial activity was also seen on water extract with MIC of 1.48 mg/ml. *B. africana* demonstrated a worthy noting activity with ethanol extract which had 0.768 mg/ml, and acetone and water extract also demonstrated good antimicrobial activity. *K. pneumonia* which was only inhibited by ethanol extract on sensitivity test, in MIC assay *B. africana* revealed best antimicrobial activity with values ranging from 1.31 – 2.30 mg/ml with all extracts. Discrepancies due to concentration of extract solvent, in this case were caused DMSO dilution, because just like *D. angustofolia* and *A. gramineus* organic extracts, *B. africana* organic extracts couldn't be dissolved in 10% DMSO as a result 100% DMSO was used instead, which explains the possibility of polar compounds or molecules which were not able to diffuse in solid agar media and yet were able to show inhibitory activity with microtiter technique. In this current study it was observed that results obtained

through agar well diffusion for antimicrobial sensitivity and microtiter dilution method for MIC, gave incongruent and confusing result when organic plant extracts dissolved with 100% DMSO were used against test organisms. Results obtained from other plants extracts in the same study like *Acorus gramineus* and *Dodonea angustofolia* whose organic extracts were also dissolved in 100% DMSO have proved that correlation of methodologies in determining plant antimicrobial activity doesn't give inconsistent results all the time. As explained by Chinyama (2009), several variables impact outcome of results, like environment, climatic conditions, choice of plant extracts, extraction method, antimicrobial test method, and test organisms. Furthermore, factors such as technique, culture medium, strain of bacteria used, and age of agar plate, plant source, whether used dried or fresh, quality of extract tested can be attributed. The explanation of possible causes of changes was concluded by saying that there's no standard method for expressing the antibacterial test result (Chinyama, 2009).

B. africana leaf methanol extract was found to be less potent inhibitor of *K. pneumoniae* in a study where South African plants with anti-Klebsiella activity for the treatment and prevention of ankylosing spondylitis were being investigated. Aqueous and methanol leaf extract of *B. africana* exhibited potent antimicrobial activity with MIC value normally below 700 mg/ml, which was contrary to the lower efficacy seen in disc diffusion screening. It was further suggested that antimicrobial compounds in these plants might be either large or low polarity and diffuse less freely in the agar gel (Cock & van Vuuren, 2014).

In previous study *B. africana* had some good antimicrobial activity against *S. Pyogenes* with range of MIC results from 3.125 – 12.5 mg/ml with the following extracts: petroleum ether, dichloromethane ether, water and ethanol. *K. pneumoniae* had MIC value ranging from 1.56 - >12.5 mg/ml with the same extracts above, and *S. aureus* had noteworthy activity with 80% ethanol and dichloromethane ether with MIC value of 0.39 mg/ml but water and petroleum ether displayed poor MIC value which is in alignment with current study whereby good antibacterial activity was observed with organic extract compared to aqueous extract (Motlhatlego, 2014).

Out of the seven plants tested in this present study *Warbugia salutaris* was found to have weak antimicrobial activity against *K. pneumoniae*, since ZOI was only observed on

ethanol extract, while water and acetone extract did not display any activity. *S. pyogenes* surprisingly was inhibited by all *Warbugia salutaris* extracts and strong inhibition was recorded on ethanol extract. Antimicrobial activity was also displayed on *A. aureus* by *W. salutaris* water and ethanol extract. Conversely, all three test pathogens were reported to be inhibited with *W. salutaris* with microdilution method having MIC values ranging from 1.72 – 7.22 mg/ml.

In a previous study Jager (2003) found that *W. salutaris* aqueous extract exhibited good antimicrobial activity against gram positive bacteria (*S. aureus ATCC 6538, Bacillus subtlis ATCC 6633*) and methanol extract showed activity against *E. coli.* Rabe and van Staden (as cited in Vuuren, 2008), stated that *Warbugia salutaris* have a compound called muzigadial, which demonstrated antibacterial activity against *S. aureus, B. subtlis, E. coli, S. epidermidis* and *M. luteus* with MIC values ranging from 12.5 – 100 mg/ml. the same applies to the current study whereby antimicrobial activity against three test strain of organisms was observed by the same plant with three different extracts.

W. salutaris was also involved in dermatological relevance plants with medicinal values research where it was found to have noteworthy antimicrobial activity with MIC values ranging in between 0.03 > 16 mg/mi against organisms that were involved in that study. Both bark and leaf parts of *Warburgia salutaris* extracts were used in that study reported antimicrobial worthy noting activities especially observed with bark extract compared to leaf extract (Mabona et al., 2013).These results were in agreement with Rabe and Van Staden (1997), report of noteworthy activity for *W. Salutaris* with methanol extract against *S. aureus*, contrary to results reported by these authors and the current study as well. Leonard and Viljoen (2015), agree with Steenkamp et-al (2005) who observed no antibacterial activity for the aqueous and methanol extracts of *W. salutaris* against *S. epidermidis* and *S. aureus*. Results obtained in these studies displayed very good antimicrobial activity compared to results obtained in this current study which justify the need for further scientific validation of this plant for clinical application. The biological activity of *W. salutaris* based on previous studies conducted, was found to be attributed to the drimane, sesquiterpernoids, including polygodial, warbuganal, muzigadial, muka

dial, and ugandensial, flavonoid and miscellaneous compounds present (Leonard & Viljoen, 2015).

5.3 Combination studies

In-vitro interactions between extracts of *A. gramineus*, *W. salutaris*, *T. sericea*, *D. angustofolia*, *K. africana*, *B. africana* and *E. camaldulensis* were determined using agar well diffusion assay and microdilution method.

As shown in table 3, *K. africana* and *T. sericea* demonstrated some antimicrobial activity with all extracts, and ZOI ranging from 23-27 mm which was not far from *K. africana* and *E. camaldulensis* which had 19.25-25.3 mm. Their MIC's exhibited noteworthy antimicrobial activity for *S. aureus* and *K. pneumoniae* with acetone extract, but *S. pyogenes* showed noteworthy activity with MIC of 0.656 mg/ml with both acetone and ethanol with *K. africana* and *T. sericea* combination. In contrast *K. africana* and *E. camaldulensis* showed noteworthy antimicrobial activity against *S. aureus* with acetone extracts as well as on *S. pyogenes* with acetone and water extracts. The rest of plant extracts also displayed some antimicrobial activity with all three test bacterial pathogens. Since results for some combinations were equal or equivalent to single plant tested FIC's was done in order to calculate interactions between these plant combinations. *T. sericea* and *K. africana* only displayed additive outcome for *S. aureus* with acetone extract whereby FIC value of 0.767 was reported. The rest displayed indifferent antagonistic interactions which explains that these plants, especially *T. sericea* works better when used individually, pertaining to ZOI and MIC values obtained on individual plant.

T. sericea leaf extract according to Talika (2012), inhibited microorganisms in combination with *L. javanica*, *C. molle* at a 1:1:1 ratio against *C. neorformans*, *K. pneumoniae*, *M. catarrhalis, S. smigematis* and *S. aureus* with ∑FIC range of 0.64 – 4.83 reported.

K. africana and *E. camaldulensis* combination demonstrated noteworthy antimicrobial activity with acetone extract against *S. aureus* and *S. pyogenes*, but aqueous extract had noteworthy activity with *S. pyogenes* as well. After calculating ∑FIC, synergistic interactions ware only observed with acetone extract against *S. aureus*, while aqueous

extract displayed additive interaction against *S. pyogenes*, the rest had non-interactive interactions.

Combination of *Dodonea salutaris* + *Ballota africana* and *Ballota africana* + *Warburgia salutaris* in sensitivity testing did not show any antimicrobial activity against *K. pneumoniae*, same with aqueous extract which did not display any activity against *S. pyogenes* with both combinations. Only aqueous extract of Dodonea and Ballota had some antimicrobial activity, while Ballota and Warbugia aqueous extract did not display any activity.

MIC value of *Ballota africana* and *Dodonea angustofolia* combination demonstrated noteworthy antimicrobial activity with ethanol extract in all three organisms while the remaining extracts showed good antimicrobial activity as well. *Ballota africana* and *Warbugia salutaris* had microbial activity with all organisms with MIC values ranging from 1.313 – 5.25 mg/ml. ΣFIC's showed synergistic interactions with *Ballota africana* and *Dodonea angustofolia* ethanol extract for *K. pneumoniae*, and additive interactions were observed with ethanol for *S. aureus* and acetone for *K. pneumoniae*. *Ballota africana* and *Warbugia salutaris* combination had additive interaction with acetone extract for S. aureus but the rest of the extracts had indifference reaction.

B. africana is traditionally used in combination with *Sativa* species to treat measles and fevers in "*khoi khoi*" and "*san"* remedy, while in other circumstances it is combined with *Valeriana capensisor* or *Stachys hispida* for treatment of insomnia or nervous stress ("Ballota africana herba," n.d.).

The combination of *Warburgia salutaris* and *Dodonea angustofolia* interacted synergistically against all three organisms at least with one or two plant extract; for *S. aureus* water and acetone displayed synergy interactions; for *S. pyogenes* acetone extract and for *K. pneumoniae* ethanol extract showed synergy interactions. While some remaining extracts in the combination displayed additive interactions, antagonistic interaction was highly observed with water extract for *K. pneumoniae*. *Warbugia salutaris* and *Dodonea angustofolia* combination was more potent inhibitor against all organisms involved in this study than all other plant combination interaction. On sensitivity testing

however, aqueous extract on both *S. aureus* and *K. pneumoniae* did not show any antibacterial activity, as well as acetone extract on *K. pneumoniae*.

Kigelia africana and *Warburgia salutaris*, *Kigelia africana* and *Acorus gramineus* combinations also showed some inhibition based on zones of inhibition ranging from 12-23 mm and 12-15.2 mm respectively, and MIC's result also demonstrated good antimicrobial activity. ΣFIC result of *Kigelia africana* and *Warbugia salutaris* acetone extract displayed additive interaction for *S. aureus*, but rest of the ΣFIC's values showed indifferent and antagonistic results. *Kigelia africana* and *Acorus gramineus* had ΣFIC values of 0.975-1 indicating additive interaction with water extract for *S. aureus* and *S. pyogenes* as well as ethanol extract for *S. pyogenes*. Rest of results with all plant extracts for the three organisms displayed indifferent interactions.

Eucalyptus camaldulensis and *Acorus gramineus* had good antimicrobial activity, sensitivity showed ZOI ranging from 19.8-27 mm. Noteworthy activity on MIC's was observed with water and acetone extract against *K. pneumoniae* with reported MIC value of 0.656 mg/ml, and the other extracts also had effective antimicrobial activity with all three bacterial pathogens. The calculated ΣFIC as revealed in table 5, depicted synergistic interaction with acetone extract on *K. pneumoniae* and additive interaction was observed with water and acetone extract for *K. pneumoniae* as well as acetone extract for *S. aureus*.

Terminalia sericea and *Eucalyptus camaldulensis*, *Terminalia sericea* and *Dodonea angustofolia, Terminalia sericea* and *Ballota africana* showed good antibacterial activity against all three bacterial pathogens with ZOI ranging from 21-35 mm and MIC values from 0.219-2.625 mg/ml. Noteworthy antibacterial activity was observed with organic extracts of all this three plant combinations with tested organisms, while MIC values for aqueous extract were potent with the weakest MIC value of 2.625 mg/ml reported. *S. aureus* and *K. pneumoniae* showed additive interactions with acetone and ethanol extracts respectively, except *Terminalia sericea* and *Ballota africana* combination ethanol extract which displayed synergistic interactions. Water extract of all these three combinations displayed indifferent interaction with test organisms (ΣFIC value of 1.3132-2.265).

Dodonea angustofolia and *Acorus gramineus*, *Terminalia sericea* and *Acorus gramineus*, and *Ballota africana* and *Acorus gramineus* demonstrated good antimicrobial activity with all three test pathogens although aqueous extract did not show any activity with *Acorus gramineus* and *Dodonea angustofolia* against *K. pneumoniae* also *Ballota africana* and *Acorus gramineus* on *S. aureus.*

Terminalia sericea and *Acorus gramineus* aqueous extract did not show any inhibition for *K. pneumoniae* as well. *Dodonea angustofolia* and *Acorus gramineus* exerted noteworthy antibacterial activity effects against *K. pneumoniae* with all extracts but some good antibacterial activity was also observed on *S. aureus* and *S. pyogenes*. Similar results were seen with calculated ΣFIC which showed synergistic interactions with organic extracts for *K. pneumoniae* and additive interactions were observed with aqueous extract with the same organism.

Additive interaction with *Dodonea angustofolia* and *Acorus gramineus* was also displayed with ethanol extracts against *S. aureus* and *S. pyogenes*, while the rest of extracts showed indifferent effects with the same organisms. *Ballota africana* and *Acorus gramineus* had noteworthy antibacterial activity against *S. pyogenes* with ethanol extract, but synergistic interactions were obtained against *K. pneumoniae* and additive interactions were seen with acetone extract against *S. aureus* and *K. pneumoniae*. The MIC values of *Terminalia sericea* and *Acorus gramineus* as presented in table 5, showed good antibacterial activity against three test organisms with MIC values ranging from 0.164 – 2.625 mg/ml. synergistic interactions were obtained against *K. pneumoniae* with ethanol extract (ΣFIC value of 0.181). *S. aureus* and *K. pneumoniae* exhibited antagonistic interaction with aqueous extract, and indifferent interaction were observed with the rest of the plant extracts.

Ballota africana and *Kigelia africana* combination showed highest microbial susceptibility with test organism which was in congruity with *Warbugia salutaris* and *Eucalyptus camaldulensis* having equivalent inhibition patterns. *Ballota africana* and *Kigelia africana* did not had any inhibition against *K. pneumoniae* with water extract. *Ballota africana* and *Kigelia africana* noteworthy antimicrobial activity was seen with acetone extract against

S. pyogenes, whereby mean MIC value ranging from 1.313 – 2.625 mg/ml were obtained with the rest of extracts combination against all three test organism.

In polyherbal preparation, ADD- 199, *K. africana* was used in combination with three other plants*: Maytenus senegalensis, Annona senegalensis,* and *Lannea welwitschii.* This combination was found to have antidiabetic and antioxidant activities (Atawodi & Olowoniyi, 2015). In another study *K. africana* was combined with *Hypoxis hemerocallidea* and tested against *C. albicans, U. urealytcum, O. ureolytica, G. vaginalis, N. gonorrhoea* and good antimicrobial results were displayed with MIC values ranging from 0.75 - >16 mg/ml with ∑FICs of 0.47 – 7.88*.* In the same study *K. africana* combined with *P. africanum, O. engleri and S. seratuloides* yielded MIC range of 0.75 - >16mg/ml and ∑FIC value of 0.31- 32 against the mentioned sexually transmitted infectious agents (van Vuuren & Naidoo, 2013).

Warbugia salutaris and *Eucalyptus camaldulensis* combination was also active in inhibiting all pathogens tested with mean MIC value ranging from 1.313 – 3.5 mg/ml and was considered good antimicrobial inhibitor. ΣFIC values calculated from the combination of *Ballota africana* and *Kigelia africana*, *Warbugia salutaris* and *Eucalyptus camaldulensis* against all pathogens tested ranged from 0.596 – 7.567, and from 0.993 – 4.25 respectively. As shown with results above *Ballota africana* and *Kigelia africana* combination had additive interactions for *S. aureus* with acetone extract, *K. pneumoniae* with ethanol extract, and for *S. pyogenes* with acetone extract. *Warbugia salutaris* and *Eucalyptus camaldulensis* combination ethanol extract also displayed additive interactions for *S. aureus*. Antagonistic interactions were observed with *Warbugia salutaris* and *Eucalyptus camaldulensis* acetone extract for *S. pyogenes*, and *Ballota africana* and *Kigelia africana* ethanol extract for *S. pyogenes* as well.

Sensitivity test for *Acorus gramineus* and *Warbugia salutaris* combination showed some good antimicrobial activity with organic extract compared to aqueous extract. The combination showed great antimicrobial activity against all three test organism with range of mean MIC value of 1.313 – 5.25 mg/ml. ΣFIC calculated from Acorus *gramineus* and *Warbugia salutaris* combination against test organism in this study ranged from 0.341 – 3.127.

This indicate that this combination had the synergistic interaction for K. pneumoniae with ethanol extract. Two pathogens namely *K. pneumoniae* and *S. aureus* showed additive interactions with acetone (ΣFIC 0.929) and water (ΣFIC 1.0) extracts of this combination respectively. The rest of the extracts demonstrated indifferent interactions against organisms tested.

W. salutaris has been reported by Hutchings (as cited in Mabona, 2013) that when combined with *Hibiscus surattensis* it is a useful treatment for sores and skin imitations. *W. salutaris* is smoked with *Cannabis sativa* for upper respiratory tract infections. Bark or root powder is known to be smoked with other plants and the smoke is inhaled for expectorant and/or dry cough treatment (Leonard & Viljoen, 2015).

When combined *Dodonea angustofolia* and *Eucalyptus camaldulensis* extracts showed some good antimicrobial activity against pneumonia pathogens with ZOI ranging from 21 – 25 mm. Noteworthy antibacterial activity was observed with organic extracts (acetone and ethanol) for *K. pneumoniae*, and with ethanol extract for *S. aureus*. ΣFIC value of 0.195 and 0.359 were noted for *S. aureus* with ethanol extract and *K. pneumoniae* with acetone extract respectively indicating the synergistic interactions. An additive interaction was seen for *S. pyogenes* and *K. pneumoniae* with ethanol extract. The rest of the *Dodonea Eucalyptus* extracts results displayed indifferent interaction with the pathogens tested, no antagonistic interactions was observed.

S. aureus was inhibited with all *Kigelia africana* and *Dodonea angustofolia* extracts. *S. pyogenes* growth was inhibited with water and acetone extract, whilst *K. pneumoniae* was inhibited by only water extract. The MIC values observed as shown in table 5, combination of *Kigelia africana* and *Dodonea angustofolia* showed good antimicrobial activity with all three organisms even with those extracts combination which did not show any activity in initial sensitivity testing. Noteworthy and actually best antimicrobial activities were seen for this combination against *S. pyogenes* with acetone extract with mean MIC value of 0.328 mg/ml and also for *K. pneumoniae* MIC value 0.875 mg/ml. But rest of the extracts in this combination can be classified as good inhibitors to all test organisms with highest MIC reported as 2.625mg/ml. ΣFIC calculated showed synergistic interaction for all the pathogens tested with acetone extract ranging from 0.167 – 0.375. An additive interaction

was noted against *S. aureus* with ethanol extract with a ΣFIC value of 0.714. Indifferent interaction of the combination was displayed with the rest of the pathogens tested.

The combination of *Ballota africana* and *Eucalyptus camaldulensis* combination showed generally good antimicrobial activity against the test organism with ZOI ranging from 18 – 35 mm. Noteworthy antibacterial activity was seen against *S. pyogenes* and *K. pneumoniae* with ethanol and acetone extract respectively. ΣFIC values for the combination against *K. pneumoniae* displayed synergistic interaction with acetone extract. Based on ΣFIC values *S. aureus* and *S. pyogenes* tested with aqueous extract, additive interactions were observed with this combination. Indifferent interactions were seen against test organism with the rest of the plant extract, with exception of acetone extract against *S. pyogenes* which exhibited antagonistic interaction.

The antimicrobial activity of *Terminalia sericea* and *Warbugia salutaris* in combination was most effective with ZOI ranging from 23.3 – 29 mm, though acetone extract did not show any activity against *S. pyogenes*. The best activity was observed with all pathogens tested with organic extract (ethanol and acetone) of this combination at mean MIC value range of 0.328 – 0.875 mg/ml. All organisms tested had some antimicrobial activity with aqueous extracts and MIC of 1.313 mg/ml was reported in all of them. This combination showed additive interactions for two pathogens namely *S. aureus* and *K. pneumoniae* with acetone and ethanol extracts registering ΣFIC values 0.973 and 0.649 respectively. Indifferent interaction was observed with the rest of the test organisms except from ethanol extract against *S. pyogenes* which displayed antagonistic interaction.

In medicinal plants combinations, synergism mechanism may be attributed to a number of factors such as: complex multi-target effects, pharmacokinetic or physiochemical properties, neutralization principles or even therapeutic approaches (Van Vuuren et al., 2011). Although only few of these plants were reported to be used in combination, majority hasn't been reported yet.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The study had a number of objectives that were centred in identifying and processing indigenous medicinal plants that were used in investigating their *in-vitro* antimicrobial activity against three pneumonia pathogens in individual and combination studies. The main objectives of this work were achieved and reported.

In chapter one, the parameters, aims and objectives of the study were introduced. Pneumonia being one of the clinically important infectious disease, formed the centre of this study. Pneumonia has been reported as one of the leading causes of mortality and morbidity worldwide. Moreover, pneumonia is known to affect mostly the under-five, elderly, immunosuppressed or immune-compromised individuals. Different interventions, management and treatment have been put in place. Recently, the insurgency of bacterial resistance to antimicrobials due to inappropriate use of antimicrobials, lack of control measures in hospitals and lack of trained laboratory personnel to perform sensitivity testing. Furthermore, bacterial resistance can be caused by unavailability of guidelines on selection of drugs and information on drug resistance that is not communicated to medical practioners/clinicians (Cheesebrough, 2006).

For centuries, medicinal plants have been used as remedies for various human ailments (Kamanga, 2013). However, use of medicinal plants known to be of native or traditional background, yet remarkable numbers of conventional/synthetic drugs are of plant origin. The purpose of this study was to find antimicrobial efficacy of selected indigenous plants used independently and in combinations for bacterial pneumonia treatment. Based on folk knowledge and literature, the following plants; *Terminalia Sericea*, *Dodonea anguistifolia*, *Acorus gramineus*, *Eucalyptus camaldulensis*, *Kigelia africana*, *Ballota africana* and *Warbugia salutaris* were reported to treat pneumonia. In the current study, these plants were used individually and in combination against the test organisms namely; *S. aureus*, *S. pyogenes* and *K. pneumoniae*. Signs, symptoms, diagnosis, treatment, management and prevention of pneumonia infections were discussed in chapter two. The chemical composition and properties of individual sample materials for

both plants and organisms were presented. Mode of action of antibiotics was also scrutinized to draw a picture of the possible ways on how medicinal plants work. A number of extraction methods and chemical compounds that were isolated from previous studies were listed and discussed. Active plant compounds which were discussed in this study are known to be the reason for their antimicrobial activity. However, some of them were not present in the plants used in this study.

Chapter three focuses on materials and methods used to conduct and implement this study. The organisms and materials used for culture and sensitivity studies were provided by NMMU. Plant samples were collected from *Nsalu* village, Lilongwe in Malawi and Port Elizabeth, South Africa. Extraction solvents, apparatus, equipment and materials were provided by NMMU's School of Biomedical Sciences, Microbiology department. Antimicrobial assays used to analyse the medicinal plants under study were introduced. Step-wisely, plant preparation, extraction methods and solvents and reconstitution solvents were described. 10% and 100% DMSO reconstitution solvent and INT colour indicator for bacterial growth where a pink or purple colour change indicated no bacterial inhibition in microtiter plate dilution method. Techniques such as ΣFIC for calculating interaction in combination studies were introduced.

Chapter four presents the experimental results obtained after performing different tests on individual and combined plant extracts. Results obtained from calculating the mean values of zone of inhibition (ZOI) in mm and minimum inhibition concentration (MIC) in mg/ml were reported. According to results, organic extracts had relatively good antimicrobial activity and larger ZOIs as compared to aqueous extracts. MIC mean values were calculated for individual and combined plant extracts and then used to determine the ΣFIC values. Based on the sets of calculated values, plant interactions were interpreted as either synergism, additive, indifferent or antagonistic interactions. Plant extracts had varying antimicrobial effects. For instance, plants such as *Terminalia sericea*, *Ballota africana* and *Eucalyptus camaldulensis* displayed good antimicrobial activity for the individual studies. *Dodonea angustofolia* and *Warbugia salutaris* which had small ZOIs in individual studies, showed good antimicrobial activity in combination studies. Combination of *D. angustofolia and W. salutaris* had significant ΣFIC value for all

test organisms ranging from 0.0491- 0.829 which indicated synergistic and additives interactions, except water extract tested for *K. pneumoniae* had an antagonistic interaction with ΣFIC of 11.05. A number of plants demonstrated additive reaction on either water, ethanol or acetone extract on combinations such as *Terminalia sericea* and *Kigelia africana*, *Kigelia africana* and *Acorus gramineus*, *Ballota africana* and *Warburgia salutaris* and *Warbugia salutaris* and *Eucalyptus camaldulensis*. However, the rest of their plant extract combination showed indifferent and antagonistic interactions. All medicinal plants involved in this study showed some antibacterial activity for all the three test organisms. It was also noted that organic extracts had greater effect in inhibiting bacterial growth than water extract. This is justified by Jouda (2013) where organic solvent is best for extracting active compounds from plants in relation to distilled water.

6.2 Conclusion

The purpose of this study was to validate use of indigenous medicinal plants in individual and combination studies for treatment of bacterial pneumonia. The study substantiated the reasoning that the use of combined medicinal plants enhances antimicrobial activity. The results obtained from this study justify the use of plants in combinations, since results displaying synergism and additive interactions on plants were less effective when used individually.

The study evaluated the antimicrobial activities of seven indigenous medicinal plants against three test organisms when used individually and in combination. Organic extracts displayed good antimicrobial activity compared to aqueous extract pertaining to ZOIs measured and MICs on both individual and combination studies on all test organisms. Despite outstanding activities with organic extracts, aqueous extracts displayed some good activity in some individual and combined plant extracts which agrees with traditional use in form of infusion and decoction (Suliman, 2011).

The current antimicrobial study validates use of medicinal plants for treatment of pneumonia. *Terminalia sericea* aqueous and organic extracts displayed noteworthy antimicrobial activity against all three test organism on individual studies with ZOIs ranging from 22-27.75 mm, and MICs of 0.0144-0.607 mg/ml. But the highest ZOI (35 mm) was observed with *Ballota africana* ethanol extract for *S. pyogenes.* Other plants

had noteworthy activity with either one or two extracts against tested organism, while others against at least one of the tested three organisms. *Warbugia salutaris* on the other hand displayed weak antimicrobial activities against all three tested organisms with both well diffusion and microtiter plate for all extracts.

On combination studies, a number of plant combinations displayed good antimicrobial activity with large ZOIs and lowest MICs. *T. sericea* and E. *camaldulensis* extracts had noteworthy antimicrobial activity with all three test pathogens with ZOI of 26.75-30 mm. Nevertheless, highest ZOIs was shown in *B. africana* and *W. salutaris* organic extract combination for *S. aureus* and *S. pyogenes* ranging from 33-35 mm, although 35 mm was also observed in *B. africana* and *E. camaldulensis* ethanol extract against *S. aureus.* Lowest MICs in combinations was observed with *T. sericea* and *A. gramineus* ethanol extract against *K. pneumoniae.* However*, T. sericea* and *E*. *camaldulensis*, *B. africana* and *T. sericea*, *T. sericea* and *D. angustofolia, T. sericea* and *W. salutaris* had noteworthy antimicrobial activity for all tested organisms with noted dominance on organic extracts.

Significant plant interactions against test pathogens were noted for *W. salutaris* and *D. angustofolia combination* with ∑FICs values ranging from 0.049-0.829 for all extracts, except 11.05 which was calculated for *K. pneumoniae* with aqueous extract where antagonistic interaction was observed. Antagonistic effect was noted in *T. sericea* and *E. camaldulensis* ethanol combination with ∑FIC value of 15.51. Many combinations had synergism or additive interaction with either one or two extracts against one of the test organism. Synergism mechanism may be attributed to complex multi-target effects, pharmacokinetic or physiochemical properties, neutralization principles or even therapeutic approaches (Van Vuuren et al., 2011). Although only few of these plants were reported to be used in combination, majority hasn't been reported yet.

This study validates the use of medicinal plants in combination to improve efficacy in treatment of pneumonia. Plants used in this study can therefore be used as a primary health care for pneumonia management. Medicinal plants can be used in preference to conventitional drugs, since they are able to treat more than one ailment which is more advantegous compared to conventional drugs which target specific conditions. Based

on availability and cost effectiveness of medicinal plants, it can benefit less privileged people and those living in remote areas far from health facilities.

6.3 Recommendations

The study has paveway as a platform for further analysis of intricate uses of medicinal plants in combination. However, scientific validation of the use of these plants in combination has been established creating room for future studies in the treatment of pneumonia as well as for formulating new antimicrobials. Medicinal plants have been observed to contain a complex of bioactive compounds, which prevent growth of organisms through various mechanisms than that of conventional antibiotics, hence posing a remarkable use in treating resistance pathogens. Results obtained from this work might be enough to serve as a platform for further studies in isolating and identifying active compounds, since varying activities of medicinal plants are actually related to variery contest of their active compounds. Also, toxicity studies to be performed to determine the safety of possible adverse effect in administrating this medicinal plants. In-vivo clinical trials should be carried out in future studies, to find us possible use of these plant extracts in the treatment of bacterial pneumonia since what happen in-vitro does not exactly portray what happens in in-vivo. In order to determine plant interactions, future studies should address the mechanism of action of synergistic interactions, which will include receptor site modification, enzymatic degradation, and accumulation of antibiotics within bacteria cell, decreased outer membrane permeability and efflux pumps.

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