Evaluation of plants used in African Traditional Medicine for asthma and related conditions

Katlego Ellena Motlhatlego

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In the Research Centre for Plant Growth and Development
School of Life Sciences
University of KwaZulu-Natal, Pietermaritzburg

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I, Katlego Ellena Motlhatlego, student number: 212556082 declare that

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We hereby declare that we acted as supervisors for this MSc student. Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Sciences Higher Degrees Office for examination by the University appointed Examiners.

SUPERVISOR: PROFESSOR J. VAN STADEN

CO-SUPERVISOR: PROFESSOR J.F. FINNIE
Conference contributions from this research


Dedication

I dedicate this work to the woman who has been my source of courage and inspiration, my mother, Juliana Motthatlego.

For you created my inmost being; you knit me together in my mother’s womb. I praise you because I am fearfully and wonderfully made; your works are wonderful, I know it with all my heart.

(Psalm 139:13-14)

Before I formed you in the womb I knew you, before you were born I set you apart; I appointed you as a prophet to the nations.

(Jeremiah 1:5)

The LORD will work out HIS plans for my life for your faithful love, O LORD, endures forever. Don’t abandon me for you made me.

(Psalm 138:8)
Acknowledgements

Firstly I would like to thank GOD the Almighty Father. When I think of how far you have carried me even when I did not deserve it, you still proved to be an exceptional and wonderful GOD to me. Everything I am I release to YOU. In your presence there is fullness of joy and you constantly supply me with all the favour of your grace. It is neither by my might nor power but it is by the spirit of the living GOD that I am able. YOU reign in power and majesty, and without you I am truly nothing. I sing with pride “Kubo bonke oThixo akekho onjengawe kuba inceba zakhe zimi nguna phakade”.

My dearest Mom, you have always allowed me to see beyond the finish line. Regardless of how hard the journey of life may had been, you carried parenthood as a privilege and managed to give me a smile at all cost. It is at this moment when your favourite hymn “Tsela e thata jwang e ke e tsamayang loeto lo tla fela leng ke tsene kananeng” touches my fragile heart. This degree is dedicated to you Mme wa me. Days when I was rushed to hospitals in the middle of the cold windy nights because of asthma attacks, little did I know that GOD had a plan over my life and would use me as a vessel of honour aiming for drug discovery and development for asthma. My childhood memory takes me back to how you ensured that there was always honey and cinnamon at home because of the knowledge that it can possibly provide a cure for asthma. Even though you did not have a scientific perspective, I greatly benefitted from the honey remedy and got to appreciate how you always went an extra mile to understand the various factors that trigger an asthma attack. GOD entrusted you with parenthood and no matter how hard it may have been seeing me struggle to breathe while wheezing with a very tight chest, you never gave up on me. Holding my hand every now and then for my regular check-up at my pediatrician (Dr C.K. Masiangoako) and understanding the type of diet for eczema and asthma meant so much to me. This study is really driven by the passion to bring a better treatment and I shall do more in the future to live this purpose. My GOD has made me the head and not the tail and truly has a plan to prosper me as HE promised (Deuteronomy 28:13; Jeremiah 29:11). GOD is not a man so HE does not lie, and HE hastens HIS word to perform it (Jeremiah 1:12; Numbers 23:19).
Mom your words of encouragement “You must always pray ngwana’ke. Have patience and determination for things always work out” help me to keep my head up high. Ke a leboga Mama’ka for being a blessing in my life. You are the best and ke a go rata!

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ugogo Emily ‘MaNdimande’ Kheswa Your memory lives. The time I spent with you has birthed a purposeful driven-life. Every Saturday I wake up with a smile for the two hours I spend with the elderly at Marian Villa Home for Aged here in Pietermaritzburg is a pursuit of wisdom. Indeed the glory of young men is their strength and the beauty of old men is the gray head (Proverbs 20:29). Moreover, doing motivational speaking for the blind children at Bawinile Disability Development Centre has been a divine and fulfilling experience as they are a reminder that we are special in the eyes of GOD. Without a doubt we are GOD’s workmanship (Ephesians 2:10). To my spiritual parents, Jan and Hilda Van Niekerk, thank you for your love and guidance. Thank you for believing that dynamite comes in small packages. Just being there for me and see me grow in GOD’s presence makes me smile. Ek is baie lief vir julle twee my geestelike ouers. To Mr and Mrs Brien, your warm love and encouragement kept me hopeful. I’ll forever be grateful. To HIS Grace Tabernacle family I do not know how to thank you because fellowshipping with you has been a tremendous opportunity. May GOD abundantly bless you!

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“Walking with a friend in the dark is better than walking alone in the light” and with these words I would love to say I appreciate you being there through thick and thin.

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Abstract

Traditional medicine is a form of discipline that has been applied within most South African societies with the objective of enhancing the physical and psychological health system in the country. Asthma is a complex inflammatory disease that involves the narrowing of the airways. The prevalence of asthma is increasing worldwide, and this chronic disease has been identified as a significant cause of morbidity and mortality. Asthma poses a major threat to health across the population of South Africa. The adverse effects of current treatments have encouraged the use of traditional medicine.

The primary aim of the research study was to evaluate the efficacy of plants used in African traditional medicine against asthma and chest infections. This was achieved by screening Adansonia digitata, Ballota africana, Catha edulis, Datura stramonium, Pelargonium sidoides, Siphonochilus aethiopicus, Xerophyta retinervis and Zantedeschia aethiopica for their pharmacological properties against key bacteria; Staphylococcus aureus (ATCC 12600), Klebsiella pneumonia (ATCC 13883), Streptococcus pyogenes (ATCC 12344) and Haemophilus parainfluenzae (ATCC 7901) as well as the fungus Candida albicans (ATCC 10231) these microorganisms are known to cause chest infections. In the microdilution antibacterial assay, the crude extracts of the screened medicinal plants showed activity at minimal inhibitory concentrations (MICs) ranging from 0.098 to >12.5 mg/ml. In the disc-diffusion assay, only the ethanol extract of stems from Siphonochilus aethiopicus and water extract of leaves from Zantedeschia aethiopica showed zones of inhibition of 13.24 and 21.10 mm. All the other screened extracts showed no zones of inhibition, which may possibly indicate that plants were ineffective against Haemophilus parainfluenzae. One or more extracts from the tested plants were effective against one or both Gram-positive bacteria investigated in the study. There was no good antifungal activity shown in the study as the MIC and minimal fungicidal concentrations (MFCs) values were higher than 1 mg/ml.

Genotoxicity of medicinal plant extracts that showed good antibacterial activity ≤ 0.5 mg/ml was evaluated using the Salmonella microsome assay without S9 metabolic activation. Two strains of Salmonella TA98 and TA102 were used in the Ames test.
Most tested extracts were non-mutagenic in the Ames test except for the *Siphonochilus aethiopicus* roots which showed a dose dependent increase.

The ethanolic crude extracts were screened in an immunological assay to determine the level of competitive binding to the receptors for the treatment of asthma and related conditions. Histamine is intimately associated with allergies. *Datura stramonium* flowers and fruits experienced remarkable histamine binding of approximately 97% at both concentrations (400 and 800 µg/ml). The immunological activity may be attributed to the various phytochemical constituents in the crude extracts. *Ballota africana* leaves and stems, *Datura stramonium* flowers and fruits, roots and stems as well as *Zantedeschia aethiopica* leaves showed excellent affinity with histamine ranging between 88 and 97% and these medicinal plants could potentially serve as a new effective antihistamine when compared to the currently available pharmaceuticals. Most of the medicinal plants tested may potentially provide remedies for asthma and related conditions such as eczema, rhinitis (hayfever), anaphylaxis, sinusitis, chronic obstructive pulmonary disease (COPD), emphysema, bronchiectasis and bronchitis.
# Table of Contents

College of Agriculture, Engineering and Sciences Declaration-Plagiarism  
i
Declaration by Supervisors  
ii
Conference contributions from this research  
iii
Dedication  
iv
Acknowledgements  
v
Abstract  
ix
Table of Contents  
xi
List of Figures  
xiv
List of Tables  
xv

1. Literature review  

1.1 Introduction  

1.1.1 What is Asthma?  

1.1.2 Worldwide prevalence of asthma  

1.1.3 The impact of asthma in South Africa  

1.1.4 The pharmacotherapy of asthma  

1.2 Problem Identification  

1.3 Traditional medicine in South Africa  

1.4 Medicinal plants  

1.4.1 The significance of medicinal plants  

1.4.2 Drug discovery from medicinal plants  

1.4.3 Conservation of medicinal plants  

1.4.4 The value of the Indigenous Knowledge System for the bioeconomy /Economic development from the local production of medicinal plants in South Africa  

1.5 Research rationale and motivation  

1.6 Aims and objectives  

1
1.7 Biological description and geographical distribution of the candidate plants

1.7.1 Adansonia digitata
1.7.2 Ballota africana
1.7.3 Catha edulis
1.7.4 Datura stramonium
1.7.5 Pelargonium sidoides
1.7.6 Siphonochilus aethiopicus
1.7.7 Xerophyta retinervis
1.7.8 Zantedeschia aethiopica

2. Antimicrobial activity

2.1 Introduction
2.2 Bacteria and fungus in asthma and chest infections
  2.2.1 Streptococcus pyogenes
  2.2.2 Staphylococcus aureus
  2.2.3 Klebsiella pneumoniae
  2.2.4 Haemophilus parainfluenzae
  2.2.5 Candida albicans
2.3 Treatment of microbial infections associated with asthma
2.4 Materials and methods
  2.4.1 Selection and collection of candidate plants
  2.4.2 Extract preparation
  2.4.3 Source of Bacterial strains
  2.4.4 Preparations of media and organisms
  2.4.5 Preparation of plant extracts for the microdilution assay
  2.4.6 Experimental procedure
2.5 Results and discussion
2.6 Conclusions
3. Mutagenic activity

3.1 Introduction

3.2 Genotoxicity testing methods

3.3 Materials and methods

3.3.1 Preparation of plant extracts

3.3.2 Mutagenic evaluation of selected plant extracts using the Ames assay

3.4 Results and discussion

3.5 Conclusions

4. Screening for Immunological activity

4.1 Introduction

4.2 The role of histamine in asthma

4.2.1 Functional properties of histamine during an allergic encounter

4.2.2 Histamine receptors and antagonists involved in the process of inflammation

4.3 Immunological testing methods

4.4 Materials and methods

4.4.1 Anti-Histamine Assay

4.5 Results and Discussion

4.6 Conclusions

5. General Conclusions

References
List of Figures

Figure 1.1  Demonstration of the cross-sections of a normal and obstructed airway (an airway when asthma symptoms arise) (ENCYCLOPEDIA BRITANNICA, 2001) 2

Figure 1.3  *Adansonia digitata* L. 17

Figure 1.4  *Ballota africana* (L.) Benth. 18

Figure 1.5  *Catha edulis* (vahl) Endl. 19

Figure 1.6  *Datura stramonium* L. 20

Figure 1.7  *Pelargonium sidoides* DC. 21

Figure 1.8  *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt 22

Figure 1.9  *Xerophyta retinervis* Baker 23

Figure 1.10  *Zantedeschia aethiopica* (L.) Spreng. 24

Figure 4.1 Demonstration of the role of histamine in inflammation (DUNFORD and HOLGATE, 2010) 70

Figure 4.2 2D Chemical structure of Loratadine 72

Figure 4.3 Determination of the level of histamine receptor binding to the medicinal plants used for the treatment of asthma and related conditions 74
List of Tables

Table 2.1 Medicinal plants used to treat asthma-related ailments and selected for investigation 32

Table 2.2 Percentage yield related to dry weight of extracts obtained using the described extraction process 40

Table 2.3: Antibacterial activity of the selected medicinal plants used for the treatment of asthma and related conditions 42

Table 2.4: Antifungal minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of medicinal plants used for the treatment of asthma and related conditions 48

Table 3.1 Number of revertant colonies of Salmonella typhimurium strains TA98 and TA102 induced by bioactive extracts used for the treatment of asthma and related conditions 64
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4NQO:</td>
<td>4-nitroquinoline–N- oxide</td>
</tr>
<tr>
<td>AES:</td>
<td>Agriculture, Engineering and Science</td>
</tr>
<tr>
<td>ATCC:</td>
<td>American Type Culture Collection</td>
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<tr>
<td>CBD:</td>
<td>Convention Biological Diversity</td>
</tr>
<tr>
<td>COPD:</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>CSIR:</td>
<td>Council for Scientific and Industrial Research</td>
</tr>
<tr>
<td>DCM:</td>
<td>Dichloromethane</td>
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<tr>
<td>DNA:</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DMSO:</td>
<td>Dimethyl Sulphoxide</td>
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<tr>
<td>DPAP:</td>
<td>Division of Pulmonary and Allergy Products</td>
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<tr>
<td>ELISA:</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<tr>
<td>EtOH:</td>
<td>Ethanol</td>
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<tr>
<td>FDA:</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>HIV/AIDS:</td>
<td>Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome</td>
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<tr>
<td>HNMT:</td>
<td>N-methyl-transferase</td>
</tr>
<tr>
<td>HTMAP:</td>
<td><em>Haemophilus</em> Test Media Agar Plates</td>
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<tr>
<td>ICSs:</td>
<td>Inhaled Corticosteroids</td>
</tr>
<tr>
<td>INT:</td>
<td><em>p</em>-iodonitrotetrazolium violet</td>
</tr>
<tr>
<td>IgE:</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>LABAs:</td>
<td>Long-Acting Inhaled β₂-Agonists</td>
</tr>
<tr>
<td>LTRAs:</td>
<td>Leukotriene Receptor Antagonists</td>
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<tr>
<td>MIC:</td>
<td>Minimal Inhibitory Concentration</td>
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<tr>
<td>MFC:</td>
<td>Minimum Fungicidal Concentration</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MH</td>
<td>Mueller-Hinton</td>
</tr>
<tr>
<td>MHB</td>
<td>Mueller-Hinton Broth</td>
</tr>
<tr>
<td>MP</td>
<td>Mononuclear Phagocyte</td>
</tr>
<tr>
<td>NAEP</td>
<td>National Asthma Education Programme</td>
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<tr>
<td>NRCATM</td>
<td>National Reference Centre for African Traditional Medicine</td>
</tr>
<tr>
<td>NRF</td>
<td>National Research Foundation</td>
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<tr>
<td>OD</td>
<td>Optical density</td>
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<tr>
<td>PE</td>
<td>Petroleum ether</td>
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<tr>
<td>RAST</td>
<td>Radioallergosorbent test</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>SABAs</td>
<td>Short-Acting Inhaled β₂-Agonists</td>
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<td>SBAP</td>
<td>Sheep Blood Agar Plates</td>
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<tr>
<td>THB</td>
<td>Todd-Hewitt broth</td>
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<tr>
<td>TRIPS</td>
<td>Trade Related Aspects of Intellectual Property Rights</td>
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<tr>
<td>UKZN</td>
<td>University of KwaZulu-Natal</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>YM</td>
<td>Yeast Malt</td>
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<td>YMB</td>
<td>Yeast Malt Broth</td>
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1. Literature review

1.1 Introduction

Respiration is one of the body’s homeostatic mechanisms. The respiratory system is a life-sustaining system of the body and primarily aims at transporting oxygen from the atmosphere to the body tissues, remove carbon dioxide waste, eliminate toxic waste, regulate temperature and stabilize the blood acid-alkaline balance (pH) (PATEL and GWILT, 2008). The lungs are the largest part of the respiratory system and provide a defence against bacterial, viral and other infectious agents. Infections and genetic factors that directly or indirectly affect lung development can cause symptoms of respiratory disease.

Respiratory diseases are major causes of ill health and school absence in children, and are among the leading causes of death worldwide (BURR et al., 1999; OPITZ et al., 2010). Respiratory diseases include asthma, bronchiolitis, common cold, influenza, Chronic Obstructive Pulmonary Disease (COPD) as well as the diseases of the upper and lower respiratory systems (PATEL and GWILT, 2008). Often common in childhood, respiratory diseases may lead to chronic disease in adult life, particularly in developing countries. AIT-KHALED et al. (2001) and BOUSQUET et al. (2003) have highlighted how chronic respiratory diseases represent a challenge to public health due to their increasing frequency and severity, and how the projected trends have an economic impact. In the past three to four decades, the world’s average surface temperature has increased by 0.6 ± 0.2°C and this has heightened health challenges to the world (BEGGS and BAMBRICK, 2006). Respiratory diseases have increased due to global warming because the environment and ecosystems have been damaged thereby causing an imbalance in living organisms such as germs impacting on air pollution.
1.1.1 What is Asthma?

The word asthma is of Greek origin meaning “panting” or “groaning” (SAUNDERS, 1993). Asthma has been identified as one of the most common respiratory complaints worldwide and is known to be triggered by various factors particularly allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals and histamine (KELLY and SORKNES, 2005; MASOLI et al., 2004). The characteristic symptoms include wheezing, shortness of breath, cough and tightness of the chest (BJERMER, 2007; CHAPMAN et al., 2005; EDMONDSTONE, 2000; WARD et al., 2002).

Figure 1.1 Demonstration of the cross-sections of a normal and obstructed airway (an airway when asthma symptoms arise) (ENCYCLOPEDIA BRITANNICA, 2001)
Asthma is defined as a chronic inflammatory disorder of the airways characterized by variable airway obstruction and airway hyper-responsiveness of smooth muscle (CHAPMAN et al., 2005; GIBBON, 2005; JABER, 2002; TAKIZAWA, 2007; WARD et al., 2002) as demonstrated in Figure 1.1. There are two types of asthma, namely allergic asthma and occupational asthma (VERSTRAELEN et al., 2008). The difference between the two forms of asthma is that allergic asthma is triggered by an allergy while occupational asthma occurs in response to a trigger in the workplace. According to VERSTRAELEN et al. (2008), asthma is regarded as being work-related when there is an association between symptoms and work. The most common form of asthma is the allergic type. Asthma triggered by an allergic reaction is caused by atopy, which is an immune disorder where the production of immunoglobulin E (IgE) to inhaled antigens leads to bronchial mucosal inflammation (ADRA et al., 1999). Not only is asthma linked to an increase in atopic sensitization but is also equivalent to similar increases in other allergic disorders such as eczema and rhinitis (GARRIDO et al., 2010; MASOLI et al., 2004; VAN DEN OORD and SHEIKH, 2009).

1.1.2 Worldwide prevalence of asthma

Asthma discerns no prejudice and affects people from all social, cultural and ethnic backgrounds (GREEN et al., 2008). According to ISAAC (1998), asthma affects one child in seven in some societies and approximately 15 million individuals worldwide. In 2011 the World Health Organization (WHO) estimated that 235 million people suffered from asthma. Asthma has been reported to be a significant cause of both morbidity and mortality (BOUSQUET et al., 2005; BRAMAN, 2006). An increase in economic burden upon patients with asthma has also been reported where costs are mainly due to hospitalizations and medications (BAHADORI et al., 2009). Moreover BRAMAN (2006) has emphasized how the prevalence of asthma increases by 50% every decade. According to MASOLI et al. (2004), asthma causes 250 000 deaths per year worldwide. This demonstrates that asthma is a serious global health problem.
1.1.3 The impact of asthma in South Africa

South Africa is regarded as a middle income country with a relatively high degree of industrialisation, a sizeable affluent class and large scale urban poverty and rural underdevelopment (EHRLICH et al., 2005). In South Africa, asthma has been ranked as the eighth leading contributor to the burden of disease and the second most important chronic disease after Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) (BRADSHAW et al., 2007). South Africa has been ranked the country with the third highest mortality rate for asthma (www.nationmaster.com/graph/mor_ast-mortality-asthma) and this indicates how the burden of non-communicable diseases is resulting in the demand for care for chronic diseases (MAYOSI et al., 2009). Asthma poses a major threat to human health across the spectrum of this developing country as this chronic respiratory disease is often not considered a severe condition as opposed to diabetes mellitus and cardiovascular disease that usually receive more attention (MASH et al., 2010). The low public health priority has therefore resulted in a deficiency of data on morbidity and mortality and the burden of asthma in the country (ZAR et al., 2001). In South Africa, most studies on asthma had been confined to the Western Cape and Eastern Cape Provinces and it is important to carry out studies in other provinces (EHRLICH and JITHOO, 2005). A National Asthma Education Programme (NAEP) has been established in South Africa with the objective of disseminating impartial information about asthma (www.asthma.com). Its infrastructure embodies doctors, nurses, allied health professionals, medical technologists, pharmacists as well as pharmacy industry members. This non-profit organisation is involved in events such as World Asthma Day (the first Tuesday of May), workshops and development meetings for professional members, congresses and fundraising. This shows that there is an effort towards educating people about asthma and gives hope to the South African community in learning to shift attitudes towards combating the burden of this chronic disease.
1.1.4 The pharmacotherapy of asthma

Asthma is closely associated with an interaction between strong genetic and environmental factors (VERCELLI, 2008). The pharmacotherapy of asthma is reported to be complex (ZDANOWICZ, 2007). The complexity of this heritable disease illustrates that there are a number of genes that contribute toward a person’s susceptibility to the disease (CHAPMAN et al., 2005).

Asthma cannot be cured but its management plays an important role in achieving the primary goals of treatment by avoiding asthma attacks, reducing inflammation and preventing lung damage. The ability to control asthma depends on prevention of allergic triggers and taking medication as prescribed. The medication is prescribed for two purposes, which are to stop an immediate attack and to control inflammation and reduce lung damage over the long term. Pharmacotherapy, particularly inhaled therapy, remains the cornerstone of asthma management with the result of improved symptom control and quality of life (MOTALA et al., 2009, NAEPP, 1997). Consequently, the appropriate medication and delivery devices form an integral component of asthma control, essential in order to cater for the needs of the patients as well as their circumstances (MOTALA et al., 2009). According to MACHIRA et al. (2011) the choice of an inhaler device is dependent on factors such as efficacy of drug delivery, cost, safety, ease of use, convenience as well as efficacy in a specific age group. Pharmacotherapy had taken an unconventional step where the goals of asthma treatment as outlined by MOTALA et al. (2009) anticipate patients to lead normal lives with no symptoms, ability to participate in all extramural activities, normal functioning of the lungs and no side effects. The drugs that are currently available for the treatment of asthma are classified as relievers and controllers (BATEMAN et al., 2008; PATEL and GWILT, 2008). Figure 1.2 demonstrates the inhaling devices used for asthma treatment.

Relievers are short-acting bronchodilators with rapid onset of action with the objective of providing acute relief from symptoms and include Short-Acting Inhaled ß2-Agonists (SABAs), anticholinergics (ipratropium bromide including Combivent and Duovent) and short acting xanthines. SABAs include Salbutamol (Asthavent,
Venteze, Ventolin), Terbutaline (Bricanyl) and Fenoterol (Berotec) (PATEL and GWILT, 2008). Controllers often referred to as preventers, have sustainable bronchodilatory action; however it has unproven anti-inflammatory action. Controller therapy is recommended for all patients with persistent asthma. Controllers are found in three forms namely Inhaled Corticosteroids (ICSs), Long-Acting Inhaled β₂-Agonists (LABAs) and Leukotriene Receptor Antagonists (LTRAs). Inhaled corticosteroids have been reported to be the most effective controller therapy for asthma as they have reduced the number of asthma-related hospitalisations, improved lung function, reduced the frequency of exacerbations, improved quality of life and reduced bronchial hyperresponsiveness. ICSs include Beclamethasome (Beclate, Becotide, Becloforte, Clenil, Qvar, Aerobec, Viarox), Fluticasone (Flixotide, Flomist), Budesonide (Budelam, Inflammide, Pulmicort), and Ciclesonide. Long-acting inhaled β₂-agonists include Formoterol (Oxis, Foradil and Foratec), Salmeterol (Serevent) and in combination with steroids (Seretide, Symbicord). Leukotriene receptor antagonists include Montelukast (Singulair) and Zafirlkast (Accolate) (PATEL and GWILT, 2008).

In February 2010 the director of the Food and Drug Administration (FDA)’s division of Pulmonary and Allergy Products (DPAP), Dr. Badrul Chowdhury, advised that LABAs should never be used as a monotherapy in the treatment of asthma in children or adults but in combination with inhaled steroids. This is due to the review of the available clinical trials that disclosed an increased risk of serious asthma exacerbations and death. Long-acting inhaled β₂-agonists are offered as single ingredient products or in combination with a corticosteroid medication. The motive behind the availability of single-ingredient treatment is because not all asthma controller medications are able to be made into a combination product. The director of FDA’s Office of Paediatric therapeutics, Dr. Dianne Murphy, emphasized that parents need to know that their child should not be on a LABA alone and this is based on the risks of hospitalisation and poor outcomes that are of particular concern for children. It is fulfilling to see that FDA urges safe use of inhaled asthma medicine (www.webmd.com/asthma/news/20100219/fda-limits-long-acting-asthma-inhalers).
Leukotriene Receptor Antagonists may be referred to as anti-inflammatory preventers as they constitute a preventative therapy for asthma. They have become an accepted treatment option for childhood asthma. This is because their pro-inflammatory actions are emphasised on the basis of their ability to increase vascular permeability and to cause influx of eosinophils (PATEL and GWILT, 2008). As opposed to LABAs that take about 12 hours to become active, LTRAs have a rapid onset of action (within one to three hours). The LTRS mechanism occurs by blocking a chemical reaction that can lead to inflammation in the airways.

**Figure 1.2** Representation of the asthma drug therapy (a) Relievers and (b) Controllers
1.2 Problem Identification

The prevalence of asthma is increasing dramatically regardless of the major changes in monitoring and treatment of the disease. Currently available bronchodilators and anti-inflammatory treatment requires a lifetime of therapy although a subset of patients remains symptomatic, and can have side effects such as headaches, heart palpitations, sinus tachycardia, anxiety, tremors and increased blood pressure (BERTRAND, 2000; PATEL and GWILT, 2008). Other side effects include diabetes mellitus, nausea, throat irritation, dyspepsia, fungal infection, adrenal suppression, insomnia, reflex cough and bronchospasm as well as dysphonia (hoarseness). The drug salbutamol in inhalers can induce hyperglycemia and hypokalemia and it is therefore advisable that patients do not take a dose of more than 5 mg (www.ch.ic.ac.uk/local/projects/mohataren/Files/risks.htm). Asthma is a costly disease because the disease results in loss of productivity caused by absenteeism from work (GREEN et al., 1998).

Generally the widespread use of antibiotics is not recommended to treat asthma (CAMARGO et al., 2009). The use of the existing medication is maintained as they can adequately control symptoms in most asthma patients so the overuse of antibiotics could lead to more challenges with the growth of drug-resistant bacteria which is already a concern worldwide. Most people refrain from using the existing medication because of the side effects and are thus making use of promising traditional plant medicines.

1.3 Traditional medicine in South Africa

Traditional medicine is defined by the WHO as “the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures” (WHO, 2000). African traditional plant use can be viewed as the pharmaceutical sector of rural areas that is imperative to health and is dependent entirely on natural biodiversity. This sector primarily aims to prevent, diagnose, improve or treat physical and mental illness. African traditional medicine is regarded to be the oldest and the most diverse of all medicine systems (GURIB-FAKIM, 2006).
Traditional medicine is a form of discipline that has been applied within most South African societies with the objective of enhancing the physical and psychological health system in the country (RABE and VAN STADEN, 1997). Moreover CHINSAMY et al. (2011) has revealed that the field of traditional medicine plays an essential role in African cultural beliefs. As compared to Chinese and Indian traditional medicines which have been extensively documented (CRAGG and NEWMAN, 2001), African herbalists are known to convey information through generations by oral means and as a result an entire archive could end at the death of older generations (KAROU et al., 2007; OGBOLE et al., 2010). Ethnobotany therefore plays a major role in documenting the native knowledge of the use of plants for medicinal purposes and further enables researchers to shift such knowledge towards the era of drug discovery and development (HEINRICH, 2000). Moreover, the documentation of this native knowledge is important for the conservation and utilization of biological resources (MUTHU et al., 2006).

A study conducted by THORNE (2000) outlined that there are about 250 000 flowering plants in the world and furthermore SCHIPPMANN et al. (2002) showed that more than 50 000 of the world’s plant species are used for medicinal purposes. The WHO has reported that 80% of the African and Asian populations still prefer to use traditional remedies rather than the modern medicine for primary healthcare (OLSEN, 1998). According to mid-2011 population estimates from Statistics South Africa, the country has approximately 51 million people (STATISTICS SOUTH AFRICA, 2011). In 2013 the population of the country was estimated to be 53 million (STATISTICS SOUTH AFRICA, 2013). South Africa has an extremely rich plant flora of over 30 000 species of higher plants and is known to harbour prominent foci of plant diversity in the world and an extraordinary high level of endemism (TAYLOR et al., 2001; VAN STADEN, 2008; VAN WYK and SMITH, 2001). Of the total number of species in South Africa, 3000 species are used as medicine (TAYLOR et al., 2001; VAN WYK and SMITH, 2001). This high diversity and endemism has allowed the use of different plant species to treat several diseases particularly within the rural population where western medicine is either inaccessible or expensive (McGAW et al., 2005). Furthermore VAN WYK (2008) highlighted that this diversity precisely signifies a valuable resource for both commercial development and basic scientific
study. South Africa aspires to be one of the top three emerging economies in the global pharmaceutical industry on the basis of an expansive innovation system using the country’s indigenous knowledge and rich biodiversity (DEPARTMENT OF SCIENCE AND TECHNOLOGY, 2007).

South Africa’s 1996 Bill of Rights from section 27 of the Constitution emphasizes access to health care services (STATUTES OF THE REPUBLIC OF SOUTH AFRICA, 1996) and this means rural communities currently have access to medical resources in clinics and hospitals. However, the use of African traditional medicine is rapidly growing and 70% of the South African population is making use of this herbal system (WEIDEMAN, 2005) because traditional medicine is believed to treat a range of health problems that western medicine does not treat adequately and may be helpful in removing the possible psychological component of asthma (FAWIBE, 2008). Traditional healing is an integral and recognised part of health care in South Africa (ANC, 1994). Due to the ease of accessibility and economic benefit of traditional medicine, the large rural population of a country is more disposed to the specified treatment as compared to the urban population (BANQUAR, 1993). Traditional healing systems could become an important resource for the primary healthcare system in hospitals and other medical centres in the country because there are eight times as many traditional healers as compared to medical practitioners (200 000: 25 000) (KALE, 1995). Even though traditional methods of healing remains a primary way of managing asthma in the African continent, the drawback is that people who use traditional medicines do not often understand the scientific rationale i.e. the active compounds.

1.4 Medicinal plants

1.4.1 The significance of medicinal plants

Human life has been dependent on plants for food, clothing and shelter for years (SALIM et al., 2008). Plants have been exploited for their medicinal purposes for thousands of years (SAMUELSSON, 2004) and as a result dependence upon them has become prevalent in developing countries where traditional medicines play a major role in health care (FARNSWORTH, 1994; SAMUELSSON, 2004;
SRIVASTAVA et al., 1996). Medicinal plants have been referred to as the ‘backbone’ of traditional medicine (CIBA FOUNDATION SYMPOSIUM 185, 1994). Medicinal plants have been used for centuries to treat human diseases and are sold in the informal and commercial sectors of the South African economy. In South Africa, the preparations from buchu [Agathosma betulina (P.J. Bergius) Pillans], Cape Aloes (Aloe ferox Mill.) and devil’s claw [Harpagophytum procumbens (Burch.) DC. ex Meisn.] are produced commercially and have significantly contributed to the world of medicine (VAN WYK et al., 1997). There are several publications in the ethnobotanical field (HUTCHINGS et al., 1996; VAN WYK et al., 1997; WATT and BREYER-BRANDWIJK, 1962) enabling a broader perspective in understanding the botanical background and medicinal use of various plants. Currently South Africa is privileged as intense ethnomedicinal research is being conducted by universities, councils, pharmaceutical companies and government. It is interesting to know that the South African Department of Science and Technology aims to urgently confront the country’s failure to commercialise the results of scientific research (DEPARTMENT OF SCIENCE AND TECHNOLOGY, 2007). This urgent need makes it possible that in future our country will be able to unveil South African plant-based drugs onto the international pharmaceutical podium.

1.4.2 Drug discovery from medicinal plants

Nature is an important source of medicinal agents and produces a vast number of complex molecules, some of which make useful drugs (CRAGG and NEWMAN, 2001). A drug has been defined by RATES (2001) as “a pharmacologically active compound, which is a component of a medicine, irrespective of its natural, biotechnological or synthetic origin”. Natural products from plants, animals and minerals have been used to treat and prevent human diseases (CHIN et al., 2006; VERMA and SINGH, 2008). The pharmaceutical industry has considered medicinal plants as a source of active compounds that can be used to prepare synthetic medicine. The application of ethnomedicinal medicine remains one of the reliable approaches for discovering potent new compounds (FABRICANT and FARNSWORTH, 2001).
Over the years numerous therapeutic agents have been discovered from plants through (i) isolation of bioactive compounds that are directly used as patented drugs, and (ii) production of drugs that serve as leads for the next generation of drugs (FABRICANT and FARNSWORTH, 2001). While the improvement of natural product-derived compounds is vital as an invaluable source of medicines for humans (SALIM et al., 2008), the process of drug discovery encounters various challenges (JACHAK and SAKLANI, 2007). This process takes approximately ten years and costs more than 800 million dollars (DICKSON and GAGNON, 2004) therefore it is significant for scientists to introduce a better and faster strategy that will enable the drug development phase to keep pace with other drug discovery efforts.

1.4.3 Conservation of medicinal plants

With the knowledge that medicinal plants are renewable natural resources, it is evident how essential conservation and sustainable use are to biodiversity. This is simply because the high demand from harvesting of medicinal plants has resulted in misuse and thereby forming a serious threat to biodiversity. Humans have been regarded to be the dominant influence on biodiversity (HEYWOOD and IRIONDO, 2003; HUNDE, 2007). Industrialization, mining, agriculture and particularly urbanization are known to contribute to the threat to South African biodiversity (MATSILIZA and BARKER, 2001; ZSCHOCKE et al., 2000). South Africa therefore holds the responsibility of ensuring that they play a practical role in protecting the global diversity (PRESTON and SIEGFRIED, 1995).

Conservation has been defined by Ezemvelo KZN Wildlife (www.kznwildlife.com) as “the rate of use of a biological resource that ensures that the same or a greater quantity of that resource will be available in the future”. Equivalent to this definition, OKIGBO et al. (2008) has emphasized that the process of conservation involves a careful preservation and planned management of a natural resource. In essence if medicinal plants are not adequately conserved, their over-exploitation would lead to the resource being “finished” thereby offering no benefits to future generations. As much as potential medicinal plants remain of great concern to the country as they harbour active constituents required to overcome challenges experienced with the modern therapeutic system, the plants that have not been scientifically evaluated
and documented are greatly endangered. Nature is not patentable but economic rationale to conserve the medicinal plants should be taken into account and thus implementation and protection of intellectual rights need to be put in place. Intellectual property rights aid in the conservation of biodiversity while promoting sustainable development (GOLLIN, 1993). This implies that the power to control a habitat enables proprietary rights for indigenous knowledge concerning the development of its biological resources and this constitutes economic benefits from those resources (DOWNES, 2000; GEORGE and VAN STADEN, 2000). This would therefore serve as a motivation to shift focus towards conservation instead of allowing the resources to be wiped out.

The National Environmental Management Biodiversity Act 10 of 2004 supports the management and conservation of biodiversity and the application of bioprospecting of indigenous biological resources (SOUTH AFRICAN BIODIVERSITY ACT, 2004). Within this Act, the South African National Biodiversity Institute is responsible for monitoring the country’s biodiversity. The South African Department of Trade and Industry implemented policy and Bill of protection of Indigenous knowledge systems using the Intellectual Property System. South African Patents Act 57 of 1978 (as amended by Patents Amendment Act 58 of 2002) has been linked to the Biodiversity Act and was proposed with the objective to protect indigenous knowledge systems. It is a challenge to the population of developing countries such as South Africa that hands down information through the generations to protect their indigenous knowledge by conventional intellectual property rights as required by the Trade Related Aspects of Intellectual Property Rights (TRIPS). In aligning the Patents Act with what is required by the International Convention Biological Diversity (CBD) and the National Environmental Management Biodiversity Bill, the Patents Amendment Bill has been envisioned to empower holders of the indigenous knowledge through an introduction of compulsory disclosure requirements on the origin of traditional knowledge upon which a patent is based (www.sahealthinfo.org/traditionalmeds/traditionalpart2.pdf). Moreover the National Reference Centre for African Traditional Medicine (NRCATM) also works towards safeguarding the intellectual property of Traditional healers and communities (www.sahealthinfo.org/traditionalmeds/traditionalpart2.pdf). This
indicates that this approach would hopefully endow an economical value to the indigenous knowledge of the people in South Africa.

1.4.4 The value of the Indigenous Knowledge System for the bioeconomy /Economic development from the local production of medicinal plants in South Africa

Nearly 25% of the drugs prescribed worldwide are plant derivatives (RATES, 2001). Valuing the indigenous knowledge system enables maintenance of cultural identity and conservation of the biodiversity. Moreover, preservation and respect of cultural heritages have been upheld while accumulating revenue from tradition medicine (ARAZEEM, 2011). As reported by MANDER et al. (2007), the trade in traditional medicine is being mostly conducted as an informal industry. Furthermore, the trade in South Africa is estimated to be worth R2.9 billion per annum. It has been reported that the extensive indigenous medicinal plant trade occurs mostly in the KwaZulu-Natal Province as it is worth approximately R62 million per annum (TIKZN, 2013). With the estimation that there are 53 million people in the country (STATISTICS SOUTH AFRICA, 2013) and MANDER et al. (2007) reporting that there are 27 million consumers of traditional medicine, this reflects that the African traditional medicine market is rapidly growing. This also reflects how the use and trade of traditional medicine has become a discipline of interest within the social and economic sciences.

The traditional medicinal trade is important in the informal economy and the arising question would be if the traditional healers are making a profit in order to sustain themselves. It would be a dynamic platform to allow the minds of the traditional healers to be shaped towards entrepreneurship because they could fundamentally contribute to the development of the economic state through the indigenous knowledge system. One of the opportunities that exist in the indigenous medicinal sector within the department of trade and investment is the strategy to promote the development of business skills (TIKZN, 2013). This means that the government will provide support to our traditional healers to sell their indigenous plant products on
the formal market and this trade will in return allow them to sustain their families and
the community as a whole. In this regard the trade of medicinal plants will alleviate
poverty and spur economic growth of our country (www.sustainable-
commerce.co.za).

The Bioprospecting Programme from the Council for Scientific and Industrial
Research (CSIR) forms a policy that focuses on benefit sharing with owners of
indigenous knowledge. This justly promotes equitable benefits and this is observed
through a consensus reached between the CSIR and traditional healers during the
year 2003 to commercialize medicinal plant products developed by the organization
(LAIRD, 2002).

1.5 Research rationale and motivation

Corticosteroids are the most prescribed agents for asthma and have been shown to
inhibit infiltrating cells, cytokine production and the production of a number of other
neurogenic and growth associated factors (GREENFEDER and ANTHES, 2002). Current asthma therapeutic approaches aim to improve lung function through bronchodilation (GREENFEDER and ANTHES, 2002). The need for new and improved treatments for asthma thus becomes a necessity to provide better therapeutic value by lessening side effects and increasing the effectiveness of the treatment. It is important to note that the medicinal properties of plants are due to the presence of active compounds. The study of herbal medicines can open the door to drug discovery.

From a personal childhood experience with asthma, this study anticipates to bring a
positive effect from the pharmacological evaluation of the plants as traditional
medicine is thought to treat a range of health problems. The major advantage of the
use of herbal medicines is that they may have more than one mode of action as
opposed to western single compound pharmaceuticals (YONG and LOH, 2004). Medicinal plant drug discovery may offer new and important leads against pharmacological targets of asthma. Just to name a few, the candidate plants that have shown at least some promise in treating asthma symptoms include butterbur [Petasites hybridus (L.) G.Gaertn., B.Mey. & Scherb.], dried Ivy (Hedera helix L.)

A study by HOFMANN et al. (2003) showed that dried ivy leaf extracts improve respiratory functions of children with chronic bronchial asthma. Moreover, preparations of the ivy leaves are specifically used for treatment in children due to the secretolytic and bronchospasmolytic effects (KRAFT, 2004). Concentrated ginkgo leaf extract has been reported to be an effective anti-airway inflammation drug (LI et al., 1997). A study by DANESCH (2004) suggests that the *Petasites hybridus* (L.) extract Petadolex is an effective and safe therapy for the treatment of asthma. Literature is available on various plants that have showed anti-asthmatic properties (DIVYA et al., 2011; KUMAR et al., 2012; VELRAJ et al., 2013; VIJAYAPANDI et al., 2013). Natural products isolated from medicinal plants remain an essential component in the search for new medicines. In the present study the candidate plants *Adansonia digitata* L., *Ballota africana* (L.) Benth., *Catha edulis* (vahl) Endl., *Datura stramonium* L., *Pelargonium sidoides* DC., *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt, *Xerophyta retinervis* Baker and *Zantedeschia aethiopica* (L.) Spreng. will be evaluated with the purpose of determining biological activity for the treatment of asthma. The selection of the candidate plants is explained in Section 2.4.1.

1.6 Aims and objectives

The aim of the present study was to evaluate the efficacy of plants used in traditional medicine against asthma. This was achieved through several experiments based on the following:

- Identification of candidate plants and a literature survey of their pharmacology and phytochemistry.
- Antimicrobial assay using microdilution and disc-diffusion techniques
- Mutagenic assay (The *Salmonella typhimurium* microsome assay)
- Antihistamine immunological assay

The significance of the study is to
- Evaluate the claimed potency
- Give scientific evidence that these plants are effective against asthma and related conditions

1.7 Biological description and geographical distribution of the candidate plants

1.7.1 *Adansonia digitata*

![Adansonia digitata](https://www.commons.wikimedia.org/wiki/File:Adansonia_digitata.jpg)

**Figure 1.3 Adansonia digitata L.**


*Adansonia digitata* L. is commonly known as the baobab tree and is especially known for its exceptional height and width. This tree is geographically distributed in areas of South Africa, Botswana, Namibia, Mozambique and other tropical African countries where suitable habitats occur ([www.plantzafrica.com/plantab/adansondigit.htm](http://www.plantzafrica.com/plantab/adansondigit.htm)). It has a height of 10-15 m and the circumference of the trunk in large specimens is estimated to be 28 m ([GEBAUER et al., 2002; PALGRAVE, 1977](http://www.plantzafrica.com/plantab/adansondigit.htm)) and its branches are thick, wide and stout. The growth of this tree occurs in sandy-textured soils but can be found on
rocky areas. This tree is characterized by a pinkish-grey, reddish brown, smooth and heavy folded bark (PALGRAVE, 1977). The flowers are white and large. The flowers have been reported to seldom survive for more than 24 hours and are pollinated by bats, insects and wind (SIDIBE and WILLIAMS, 2002).

1.7.2 Ballota africana

Figure 1.4 Ballota africana (L.) Benth.

Image retrieved from
www.google.co.za/search?q=image+ballota+africana&biw=1242&bih=607&tbm=isch&tbo=u&source=univ&sa=X&ei=Tdw5U6nZJIre7Aaw-YHwCA&ved=0CCkQsAQ#facrc= &imgdii=zDtO_fmNy2ixlM%3A%3Bdq69xzMvg_xSuM%3BzDtO_fmNy2ixlM%3A&imgrc=zDtO_fmNy2ixlM%253A%253B89gug7nKD9ZgRM%3Bhttp%253A%252F%252Fwww.plantzafrica.com%252Fplanttab%252Fplimag esab%252Fballotafric2.jpg%3Bhttp%253A%252F%252Fwww.plantzafrica.com%252Fplantab%252Fballotafric.htm%3B290%3B231

Ballota africana (L.) Benth. is commonly known as kattekrui and is described as a rigid plant of up to 1.2 m in height (VAN WYK et al., 1997). Ballota africana is geographically distributed on rocky flats and lower slopes from Nieuwoudtville to the Cape Peninsula, Caledon, the Karoo and Namaqualand. The stem of this plant is characterized by the production of densely hairy, opposite leaves with a rounded
apex and a noticeable pattern of recessed veins. Its flowers are usually pink, purple or mauve and are seasonally formed around May to November appearing in dense clusters above each leaf pair. The seeds are about 2 mm in diameter and have a shiny black distinctiveness (www.plantzafrica.com/medmonographs/ballotafric.pdf).

1.7.3 *Catha edulis*

![Figure 1.5 *Catha edulis* (vahl) Endl.](image)

*Catha edulis* (vahl) Endl. is an evergreen small tree of the family Celastraceae characterized by its cultivation in certain regions of Africa and Southern Arabia (BARKWAN *et al.*, 2001; HASSAN *et al.*, 2007; LEMESSA, 2001). *Catha edulis* grows to a height of 2-10 metres. The leaves are simple with a glossy green colour above and paler below (LEMESSA, 2001; VAN WYK *et al.*, 1997). The leaves are distinguished by a firm texture and an evenly toothed margin.
1.7.4 *Datura stramonium*

![Image of Datura stramonium](image)

*Figure 1.6  * **Datura stramonium** L.

*Datura stramonium* L. is a woody-stalked, leafy herb growing to an average height of 1.5 m (STACE, 1997). This weed is known to produce spiny seed pods and white or purplish tubular flowers (HENDERSON and ANDERSON, 1996). According to VAN WYK et al. (1997), development of these flowers is followed by four-locular fruit capsules where the spiny seeds are found. It is an annual that blossoms in spring and summer. Although it is widely spread in the United States, *Datura stramonium* has become a cosmopolitan weed and is now also widely spread in South Africa (HENDERSON and ANDERSON, 1996). The leaves are soft, irregularly toothed and are divergent with an unpleasant smell when crushed (VAN WYK et al., 1997).
1.7.5 *Pelargonium sidoides*

*Pelargonium sidoides* DC. is described as a medicinal plant with woody roots. Its long-stalked leaves are clustered, mildly aromatic, heart-shaped and velvety. It is native to South Africa. This evergreen plant is widely distributed throughout Lesotho as well as the Eastern Cape, North-west, Free State, Gauteng and Mpumalanga Provinces of South Africa (DREYER and MARAIS, 2001). The growth of *Pelargonium sidoides* occurs in arid to dry climates. However, they have the ability to withstand frosts and go dormant in very cold areas or during long periods of drought. Therefore, when *Pelargonium sidoides* plants are exposed to areas with very cold winters it is sensible to keep the plants inside from late autumn to early spring to prevent them from freezing to death. *Pelargonium sidoides* is very similar to *Pelargonium reniforme* in appearance and this often raises confusion. The two species are distinguished by the colour of their flowers. Petals of *Pelargonium sidoides* are black and maroon whereas those of *Pelargonium reniforme* are pink to purplish (DREYER and MARAIS, 2001; VAN DER WALT and VORSTER, 1988).
1.7.6 *Siphonochilus aethiopicus*

*Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt is a deciduous plant with large, hairless leaves (*VAN WYK et al.*, 1997). Flowers sprout from the underground stem during late spring and early summer. This plant species was once widely distributed in KwaZulu-Natal, Mpumalanga and Northern Cape although it is now thought to be extinct in KwaZulu-Natal (*CUNNINGHAM, 1988; GORDON-GRAY et al., 1989*).
1.7.7 Xerophyta retinervis

Figure 1.9  Xerophyta retinervis Baker

*Xerophyta retinervis* Baker has been described as a peculiar and deciduous plant that grows up to 1.8 m and is characterized by long, strap-shaped leaves occurring in terminal tufts and at the tip of the stem (*POOLEY, 2005; VAN WYK et al., 1997*). Its flowers are usually mauve or white, and borne in groups on long, slender stems of approximately 150 mm and it flowers around September to November. This plant is usually found on rocky outcrops in hot dry areas and is widely scattered in grassland areas of South Africa (*POOLEY, 2005; SMITH and AYENSU, 1974; VAN WYK et al., 1997*).
1.7.8 *Zantedeschia aethiopica*

![Image](image.png)

**Figure 1.10 Zantedeschia aethiopica (L.) Spreng.**

*Zantedeschia aethiopica* (L.) Spreng. is an evergreen herb that grows 0.6-1 m in height with dark glossy green and hairless leaves. It has funnel-shaped flowers (POOLEY, 2005; VAN WYK *et al.*, 1997). This plant mainly originates in Southern Africa and is distributed over a large part of South Africa where it grows only in wet or seasonally wet places (LETTY, 1973; VAN WYK *et al.*, 1997).

Information on the plants’ medicinal uses is included in Table 2.1.
2. Antimicrobial activity

2.1 Introduction

The rich cultural and biological diversity of South Africa has contributed to successful and sustained ethnopharmacology research over the years. Ethnopharmacology is defined as “a connection of the ethnography of health and healing with the physiologic relevance of the indigenous medical practice” (ETKIN and ELISABETSKY, 2005). Ethnopharmacological studies therefore aim to provide the rationale for selection and scientific investigation of medicinal plants (WELDEGERIMA, 2009). Essentially, this approach serves as an innovative and powerful discovery engine for newer, safer and more affordable medicines (PATWARDHAN, 2005). Plants are generally regarded as complex matrices as they produce a wide range of secondary metabolites with different functional groups and polarities (INUI et al., 2012; KENNEDY and WIGHTMAN, 2011; WITZANY, 2006). A number of novel extraction techniques have been developed with the objective to shorten the extraction time, decrease solvent consumption, increase extraction yield and enhance the quality of extracts (GUPTA et al., 2012; WANG and WELLER, 2006).

The world is faced with a health hazard as a result of infectious diseases (MURUGAN and MOHAN, 2011). However, it has been reported that developing countries carry greater health challenges (SASIKUMAR et al., 2003). Medicinal plants have demonstrated various forms of antimicrobial activities due to their inherent active chemical constituents (MURUGAN and MOHAN, 2011; VIJI and MURUGESAN, 2010). Screening plants for their biological activity is important to establish the ethnobotanical knowledge for a particular disease and disorder in humans (JEYASEELAN et al., 2012; SHAHEEN et al., 2009).

According to LEMANSKE (2003), respiratory tract infections caused by bacteria have a role in the pathogenesis of asthma. Bacterial colonisation of the airways is associated with exacerbation of asthma (HOLLAMS et al., 2010; TOEWS, 2005). In
a report authored by CAZZOLA et al. (1991), 27% of asthmatic patients demonstrating exacerbation of asthma symptoms had bacteria in their sputum with Streptococcus pneumonia, Streptococcus pyogenes, Staphylococcus aureus, Moraxella catarrhalis and Haemophilus influenzae being most prevalent. Asthma is often associated with an increased risk of infection with Streptococcus pyogenes (JUHN et al., 2012). There is evidence which indicates that atopy is frequently associated with asthma, and the role that Staphylococcus aureus plays towards the exacerbation of atopic dermatitis is well established (BUNIKOWSKI et al., 2000; BURROWS et al., 1989; TASKAPAN and KUMAR, 2000). Furthermore HOLLAMS et al. (2010) studied the relationship between bacteria and asthma symptomatology and immunophenotypes by measuring titres of IgE against Streptococcus pneumonia, Staphylococcus aureus and Haemophilus influenzae. The correlation between atopy and asthma was confirmed where the IgE responses to Staphylococcus aureus enterotoxins were higher and more frequent in patients with bronchial infections. Staphylococcus aureus enterotoxin IgE antibodies have been determined as among the risk factors for asthma severity (BACHERT et al., 2012).

In addition, BISGAARD et al. (2007) emphasize colonization by Haemophilus influenzae, Moraxella catarrhalis and Streptococcus pneumoniae and its relation to asthma as some of the contributing factors to an increased risk of the disease.

Fungal infections are regarded as a major concern for public health (TRAKRANRUNGSIE, 2011). Over the years, an increased occurrence of fungal diseases has been reported and this has been shown to have an effect on morbidity and mortality in human communities (ARIF et al., 2011; GARBINO et al., 2001; PFALLER and DIEKEMA, 2007; SHAHID et al., 2009). Posing a serious threat to immunocompromised patients, Candida albicans is among the most widely occurring opportunistic fungal infections in these patients (ANGULO et al., 2002; ASCIOGLU et al., 2002, FISHER-HOCH and HUTWAGNER, 1995; GUDLAUGSSON et al., 2003). The relationship between Candida albicans and asthma and its related conditions has been established thereby enabling an investigation of the sensitisation potential since this yeast-like fungus is recognized to be a potent bronchial antigen (AKIYAMA et al., 1994; ASERO and BOTTAZZI, 2004; GUMOWSKI et al., 1987; LEE et al., 1987). Candida albicans has been described as an opportunistic pathogen responsible for various non-life threatening infections
A study by TARGONSKI et al. (1995) proposed that exposure to environmental moulds may play a role in asthma-related mortality. It is clear that there is mounting evidence in the literature demonstrating a significant relationship between microbial infections and asthma. It is against this background that the present study seeks to investigate four standard bacterial strains and one fungal strain associated with respiratory tract infections against selected medicinal plants. The four bacterial strains include Gram-positive: *Streptococcus pyogenes*, *Staphylococcus aureus*, and Gram-negative: *Klebsiella pneumoniae* and *Haemophilus parainfluenzae*, and the fungal strain: *Candida albicans*.

### 2.2 Bacteria and fungus in asthma and chest infections

#### 2.2.1 Streptococcus pyogenes

*Streptococcus pyogenes* is a spherical Gram-positive bacterium known to cause group A streptococcal infections (DE MELO et al., 2003; IKEBE et al., 2005). This human pathogen causes a variety of clinical manifestations (BALDASSARRI et al., 2006) and is reported to be involved in respiratory tract infections and has an effect on atopic conditions (GRACIA et al., 2009; YUSUF, 2012). *Streptococcus pyogenes* is a non-motile, non-spore forming coccus that occurs in long chains or pairs of cells. *Streptococcus pyogenes* is catalase-negative and is a facultative anaerobe with individual cells of 0.6 to 1.0 µm in diameter (BARON, 1996). Catalase-negative means this particular bacterium does not produce catalase. The enzyme catalase detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

#### 2.2.2 Staphylococcus aureus

*Staphylococcus aureus* is a Gram-positive bacterium that is frequently found in the human respiratory tract. This bacterium occurs as spherical catalase-positive cocci that are arranged in irregular three-dimensional grape-like clusters of cells of 1 µm in diameter (TODAR, 2009). Cluster formation is due to cell division occurring in three planes where daughter cells tend to remain in close proximity. The *Staphylococcus*
aureus clusters are non-motile, non-spore forming and non-capsulated (with the exception of rare strains that are capsulated). *Staphylococcus aureus* is ubiquitous and characterized as a facultative anaerobe. This bacterium can grow by aerobic respiration or fermentation producing lactic acid, and can grow within a temperature range of 15 and 45 °C, a pH range of 4 to 11 and at NaCl concentrations as high as 15% (TODAR, 2009). *Staphylococcus aureus* is often not pathogenic but can cause respiratory diseases and related conditions such as atopic dermatitis and sinusitis. *Staphylococcus aureus* has been identified as the leading cause of nosocomial infections (LOWY, 2003).

2.2.3 *Klebsiella pneumoniae*

*Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, facultative anaerobic, rod-shaped bacterium (GUO et al., 2012). This bacterium consists of straight rods of 1 to 2 µm in length and is covered by a polysaccharide capsule. Ubiquitous in nature, *Klebsiella pneumoniae* is found in the normal flora of the mouth, skin and intestines (PODSCHUN and ULLMANN, 1998). The rod-shaped bacterium causes opportunistic infections that are mostly observed in individuals with chronic lung disease, diabetes, weak immune systems and chronic alcoholism (PODSCHUN and ULLMANN, 1998).

2.2.4 *Haemophilus parainfluenzae*

*Haemophilus parainfluenzae* is a Gram-negative bacterium that forms part of commensals in the upper respiratory tract (TAYLOR et al., 1992). It is an opportunistic pathogen in chronic lung diseases and is believed to cause systematic diseases (MITCHELL and HILL, 2000). *Haemophilus parainfluenzae* is characterized by small, non-motile pleomorphic rods. This facultative anaerobic bacterium is positive for oxidase, nitrate reduction and H2S production. Moreover, *Haemophilus parainfluenzae* is positive for catalase, ortho-nitrophenyl-β-galactoside, ornithine decarboxylase and urease (JUNI et al., 1982). *Haemophilus parainfluenzae* is negative for indole and aesculin production (www.catalog.hardydiagnostics.com/cp_prod/content/hugo/RapidIndoleBroth.
This means that this bacterium does not have the ability to cleave indole from tryptophan and to hydrolyze esculin in the presence of bile.

2.2.5 Candida albicans

Candida albicans is a dimorphic fungus that exists as a commensal inhabitant of mucosal surfaces in most healthy individuals (MOLERO et al., 1998; SAIGAL et al., 2011). Though it is normally a harmless commensal organism, it is an opportunistic pathogen for some immunologically weak and immunocompromised people (KIM and SUDBERY, 2011; ROSENBACH et al., 2010). It is a pathogenic yeast-like fungus that grows under aerobic conditions. The fungus grows in temperature ranges of 20-40°C and pH of 2-8 (ANAND and PRASAD, 1991). Candida albicans contains a catalase (TOSADO-ACEVEDO et al., 1992).

2.3 Treatment of microbial infections associated with asthma

Antibiotics are believed to be the perfect system to combat bacteria. Commonly administered in human and veterinary medicine, antibiotics are used to treat a wide variety of infectious diseases (BHALODIA and SHUKLA, 2011). The selection of an optimal empiric antibiotic requires up-to-date knowledge on antimicrobial susceptibility of the suspected pathogen (GRACIA et al., 2009). Penicillin, erythromycin, imipenem, clindamycin, rifampin, cephalosporins, lincosamides and macrolides have been recommended for treating streptococcal infections. However, the proven efficacy and safety, narrow spectrum, and low cost of penicillin have made this antibiotic the treatment of choice for several decades (BISNO et al., 2002; DAJANI et al., 1995; DE MELO et al., 2003; GRACIA et al., 2009; HSEUH et al., 1995). According to IKEBE et al. (2005), a combination of penicillin and clindamycin has been successfully used to treat severe invasive Streptococcus pyogenes. Moreover, IKEBE et al. (2005) reported citing STEVENS et al. (1988) that penicillin is 100% effective against Streptococcus pyogenes. Neomycin is one of the antibiotics that have been recommended for the treatment of respiratory infections and skin conditions caused by Staphylococcus aureus. Neomycin belongs to a group
of aminoglycoside antibiotics containing a 4, 5-disubstituted 2-deoxystreptamine core.

Literature revealed that there is a need for the discovery of new classes of antifungal compounds to combat fungal infections (ARIF et al., 2011; RUNYORO et al., 2006). Despite the fact that there is a set of common antifungal drugs including amphotericin B, fluconazole, miconazole, itraconazole and nystatin, RUNYORO et al. (2006) have articulated how difficult it is to manage Candida infections. Amphotericin B has intrinsically served as a touchstone for treating disseminated life-threatening fungal infections for over 40 years (ELLIS, 2002). This drug is a polyene macrolide antibiotic derived from the actinmycete Streptomyces nodosus (ELLIS, 2002). Amphotericin B binds with high affinity to ergosterol (MARTIN et al., 1994).

Since the development of antibiotics to treat bacterial infections, it has been established that there is an increasing problem of antibiotic resistance and a continuing need for new solutions (BALL et al., 2004; MARTIN and ERNST, 2003). Resistance to antibiotics in pathogenic fungi has been identified as a problem of special importance in the control of infections (GULSHAN and MOYE-ROWLEY, 2007). The resistance to antifungal agents has severe implications such as morbidity and mortality (ARIF et al., 2011). Antibiotic resistance is viewed as a natural biological phenomenon and has been recognized as one of the major threats to human health worldwide (JUNIOR et al., 2009; WALKER et al., 2009). Antibiotic resistance occurs when microbes develop the ability to defend themselves against the effect of an antibiotic. The most common causes of antibiotic resistance is when patients do not complete the prescribed course in full, skipping doses of antibiotics, and not taking antibiotics at regular intervals. The resulting resistance to antibiotics has served as a motivation for recent research that has been directed towards the use of traditional medicine for the treatment and control of infections. Many plants are used worldwide in the sector of traditional medicine to treat microbial infections. It has been established that the majority of people prefer the herbal system even though natural products are not safer than the synthetic antibiotics, and there is a growing need for the development of herbal antibiotics (MARTIN and ERNST, 2003). According to MAHESH and SATISH (2008), medicinal plants represent a rich source of antimicrobial agents. The mainstream medical sector is resorting to drugs derived from plants because the traditional antibiotics eventually become ineffective.
This is motivated by the fact that drugs derived from natural sources have showed great impact on the prevention and treatment of human diseases (BHALODIA and SHUKLA, 2011). Several studies have been done where traditional medicinal plants have been investigated in search of novel antifungals (JONES et al., 2000; MOTSEI et al., 2003; WEBSTER et al., 2008). The aim of the current study is to investigate plants that may serve as antimicrobial agents combating asthma and related conditions.

2.4 Materials and methods

2.4.1 Selection and collection of candidate plants

Eight plants that are used traditionally to treat asthma and related conditions were selected based on indigenous ethnopharmacological records from the available literature (HUTCHINGS et al., 1996; VAN WYK et al., 1997; WATT and BREYER-BRANDWIJK, 1962; www.portal.isiknowledge.com). Plant materials of these species were collected from the University of KwaZulu-Natal (UKZN) Botanical Garden, UKZN Ukulinga Research Farm, Western Cape area and Pretoria region between March 2012 to March 2013 depending on the availability. Voucher specimens were prepared, identified by Dr C. Potgieter and lodged in the UKZN Bews Herbarium in Pietermaritzburg for reference purposes.
<table>
<thead>
<tr>
<th>Plant family</th>
<th>Species</th>
<th>Local name</th>
<th>Parts used</th>
<th>Place of collection</th>
<th>Voucher number</th>
<th>Traditional uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araceae</td>
<td><em>Zantedeschia aethiopica</em> (L.) Spreng.</td>
<td>Ihlukwe</td>
<td>Leaves and rhizomes</td>
<td>UKZN Botanical Garden</td>
<td>Motlhatlego 4 NU</td>
<td>Leaves used for wounds, sores and boils. Ground leaves applied to parts affected by rheumatism and gout. Boiled rhizomes mixed with honey are taken for bronchitis, asthma, heartburn and rheumatism or gargled for a sore throat.</td>
<td>VAN WYK <em>et al.</em>, 1997; WATT and BREYER-BRANDWIJK, 1962</td>
</tr>
<tr>
<td>Bombacaceae</td>
<td><em>Adansonia digitata</em> L.</td>
<td>Kremetart, Seboi, Mowana, Ximuwu, Isimuku, Muvhuyu</td>
<td>Bark</td>
<td>Venda</td>
<td>Mulaudzi 28 NU</td>
<td>Asthma and skin disorders.</td>
<td>ANANIL <em>et al.</em>, 2000; KARANDIKAR <em>et al.</em>, 1965</td>
</tr>
<tr>
<td>Family</td>
<td>Species</td>
<td>Common Name(s)</td>
<td>Part(s)</td>
<td>Collection Location</td>
<td>Uses</td>
<td>References</td>
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<tr>
<td>Celastraceae</td>
<td><em>Catha edulis</em> (vahl) Endl.</td>
<td>Umhlawazizi, umhlwazi</td>
<td>Leaves</td>
<td>UKZN Botanical Garden Mothathlego 2 NU</td>
<td>Coughs, asthma and other respiratory conditions. Relieve fatigue and sleepiness, and has a stimulating and euphoric effect.</td>
<td>CAWSTON, 1933; VAN WYK et al., 1997; WATT and BREYER-BRANDWIJK, 1962</td>
<td></td>
</tr>
<tr>
<td>Geraniaceae</td>
<td><em>Pelargonium sidoides</em> DC.</td>
<td>Kalwerbossie</td>
<td>Roots</td>
<td>UKZN Botanical Garden Moyo 08 NU</td>
<td>Gonorrhoea, diarrhoea, dysentery, colds and lung infections.</td>
<td>HUTCHINGS et al., 1996</td>
<td></td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Ballota africana</em> (L.) Benth.</td>
<td>Kattekruid</td>
<td>Leaves</td>
<td>Western Cape area J. Manning s.n.</td>
<td>Colds and influenza, asthma, bronchitis, hoarseness, heart trouble, hysteria, insomnia, typhoid fever, headaches, liver problems and piles.</td>
<td>VAN WYK et al., 1997</td>
<td></td>
</tr>
<tr>
<td>Family: Solanaceae</td>
<td><em>Datura stramonium</em> L.</td>
<td>Iloyi, iloqi, iyoli (elimhlophe)</td>
<td>Leaves</td>
<td>Ukulinga Research Farm</td>
<td>Mothlatlego 1 NU</td>
<td>Relieve asthma and reduce pain. Weak infusion used as hypnotics by the elderly and as aphrodisiacs by adults.</td>
<td>POOLEY, 2005; VAN WYK et al., 1997; WATT and BREYER-BRANDWIJK, 1962</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>More rarely the green fruit</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family: Velloziaceae</td>
<td><em>Xerophyta retinervis</em> Baker</td>
<td>Isiphemba, isiqumama</td>
<td>Roots, whole plant or stem bark</td>
<td>Pretoria region</td>
<td>McGaw 85 NU</td>
<td>Dried leaves are smoked for asthma relief or smoke from the whole plant to stop nose bleeding.</td>
<td>HUTCHINGS et al., 1996; WATT and BREYER-BRANDWIJK, 1962</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

s.n. = sine numero meaning specimen without a number
2.4.2 Extract preparation

2.4.2.1 Plant sample extraction
The plant material was oven dried at 50°C. When absolutely dry, it was ground into fine powders using an Ultra Centrifugal Mill (ZM 200, Retsch®, Germany) and stored in honey jar containers at room temperature. Ground plant samples (5 g) were sequentially extracted using 100 ml each of petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water in a sonication bath containing ice at room temperature for an hour in each solvent. The extracts were then filtered under vacuum through Whatman No. 1 filter paper. The resultant extracts were either freeze-dried or concentrated using a Büchi rotary evaporator (Germany). The concentrated extracts were further dried under a stream of cold air. Water extracts were collected and transferred into pre-labelled glass jars and freeze-dried. The total extract mass was measured and recorded as the yield percentage of each extract. The prepared crude extracts were stored in the dark at 10°C to minimize photodegradation of light-sensitive compounds.

2.4.3 Source of Bacterial strains
All the bacterial and fungal strains used in this study were recognised as part of the American Type Culture Collection (ATCC).

2.4.4 Preparations of media and organisms

2.4.4.1 Preparation of Haemophilus parainfluenzae
The *Haemophilus* Test media agar plates (HTMAP) were prepared by dissolving 21.5 g of *Haemophilus* Test Medium Base in 500 ml of distilled water. The solution was boiled to allow complete dissolution and was then sterilized by autoclaving at 121°C for 15 minutes. The solution was cooled down to 50°C and the content of one vial of *Haemophilus* Test Medium Supplement (SR0158) was added aseptically as directed by the manufacturer, mixed well and poured into sterile petri dishes.
*Haemophilus parainfluenzae* (ATCC 7901) was obtained from the supplier [Quantum Biotechnologies (Pty) Ltd]. The manufacturer's instructions were followed with regards to the inoculation of the primary culture using the KWIK STIK re-hydration method. The KWIK STIK was removed from the pack and was pinched just below the fluid meniscus of the ampule found in the cap in order to release the hydrating fluid. The pellet was held vertically and the hard surface was tapped to allow the flow of fluid through the shaft into the bottom of the unit containing the pellet. The pellet was crushed and was mixed with the fluid, through the pinching method. The swab was heavily saturated in suspension and inoculated by gently rolling over one-third of the HTMAP. The inoculated primary cultured plates were then incubated anaerobically at 37°C for 18-24 hours. For long-term storage, *Haemophilus parainfluenzae* (ATCC 7901) was prepared for cryopreservation by swabbing growth from the HTMAP into 15 ml brain heart infusion (BHI) broth supplemented with 10-20% sterile glycerol, then transferring 2 ml of this mixture into cryovials. The cryovials were stored at temperatures below -70°C.

### 2.4.4.2 Preparation of *Streptococcus pyogenes*

Sheep Blood Agar Plates (SBAP) were purchased from the supplier [Quantum Biotechnologies (Pty) Ltd]. *Streptococcus pyogenes* (ATCC 12344) was obtained from the supplier [Quantum Biotechnologies (Pty) Ltd]. The bacterium was prepared in the same manner using the KWIK STIK re-hydration method as described in Section 2.4.4.1. However, the inoculated primary cultured plates were incubated anaerobically at 37°C for 24-72 hours.

For long-term storage of *Streptococcus pyogenes*, 18.2 g of Todd-Hewitt broth was dissolved in 500 ml of distilled water. The solution was mixed well and autoclaved at 121°C for 20 minutes. The Streptococcal colonies from the sheep blood agar plate were inoculated in the Todd- Hewitt Broth and incubated at 37°C overnight under anaerobic conditions (5% CO₂). *Streptococcus pyogenes* bacteria were prepared for deep freezing by swabbing growth from the SBAP into 2 ml cryovials and twenty Eppendorf tubes containing Todd-Hewitt broth (THB) supplemented with 20% sterile glycerol. The cryovials were stored at temperatures below -70°C.
2.4.4.3 Preparation of *Staphylococcus aureus* and *Klebsiella pneumoniae*

Mueller-Hinton (MH) agar plates were prepared by dissolving 38 g of MH agar in 1000 ml of distilled water. The solution was boiled to allow complete dissolution and was then sterilized by autoclaving at 121°C for 15 minutes. The solution was cooled down to 55°C and poured into sterile petri dishes. The petri dishes containing *Staphylococcus aureus* (ATCC 12600) and *Klebsiella pneumoniae* (ATCC 13883) were taken from 4°C storage. Single colonies of each bacterium were respectively transferred into McCartney bottles each with 10 ml Oxoid Mueller-Hinton Broth (MHB) (CM0405). These bottles were then incubated in a shaking incubator overnight at 37°C. The maintenance of the bacterial vigour and viability required the bacteria to be sub-cultured every 30 days.

2.4.4.4 Preparation of *Candida albicans*

The Yeast Malt (YM) agar plates were prepared by dissolving 23 g of YM agar in 1000 ml of distilled water. The solution was boiled to allow complete dissolution and was then sterilized by autoclaving at 121°C for 15 minutes. The solution was cooled down to 55°C and poured into sterile petri dishes. *Candida albicans* (ATCC 10231) cultures were prepared in a similar manner to that of *Staphylococcus aureus* and *Klebsiella pneumoniae* (Section 2.4.4.3) with the difference in using Yeast Malt Broth (YMB) in place of MHB.

2.4.5 Preparation of plant extracts for the microdilution assay

For the microdilution method for *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Candida albicans*, the organic solvent extracts and aqueous extracts were re-suspended respectively in 80% ethanol and sterile distilled water to a final concentration of 50 mg/ml. The same procedure was followed in the case of *Streptococcus pyogenes* except that the organic solvent extracts were re-suspended in 1% dimethyl sulphoxide (DMSO). To enable complete re-suspension, solutions were sonicated for an hour. Organic solvent and aqueous extracts used for the paper
disc diffusion technique were re-suspended respectively in 1% DMSO and sterile distilled water at a final concentration of 50 mg/ml. The optical density (OD) of the saturated bacterial cultures of *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively, was measured at 600 nm to obtain a reading of approximately 1.9-2.0, which were then diluted 1:100 with MHB (200 µl of bacterial culture into 19.8 mL of broth) to give a reading between 0.4 to 0.6 (Eloff, 1998). In the case of *Streptococcus pyogenes*, cultures were measured at 600 nm to obtain a reading of 0.2 to 0.3 then followed the same method by Eloff (1998). The OD of *Candida albicans*, was measured at 530 nm to obtain a reading of 0.25 to 0.28. Four ml of sterile saline was added to 400 µl of the overnight grown culture, and the OD of 1 ml of this fungal culture was measured at 530 nm. The fungal culture was diluted 1:1000 with YMB.

2.4.6 Experimental procedure

2.4.6.1 Microdilution technique
The microdilution technique with the test organisms *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* was used to obtain the minimal inhibitory concentration (MIC) of the plant extracts (Eloff, 1998). The MIC is the lowest concentration of the medicinal plant extracts at which the microorganism does not demonstrate visible growth. All extracts were initially tested at 12.5 mg/ml in 96-well microplates and serially diluted in two-fold dilution steps to 0.098 µg/ml. Following this step, 100 µl bacterial culture was then added to each well. The antibiotic neomycin (50 µg/ml) was used as a reference in the assays for *Staphylococcus aureus* and *Klebsiella pneumoniae*, with antibiotics ampicillin and penicillin (50µg/ml) as the reference for *Streptococcus pyogenes*. Extract-free solutions were used as a blank control. The microplates were covered with parafilm and incubated overnight at 37 °C. In the case of *Streptococcus pyogenes*, plates were incubated anaerobically at 37°C for a period of 48 hours. As an indicator of bacterial growth, 50 µl p-iodonitrotetrazolium violet (INT) (Sigma 0.2 mg/l) dissolved in water was added to the wells and incubated at 37 °C for an hour. Moreover, the addition of INT for the *Streptococcus pyogenes* plates required anaerobic conditions
for an hour. The minimal inhibitory concentration (MIC) values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, as indicated as a clear well. INT allows the bacterial suspension to turn red-pink where bacterial growth is not inhibited. The assays were repeated thrice with two replicates.

The microdilution technique with the test organism Candida albicans was used to obtain the MIC of the plant extracts (Eloff, 1998). The organic and water extracts were dissolved at 50 mg/ml with 80% ethanol and water, respectively. All extracts were initially tested at 12.5 mg/ml in 96-well microplates and serially diluted in two-fold dilution steps to 0.39 mg/ml. Following this step 100 µl of the 1:1000 diluted fungal culture was then added to each well. The antibiotic Amphotericin B (50 µg/ml) was used as a reference in this assay. Extract-free solutions were used as a blank control. The microplates were covered with parafilm and incubated overnight at 37 °C. As an indicator of fungal growth, 40 µl INT dissolved in water was added to the wells and incubated at 37 °C for 24 hours. MIC values were recorded as the lowest concentration of the extract that completely inhibited fungal growth, as indicated by a clear well. The use of INT allowed the fungal suspension to turn red-pink where fungal growth is not inhibited. The assays were repeated thrice with two replicates. After recording the MIC values, Yeast Malt Broth (50 µl) was added to each clear well in the series and the plates were incubated for a further 24 hours at 37 °C. This was done with the objective of determining the minimum fungicidal concentration (MFC).

2.4.6.2 Disc diffusion
Antibacterial activity of the plants was also tested by the disc-diffusion method as described by (Davis and Stout, 1971; Rojas et al., 2001, Moussa et al., 2012). The disc-diffusion technique with the test organism Haemophilus parainfluenzae was used to detect haemolysis. The objective of this method is to detect whether the antibiotic is effective/ineffective for the pathogen and its strain (Moussa et al., 2012). A single colony of Haemophilus parainfluenzae from HTMAP was inoculated in MH broth and the culture was incubated at 37°C under anaerobic conditions for 48 hours. The OD reading of the overnight culture was taken at 600 nm. The overnight suspension of cultures was further diluted until an
OD$_{600}$ of 0.3 to 0.4 was reached (DWORKIN et al., 2006). Two hundred µl of the diluted inoculum of bacteria (fresh culture suspension) was evenly distributed to each HTMAP through the spread plate technique. The filter paper discs were prepared with the help of a punch machine and were placed into McCartney bottles for autoclaving at 121°C for 20 min. Three sterile 6 mm filter paper discs were prepared for antibacterial screening. These sterile filter paper discs were placed on blank petri dishes and were impregnated with 5 µl of filter-sterilized plant extracts, and allowed to dry before being placed onto the respective plates. Ampicillin and penicillin were used at eleven concentrations (200 µg/ml, 160 µg/ml, 120 µg/ml, 80 µg/ml, 40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml and 0.625 µg/ml) as the positive control and 1% DMSO as a negative control. The plates were allowed to dry for 15 to 30 minutes and were then incubated at 37°C for 48 hours under anaerobic conditions. The diameters of the clear zones around each disk were measured after incubation with the help of a Vernier Caliper.

### 2.5 Results and discussion

**Table 2.2 Percentage yield related to dry weight of extracts obtained using the described extraction process**

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Part</th>
<th>Percentage yield (dry weight) obtained with extraction solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PE</td>
</tr>
<tr>
<td>Adansonia digitata</td>
<td>Bark</td>
<td>0.16</td>
</tr>
<tr>
<td>Ballota africana</td>
<td>Leaves</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>0.31</td>
</tr>
<tr>
<td>Catha edulis</td>
<td>Leaves</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>0.52</td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>Leaves</td>
<td>1.03</td>
</tr>
<tr>
<td>Plant name</td>
<td>Plant part</td>
<td>PE</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>------</td>
</tr>
<tr>
<td>Pelargonium sidoides</td>
<td>Flowers and fruits</td>
<td>6.67</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>0.24</td>
</tr>
<tr>
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Table 2.3: Antibacterial activity of the selected medicinal plants used for the treatment of asthma and related conditions

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<th>Zone of inhibition (mm)</th>
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*S.p.* = *Streptococcus pyogenes*; *K.p.* = *Klebsiella pneumoniae*; *S.a.* = *Staphylococcus aureus*; *H.p.* = *Haemophilus parainfluenzae*

Values highlighted in red and bold are considered very active (MIC of <1 mg/ml and zone of inhibition in mm)
Table 2.4: Antifungal minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of medicinal plants used for the treatment of asthma and related conditions

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<td>Ballota africana</td>
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Grinding the dried plant material to fine powders increases the surface area for extraction and in so doing it increases the rate of extraction (GURJAR et al., 2012). Extraction has been established as the crucial first step in the analysis of medicinal plants (GUPTA et al., 2012; SASIDHARAN et al., 2011). The percentage yields of the extracts are presented in Table 2.2. The highest yield was obtained with the water extract of Datura stramonium flowers and fruits (27.60%) and the lowest yield was obtained from the Petroleum Ether extract of Pelargonium sidoides roots (0.05%). Generally out of the four solvents used for extraction, the highest extract yield was obtained using 80% EtOH and the lowest with PE. The trend of the percentage yield of crude extracts was 80% EtOH > Water > DCM > PE. The higher yields obtained with 80% EtOH may possibly be due to the wide range of phytochemicals extracted with the combination of this solvent and water. Siphonochilus aethiopicus rhizomes, Catha edulis leaves, and Zantedeschia aethiopica stems and leaves gave the highest yields with 80% EtOH. Water is the
universal and most common solvent used to extract plant products (MADANE et al., 2013) and the high yield is expected to correlate with high antimicrobial activity. Although many traditional healers use primarily water for extraction, plant extracts from organic solvents give more consistent antimicrobial activity compared to water extracts (MADANE et al., 2013; PAREKH et al., 2005). This is simply because most phytochemicals are more soluble in organic solvents than water (MADANE et al., 2013).

The results of the antibacterial assay expressed as MIC values are presented in Table 2.3 and range from 0.098 to >12.5 mg/ml. The results of the antifungal assay expressed as MIC and MFC values are presented in Table 2.4 and range from 1.56 to >12.5 mg/ml. According to FABRY et al. (1998), MIC values below 8 mg/ml for crude extracts are considered to have good activity. However, it has been reported that antibacterial MIC values equal or less than 1 mg/ml possess greatest activity (NDHLALA et al., 2009). This classification was applied in this study for the determination of activity of the crude extracts against the key bacteria and the fungus. It does not necessarily imply that the crude extracts with MIC values over 1 mg/ml do not have any activity. This may possibly demonstrate that a number of relatively inert substances show antibacterial activity at this high concentration (GIBBONS, 2004) or that the plant extracts may have activity against other pathogenic bacteria than those tested.

The highest antibacterial activity of 0.098 mg/ml was exhibited by the PE leaf extract of Pelargonium sidoides against Streptococcus pyogenes. The PE and ethanol extracts of Pelargonium sidoides roots recorded good activity of 0.39 mg/ml against Streptococcus pyogenes. The roots of Pelargonium sidoides have successfully been used in the treatment of asthma and other chronic respiratory conditions such as bronchitis, tonsillopharyngitis, common cold and pneumonia (LIZOGUB et al., 2007; TIMMER et al., 2009). In developing a strategic plan to solve the issue of destructive harvesting of the underground parts of plants, the use of leaves and stems has been recommended as an alternative to tubers and roots for medicinal purposes (ZSCHOCKE et al., 2000). Following the same approach, LEWU et al. (2006) recommended the substitution of roots of Pelargonium sidoides with the leaves in the treatment of bacterial infections associated with the respiratory system. As reflected
in Table 2.3, 66.7% of the crude extracts of PE, DCM, EtOH and Water used to screen the *Pelargonium sidoides* leaves responded positively to *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Staphylococcus aureus* with MIC values of good activity ranging from 0.098 and 0.78 mg/ml. This complements a study by LEWU *et al.* (2006) on the use of the leaves of *Pelargonium sidoides* in medicinal formulations as a way of achieving sustainable harvesting. *Pelargonium sidoides* decreases cough and asthma attack frequency (TAHAN and YAMAN, 2013) and the efficacy of EPs® 7630 in the treatment of respiratory tract infections has been established (KOLODZIEJ and KIDERLEN, 2007). The antibacterial properties of this plant achieved in this study therefore encourages the use of a commercial product Umckaloabo®, which is used for respiratory tract infections with the objective of reducing inflammation of the bronchi. *Pelargonium sidoides* remains an important medicinal plant in traditional medicine to treat asthma and related conditions.

The PE stem extract of *Datura stramonium*, ethanol root extract of *Siphonochilus aethiopicus* and DCM stem extract of *Zantedeschia aethiopica* exhibited the second highest antibacterial activity of 0.195 mg/ml against *Streptococcus pyogenes*. Moreover, the PE and DCM stem extracts of *Datura stramonium* had a MIC value of 0.78 mg/ml against *Staphylococcus aureus*. The PE leaf extract of *Datura stramonium* had a MIC value of 0.78 mg/ml against *Klebsiella pneumoniae*. The PE and DCM root extracts of *Datura stramonium* yielded good activity (0.78 and 0.39 mg/ml, respectively) against *Streptococcus pyogenes*.

The PE rhizome extract of *Adansonia digitata* as well as the EtOH and water leaf extracts of *Catha edulis* were shown to be active with a MIC value of 0.39 mg/ml against *Streptococcus pyogenes*. The ethanol stem extract of *Catha edulis* had a MIC value of 0.78 mg/ml against *Staphylococcus pyogenes*. The DCM and ethanol leaf extracts and DCM stem extract of *Ballota africana* showed good antibacterial activity at a MIC value of 0.39 mg/ml against *Staphylococcus aureus*.

The PE leaf extract of *Siphonochilus aethiopicus* and ethanol leaf extract of *Xerophyta retinervis* were active against *Streptococcus pyogenes* (0.39 mg/ml). The ethanol leaf extract of *Siphonochilus aethiopicus*, DCM root extract of *Siphonochilus aethiopicus* and water leaf extract of *Xerophyta retinervis* exhibited antibacterial
activity against _Streptococcus pyogenes_ (0.78 mg/ml). Ethanol and water leaf extracts, ethanol stem extract, as well as PE and DCM root extracts of _Zantedeschia aethiopica_ also exhibited antibacterial activity against _Streptococcus pyogenes_ with a MIC value of 0.78 mg/ml.

The PE extracts of _Siphonochilus aethiopicus_ roots and _Siphonochilus aethiopicus_ rhizomes yielded 0.39 and 0.78 mg/ml respectively against _Klebsiella pneumoniae_. The PE root extract of _Siphonochilus aethiopicus_ was found to be active with a MIC of 0.39 mg/ml against _Staphylococcus aureus_. The DCM extracts of _Siphonochilus aethiopicus_ stems, roots and rhizomes had an MIC value of 0.78 mg/ml against _Staphylococcus aureus_. Furthermore, the DCM and ethanol leaf extracts of _Xerophyta retinervis_ and PE rhizome extract of _Zantedeschia aethiopica_ showed good inhibition of _Staphylococcus aureus_ with an MIC value of 0.78 mg/ml.

As revealed in **Table 2.4**, there was no good antifungal activity shown in the study as the MIC and MFC values were more than 1 mg/ml. However, the PE leaf extract of _Catha edulis_ as well as the DCM leaf extract of _Ballotta africana_, _Catha edulis_ stems, roots and stems of _Datura stramonium_, _Pelargonium sidoides_ leaves, leaves and rhizomes of _Siphonochilus aethiopicus_ and _Zantedeschia aethiopica_ rhizomes yielded modest antifungal activity against _Candida albicans_ with a MIC value of 1.56 mg/ml. Furthermore, the ethanol root extract and the water stem extract of _Zantedeschia aethiopica_ recorded a MIC of 1.56 mg/ml. A study done by **NIELSEN et al. (2012)** tested the antifungal activity of methanol leaf and stem extracts of _Zantedeschia aethiopica_ using the microdilution technique with a different _Candida albicans_ strain. In their study _Zantedeschia aethiopica_ exhibited antifungal activity of 312.50 µg/ml while in this current study the PE, DCM and EtOH leaf and stem extracts of _Zantedeschia aethiopica_ yielded a MIC of 3.125 mg/ml. Furthermore, **STEENKAMP et al. (2007)** used the same _Candida albicans_ strain as the one used for this study where they tested the antifungal activity using the plate-hole diffusion technique and could not determine the MIC since the crude plant extract showed no zone of inhibition. Naturally, _Zantedeschia aethiopica_ may not possess antifungal properties against _Candida albicans_. With regards to _Siphonochilus aethiopicus_, the results complement a study by **COOPOOSAMY et al. (2010)** that less antifungal activity was shown with _Candida albicans_ when compared to other tested fungal
strains. Similarly, SIDDQUI et al. (2012) showed that Catha edulis did not have antifungal properties. The lowest MFC was obtained from the DCM extract of the rhizomes of Zantedeschia aethiopica. The fungal strain was resistant to most plant extracts screened.

The purpose of running the disk diffusion assay was to determine the sensitivity or the resistance of the anaerobic bacteria to various antimicrobial compounds. This is important in the management of infections and enables the innovation of effective drugs. For this study, the disk diffusion was done because it is the accepted standard for the particular strain. Although the screening process of a number of medicinal plants used to treat bacterial infections has been subjected to in vitro testing, it has been established that their efficacy is often restricted to being tested in controlled clinical trials (MARTIN and ERNST, 2003). Only the ethanol stem extract of Siphonochilus aethiopicus and water leaf extract of Zantedeschia aethiopica showed zones of inhibition of 13.24 and 21.10 mm, respectively. The size of the clear zone is directly proportional to how sensitive Haemophilus parainfluenzae is. All the other screened extracts showed no zone of inhibition, which suggests that plant extracts were ineffective against Haemophilus parainfluenzae. Negative controls exhibited no zone of inhibition as expected while positive controls (disc containing antibiotics, Ampicillin and Penicillin) exhibited zones of inhibition against the strain of interest for this assay.

One or more extracts from the tested plants were effective against one or both the Gram-positive bacteria investigated. Several studies have also reported that the Gram-positive bacteria are more susceptible as compared to Gram-negative bacteria (KARSHA and LAKSHMI, 2010; RABE and VAN STADEN, 1997; VLIETINCK et al., 1995; WAGATE et al., 2010). This is mainly due to the differences in their cell wall structure. Gram-negative bacteria are generally considered to be more resistant to a large number of antibiotics compared to the Gram-positive ones because of the presence of an outer membrane (NIKAIDO, 1998). The outer membrane serves as an efficient permeability barrier to large molecules and hydrophobic molecules, and also provides protection from adverse environmental conditions. Streptococcus pyogenes is an important human pathogen causing asthma and related respiratory conditions and crude extracts have been shown to be effective against this particular
pathogen. This is supported by the link between asthma and an increased risk of *Streptococcus pyogenes* where the infections due to this bacterium account for approximately 500 000 deaths (JUHN et al., 2012).

These results indicate that the medicinal plants screened in this study have antibacterial activity against bacteria often associated with asthma and related conditions. However, the challenges that are faced with *Datura stramonium* and *Siphonochilus aethiopicus* are their natural toxic state and scarcity, respectively. A study by SHAGAL et al. (2012) corroborated how important *Datura stramonium* stem-bark extract is when used ethnomedically to treat diseases caused by some pathogenic bacteria. CHARPIN et al. (1979) studied the bronchodilator effects whereby patients inhaled antiasthmatic *Datura stramonium* cigarette smoke. From this study, it was observed that the maximal bronchodilator effect of the cigarette tested was similar in most patients to that of a modern adrenergic drug, salbutamol. However, the regular use of these antiasthmatic cigarettes was not recommended. The alkaloids contained in the plant may cause chronic mucosal inflammation or be carcinogenic agents. The toxic nature and potential risks associated with the use of *Datura stramonium* have been reported to have adverse effects on the central nervous system (Gaire and Subedi, 2013) even though this plant has been used as a treatment for asthma (Gaire and Subedi, 2013; Gandevia, 1975; Jackson, 2010; Pooley, 2005; Van Wyk et al., 1997). Moreover, the hallucinogenic and euphoric effects of this plant species was verified by PRETORIUS and MARX (2006) who suggested that the species may possibly cause permanent damage to the foetus if consumed during pregnancy. Generally, intoxication from *Datura stramonium* results in an anticholinergic reaction which may leave the consumer with symptoms such as restlessness, hallucinations, blurred vision and dry mucous membranes (www.arscurandi.ca/stram.html). It has been reported that ingesting high levels of this plant may produce dangerous medical conditions such as cardiac arrhythmia, hyperpyrexia, seizures, coma, and respiratory arrest (Chan, 2002).

Although literature reports toxicity of some medicinal plants, traditional healers prescribe these plants irrespective of their toxicity. Medicinal plants act on the body by regulating and balancing its vital processes, thus traditional healers frequently use
combinations of plants to treat diseases (KAMATOU et al., 2006; OKIGBO and MMEKA, 2006). Unlike in modern drug discovery which is based on the lock-and-key system where one single compound is used to hit the target in order to combat the disease of interest (SAMS-DODD, 2005; SMITH, 2003), the diversity of plant compounds proposes a high probability of synergistic interactions among them (BIAVATTI, 2009; NELSON and KURSAR, 1999). The modulation of toxicity of Datura stramonium may possibly be obtained by combination with other plants and thereby forming some form of counteraction. Even though traditional healers have limited understanding of the scientific rationale of compounds, they seem to have a broader view in understanding the synergy from combination of different plants for various diseases and the balance between efficacy and safety. Research has been done supporting that leaves of Datura stramonium should still be regarded as an important treatment for asthma and related conditions due to its pharmacological properties as reported by SONI et al. (2012) and as demonstrated by its antibacterial activity (Table 2.3).

Antibacterial activity of crude extracts of Siphonochilus aethiopicus have previously been evaluated against Staphylococcus aureus and Klebsiella pneumoniae (STAFFORD et al., 2005). According to FOUCHE et al. (2011), there had been a narrow focus drawn on the pharmacological activities and active compounds of Siphonochilus aethiopicus whilst the plant had been included in several general surveys associated with diverse biological effects. STREET and PRINSLOO (2013) reviewed from GERICKE (2011) that the application of sesquiterpenoid siphonochilone and extracts containing siphonochilone from Siphonochilus aethiopicus has been patented for application of the anti-inflammatory activities in formulation treating asthma and allergic conditions. Furthermore, STREET and PRINSLOO (2013) reported the isolation of two new furanoterpenoid derivatives 4aaH-3,5a,8ab-trimethyl-4,4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one and 2-hydroxy-4aaH-3,5a,8ab-trimethyl-4,4a,9-tetrahydronaphtho[2,3-b]-furan-8-one from Siphonochilus aethiopicus from various sources (HOLZAPFEL et al., 2002; VAN WYK et al., 1997; VILJOEN et al., 2002). As highlighted in Table 2.1, Siphonochilus aethiopicus is traditionally used for respiratory ailments. Despite this being the case, and that it holds potential to treat asthma (Table 2.1), no studies had been carried out to pharmacologically evaluate the anti-asthmatic properties of this plant.
(FOUCHE et al., 2011). As a result, FOUCHE et al. (2011) investigated in vitro and in vivo anti-asthmatic properties of this plant, using in vitro biological assays; the phosphodiesterase (PDE IV), glucocorticoid binding and histamine H1 binding assay. The conclusion drawn from their study is that the plant may have anti-inflammatory, anti-allergic and bronchodilatory effects, thereby carrying the possibility of effectiveness against asthma-related illnesses. Good antibacterial activity results from the current study using rhizomes and stems of Siphonochilus aethiopicus compliment the success of the research conducted by FOUCHE et al. (2011). This shows that the value of this plant for the treatment of asthma and related conditions cannot be overlooked. Due to the overexploitation Siphonochilus aethiopicus no longer grows in its natural habitat (LIGHT, 2002; WINTER and BOTHÁ, 1994; ZSCHOCKE et al., 2000). This means that the need for conservation remains an important aspect so that future generations are not deprived of the benefits from this plant.

Generally antibacterial activity was good in most PE, DCM and ethanol extracts. It is worth noting that only in the case of the leaves of Catha edulis, Pelargonium sidoides, Xerophyta retinervis and Zantedeschia aethiopica did the aqueous extract exhibit good antibacterial activity at MIC values ranging between 0.39 and 0.78 mg/ml against Streptococcus pyogenes. According to RABE and VAN STADEN (1997) the traditional healers normally use water for the preparation of the plant extracts. Therefore, these results validate the use of water extracts in traditional medicine. Traditional healers do not typically have access to lipophilic solvents such as the ones used in this study, particularly PE and DCM. When administering medication, it is important to consider the type of solvent used when deciding on dosage. Traditional healers can give higher dosages of the extracts when using water as an extractant. However, in the case where lipophilic solvents are used, the dosage would have to be low.

2.6 Conclusions

Datura stramonium, Pelargonium sidoides and Zantedeschia aethiopica exhibited high antibacterial activity ranging between 0.098 and 0.195 mg/ml. These medicinal
plants could potentially provide an opportunity for a scientific lead in the isolation of pharmacologically active constituents and the biochemical analysis of plants used in African traditional medicine in the treatment of asthma.
3. Mutagenic activity

3.1 Introduction

Medicinal plants have successfully been used in African traditional medicine to treat various diseases. Like many developing countries that still prefer traditional medicine over western medicine, a large proportion of the South African population use traditional medicine for the treatment of physical and psychological health needs including respiratory disorders such as asthma. Due to the “natural state” of medicinal plants, it is assumed that they are non-toxic and are safe to use (ATERE and AJAO, 2009; BATEMAN et al., 1998; FENNELL et al., 2004; STICKEL et al., 2000). It has been reported that the WHO encourages developing countries to supplement their health programme with traditional herbal preparation as long as they are proven to be non-toxic (AKINTONWA et al., 2009).

Plants contain a vast amount of secondary metabolites. Secondary metabolites are generally considered to be defensive and are toxic to the predators. Toxicity is defined as the degree to which something is poisonous. Genotoxicity and cytotoxicity are amongst the most studied types of toxicity. Cytotoxicity is the quality of being toxic to the cells. Genotoxicity is defined as a “destructive effect on a cell’s genetic material affecting its integrity” and often leads to mutations in various cells or other bodily systems (SHAH, 2012). Human beings are generally surrounded by a variety of chemical substances that may possibly act as mutagens. Mutagens are physical or chemical agents that change the genetic material of an organism and have the ability to increase the frequency of mutations above the natural background level. Like many human genetic diseases, asthma may be attributed to point mutations. A point mutation involves the modification of a single base in the DNA or the insertion/deletion of one or more bases which could possibly result in a gain or loss of a chromosome (MORTELmans and ZEIGER, 2000).

Scientific studies have investigated the mutagenic and antimutagenic potential of several medicinal plants (FERNANDES and VARGAS, 2003; VERSCHAEVE et al., 2004; VERSCHAEVE and VAN STADEN, 2008). Furthermore, research by DE SÁ
FERREIRA and VARGAS (1999) showed the potential toxicity and carcinogenic effects of some substances present in the medicinal plants tested in their study. There is a clear correlation between mutagenicity and carcinogenicity (BARTSCH and TOMATIS, 1983; JONES et al., 1981). Green plants are generally known as a primary source of antimutagens and natural toxic agents (PLEWA and WAGNER, 1993). Potential mutagenic hazards resulting from the long-term use of medicinal plants is a concern (ELGORASHI et al., 2003) and hence safety for the continued use of medicinal plants is important in the field of traditional medicine (VERSCHAEVE et al., 2004).

3.2 Genotoxicity testing methods

Genotoxicity tests are in vitro and in vivo tests used to detect compounds that induce genetic damage by various mechanisms. These tests enable hazard identification with respect to damage to Deoxyribonucleic acid (DNA) and its manifestation in the form of gene mutations, larger scale chromosomal damage and recombination (JENA et al., 2002). According to YAHAGI et al. (1974), mutagenicity tests are considered efficient and rapid methods for screening the carcinogenicities of various kinds of chemical substances. The most widely used assays for detecting chemically induced gene mutations are those employing bacteria (GATEHOUSE, 2012). This is simply because bacteria are an integral part of the ecosystem and bacterial assays are relatively quick and simple (KAUR et al., 2012).

The Ames test is a well-known bacterial mutagenicity test. The Ames test is defined by MORTELmans and ZEIGER (2000) as “a short-test bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations”. This test is based on the plate incorporation system where reverse His⁻ → His⁺ mutations are visualised by plating Salmonella typhimurium in a histidine poor growth medium (MORTELmans and ZEIGER, 2000; VERSCHAEVE and VAN STADEN, 2008). Salmonella typhimurium contains a mutation that prevents the synthesis of histidine, which is an essential amino acid. The His⁺ mutants have the ability to form visible colonies. Different bacterial strains of Salmonella typhimurium are used to identify the types of
mutations. Mutagens can cause the mutant strains to revert back to wild type and therefore the number of growing revertant colonies is related to the mutagenic potency (MARON and AMES, 1983). In this study the mutant strains TA98 and TA102 were used. TA98 and TA102 detect frame shift mutations and base pair mutations, respectively (KAUR et al., 2012). It has been revealed that testing with TA98 without metabolic activation is sufficient to identify nearly 90% of the mutagens in a population (ZEIGER et al., 1985). The Ames test is based on the assumption that any substance that is mutagenic may also turn out to be carcinogenic. Numerical chromosome changes have been linked with tumorigenesis and therefore medicinal plant crude extracts that are positive in genotoxic tests have the potential to be carcinogenic or mutagenic in humans (JENA et al., 2002). The Ames test is the oldest and most common test and is often the most preferred method to screen substances for possible carcinogenicity because of the ease of performance and low cost.

3.3 Materials and methods

3.3.1 Preparation of plant extracts

Plant extracts (Section 2.4.2.1) were dissolved with either 10% DMSO or 50% ethanol to make up stock solutions of 50 mg/ml. The stock solutions (10 mg/ml) were filter-sterilized using 0.22 µm Millipore filter tips. Filter-sterilized stock solutions were used to prepare working solutions (5 mg/ml, 0.5 mg/ml, and 0.05 mg/ml) by simple dilutions with sterile distilled water.

3.3.2 Mutagenic evaluation of selected plant extracts using the Ames assay

The Salmonella typhimurium microsome assay was used to evaluate mutagenicity of the medicinal plant extracts that showed good antibacterial activity (≤ 0.5) mg/ml using TA 98 and TA102. The procedure was carried out according to MARON and AMES (1983). A 100 µl aliquot of bacterial culture was inoculated in 10 ml of Oxoid
No. 2 nutrient broth and the McCartney bottles were covered in foil, and incubated overnight at 37 °C. The top agar was melted and placed in a 50 °C water bath. A 100 µl aliquot of plant extract was dispensed to allocated sterile tubes. Five hundred µl of phosphate buffer (adjusted to pH 7.4) was added to each test tube. The overnight bacterial cultures (100 µl) were added to each of the test tubes. Subsequently, 2 ml of enriched top agar (100 ml of top agar and 10 ml biotin/histidine) was dispensed into each tube and the tubes were placed in a 37 °C water bath. The resulting mixture was mixed by vortexing and gently poured onto minimal plates, which were allowed to solidify and then incubated in an inverted position at 37 °C for 48 hours. The tumorigenic chemical 4-nitroquinoline–N-oxide (4NQO) of 2 µg/ml was used as a positive control for the experiment. Sterile distilled water was used as a negative control. After 48 hours incubation, the number of bacterial colonies was counted using a colony counter.

3.4 Results and discussion

Table 3.1 Number of revertant colonies of *Salmonella typhimurium* strains TA98 and TA102 induced by bioactive extracts used for the treatment of asthma and related conditions

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant part</th>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>TA strain</th>
<th>TA strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballota africana (L.) Benth.</td>
<td>Leaves</td>
<td>DCM</td>
<td>5</td>
<td>22.67 ± 4.93</td>
<td>106.00 ± 2.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>20.00 ± 4.58</td>
<td>113.33 ± 15.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>34.33 ± 6.03</td>
<td>106.00 ± 6.25</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>DCM</td>
<td>5</td>
<td>23.00 ± 10.44</td>
<td>91.00 ± 7.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>27.50 ± 2.12</td>
<td>114.00 ± 21.21</td>
</tr>
<tr>
<td>Plant Species</td>
<td>Part</td>
<td>Solvent</td>
<td>Concentration</td>
<td>Inhibitory Activity (µM)</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Catha edulis</em> (Vahl) Endl.</td>
<td>Leaves</td>
<td>EtOH</td>
<td>0.05</td>
<td>27.67 ± 4.04 (107.33 ± 5.69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>22.67 ± 4.16 (255.00 ± 38.58)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>18.67 ± 3.79 (233.67 ± 19.50)</td>
<td></td>
</tr>
<tr>
<td><em>Datura stramonium</em> L.</td>
<td>Stems</td>
<td>PE</td>
<td>5</td>
<td>21.00 ± 0.82 (92.33 ± 15.89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>16.33 ± 4.99 (99.33 ± 22.37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>18.00 ± 2.94 (102.50 ± 0.71)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>DCM</td>
<td>5</td>
<td>24.33 ± 1.25 (120.00 ± 8.89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>21.00 ± 2.83 (122.67 ± 9.50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>18.67 ± 3.68 (112.00 ± 8.89)</td>
<td></td>
</tr>
<tr>
<td><em>Pelargonium sidoides</em> DC.</td>
<td>Leaves</td>
<td>EtOH</td>
<td>5</td>
<td>33.67 ± 3.51 (223.67 ± 31.47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>28.67 ± 7.77 (294.33 ± 4.16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>23.67 ± 3.06 (253.67 ± 42.62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>EtOH</td>
<td>5</td>
<td>23.67 ± 5.13 (242.00 ± 43.84)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>22.33 ± 5.51 (282.00 ± 59.27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>24.67 ± 4.16 (300.00 ± 7.00)</td>
<td></td>
</tr>
<tr>
<td><em>Siphonochilus aethiopicus</em> (Schweinf.) B.L. Burtt</td>
<td>Roots</td>
<td>PE</td>
<td>5</td>
<td>28.33 ± 3.77 (123.67 ± 9.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>18.00 ± 2.83 (95.33 ± 0.58)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>24.33 ± 2.06 (108.00 ± 18.36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>EtOH</td>
<td>5</td>
<td>23.00 ± 15.56 (90.67 ± 7.51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>9.67 ± 1.53 (88.00 ± 4.00)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>EtOH</td>
<td>0.05</td>
<td>7.50 ± 6.36</td>
<td>77.67 ± 10.79</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>------</td>
<td>------</td>
<td>-------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Xerophyta retinervis Baker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td></td>
<td></td>
<td>24.50 ± 4.95</td>
<td>203.33 ± 9.24</td>
</tr>
<tr>
<td>4-NQO</td>
<td></td>
<td></td>
<td></td>
<td>250.67 ± 24.71</td>
<td>540.50 ± 57.28</td>
</tr>
</tbody>
</table>

The Ames test was used in this study to determine possible gene mutations caused by medicinal plant extracts without exogenous metabolic activation. The results showing the rate of reversion mutation are presented in Table 3.1. According to ZEIGER (2001) a positive response in any single bacterial strain with the presence or the absence of metabolic activation is sufficient to designate a chemical substance as a mutagen. The evaluation of data was based on the assumption if (i) the number of revertant colonies of the tested crude extract is twice or more than the number of revertant colonies produced by the negative control and/or (ii) there is any dose dependent increase in the number of colonies observed with the test sample then the extract would be considered significantly mutagenic (VERSCHAEVE and VAN STADEN, 2008). Most tested extracts were negative in the Ames test except for the *Siphonochilus aethiopicus* roots which showed a dose dependent increase. It is important to note that the number of revertant colonies induced by lower concentration of the ethanol extract of *Siphonochilus aethiopicus* roots were less than those of the negative control. This could be attributed to toxicity of the extract at
lower concentration. Further studies aimed at investigating this plant may be required. In a study conducted by STEENKAMP et al. (2005) the methanol extract of *Siphonochilus aethiopicus* produced the highest amount of DNA damage. The genotoxic properties of *Siphonochilus aethiopicus* was confirmed by TAYLOR et al. (2003) and this raises a concern for the use of this plant. The tested extracts that were negative means that they were devoid of any mutagenic properties. The test was performed without an exogenous metabolic activation system. Due to the unavailability of the S9 mixture, the crude samples that exhibited good antibacterial activity could not be tested for any possible indirect mutagenic properties.

According to REID et al. (2006), the absence of a mutagenic response by medicinal plant extracts against *Salmonella typhimurium* bacterial strains in the Ames assay is a positive step forward in determining the safe use of those plants. FRIEDMAN (2004) and STEENKAMP et al. (2004) have documented the toxicity of *Datura stramonium* and reported deaths and poisonings linked to the toxic nature of this plant over a number of years. *Datura stramonium* stems and roots exhibited good antibacterial activity against *Streptococcus pyogenes* (Table 2.3. Section 2.5), but showed no mutagenic response against *Salmonella typhimurium* bacterial strains TA98 and TA102 in the absence of S9 metabolic activation. Negative mutagenic response does not mean that they are safe. It is a confirmation that the substance is not mutagenic to the particular bacterial strain used and for the genetic endpoint tested (REID et al., 2006). To increase the likelihood of detecting a test substance capable of mutagenesis, the use two or more tester strains such as TA97, TA100, TA1535 and TA1537 is recommended. Although the *Datura stramonium* extracts have been shown to be non-mutagenic, they may be cytotoxic. The induction of cytotoxicity by *Datura stramonium* has been reported (AHMAD et al., 2009; MERZA et al., 2010).

### 3.5 Conclusions

It is recommended that further testing be performed with the S9 mix to screen for indirect acting mutagens.
4. Screening for Immunological activity

4.1 Introduction

The immune system aims to protect the body against foreign bacteria and viruses. According to JANeway et al. (2001), any substance that has the ability to provoke an immune response is said to be immunogenic. When the immune system does not function in the manner that it should, the result is an immune system disorder. Asthma is linked to an increase in atopic sensitization and is equivalent to similar increases in other allergic disorders such as eczema and rhinitis (GARRido et al., 2010; MASOLI et al., 2004; Van Den OORD and SHEIKH, 2009). Patients with asthma and related conditions experience overactive immune responses that can cause coughing, wheezing, shortness of breath and tightness of the chest. The prevalence of allergic diseases such as asthma is increasing and affects 30-40% of the population (POTTER, 2005; VAN Schoor, 2008).

Asthma is associated with airway remodelling, which refers to the structural changes that occur in both large and small airways relevant to miscellaneous diseases (BARA et al., 2010; BERGERON et al., 2010; COHEN et al., 2007). Current asthma therapeutic approaches aim to improve lung function through bronchodilation whereby inflammation is minimized in responsive individuals (GREENFEDer and ANThes, 2002; HIROTA et al., 2012). According to HIROTA et al. (2012), there is considerable uncertainty regarding the capacity of the current therapies to decrease or reverse the ongoing remodelling process. It has been reported that many people worldwide have resorted to the use of medicinal plants as immunomodulators especially in developing countries (LUBEGA et al., 2013). Investigating an immunobiological activity of medicinal plants forms an integral/fundamental base in the treatment of chronic infections and immunological disorders. It has been anticipated that research into the immunomodulatory properties and mechanisms of action of herbal medicines will provide new insights into immune function and possible avenues of immunotherapy (PLAEGER, 2003). A recent study was conducted to
evaluate the immunomodulatory/anti-inflammatory effect of medicinal plant extracts, which were found to attenuate *in vivo* and *in vitro* asthmatic reactions (AMMAR *et al.*, 2013). A study by AMMAR *et al.* (2013) advocate that medicinal plant extracts used may be useful as an adjuvant therapy for asthmatic patients in the future and this endorses the fact that herbal medicines will provide possible avenues of immunotherapy (PLAEGER, 2003).

One of the immunohistopathologic features of asthma takes account of mast cell activation. Mast cells have been reported to play a role in the pathogenesis of atopic diseases and asthma (YUSUF, 2012). The mast cells serve to alert the immune system to local infections induced by allergic reactions. According to HART (2001), mast cells are strategically placed close to blood vessels and nerves in tissues that interface with the external environment. Mast cells are known to be the key-effector cells in innate immune responses to infections and are responsible for the release of an inflammatory substance, histamine, whenever the individual is re-exposed to an allergen (YUSUF, 2012). Histamine was first identified as a potent vasoactive amine and is currently recognized for its regulatory activities in the respiratory system (COUILLIN *et al.*, 2004). Histamine has been recognized as an important mediator in airway inflammation and has been reported to elevate in the airways of asthmatic patients (BARNES *et al.*, 1998; SZCZEPAŃKIEWICZ *et al.*, 2010; WILSON *et al.*, 2000; WILSON, 2006).
4.2 The role of histamine in asthma

4.2.1 Functional properties of histamine during an allergic encounter

An allergy can be delineated as the potential for the development of immunologically mediated reactions to allergens and it has been reported that the majority of allergy-based clinical diseases are mediated by immunoglobulin E (IgE) antibodies (METZGER et al., 1986; MILGROM et al., 2001, SASAKI et al., 2000). The role that histamine plays in inflammation is outlined in Figure 4.1. Histamine plays a role in allergic reactions and does this by binding to histamine receptors (HORTON et al., 2001; SASAKI et al., 2000). At the time of exposure to the allergen, histamine is
released as soon as an antigen is bound to the membrane-associated IgE and this then enables the allergic reactions in the system to diminish. The released histamine is primarily metabolized by two enzymes namely N-methyl-transferase (HNMT) and diamine oxidase (LEE et al., 2012; SASAKI et al., 2000; SHARMA et al., 2005; SZCZEPANKIEWICZ et al., 2010). Within these metabolic pathways, HNMT is the primary pathway for histamine bio-transformation in the bronchial epithelium (OKINANGA et al., 1995).

### 4.2.2 Histamine receptors and antagonists involved in the process of inflammation

During an allergic reaction, histamine is often mediated by three types of receptors namely H1, H2 and H3 receptors (SASAKI et al., 2000). The H1 receptor is located in the cell membranes of various human tissues such as the airways. In the case of an asthma reaction, the allergic reaction in susceptible individuals is stimulated by the binding of the H1 receptor to histamine. The histamine antagonists, commonly known as antihistamines, are defined as inhibitors of histamine receptors. They function by preventing the histamine from attaching to the H1 receptor protein. H1-antihistamines have been used in the treatment of various allergic reactions and South African products include Loratadine, Deselex, Allecet and Zyrtec. For the current study, Loratadine served as a point of reference for the antihistamine (Figure 4.2).
Loratadine is the drug of choice that has demonstrated significant anti-allergic activity in humans and therefore serves as the control for this current work. Loratadine has been described as a long acting second generation antihistamine that exhibits competitive, specific and selective antagonism of H1 receptors (HARIA et al., 1994; TOWN and HOLGATE, 1990).

4.3 Immunological testing methods

Skin prick tests and blood [Radioallergosorbent test (RAST)] are widely performed tests for allergen specific IgE to demonstrate the presence of antibodies to one or several allergens (AUSTRALIAN SOCIETY CLINICAL IMMUNOLOGY and ALLERGY, 2010). It has been deduced that it is essential to determine if allergens are an important factor in an asthmatic patient and that the ability to reduce the
The amount of causative allergens provides greater chances to improve this chronic disease (AUSTRALIAN SOCIETY CLINICAL IMMUNOLOGY and ALLERGY, 2010). Several immunological assays have been established with the objective of measuring the direct antibody to its antigen. Radioimmunoassay (RIA) and Enzyme-linked Immunosorbent assay (ELISA) are direct binding assays for antibodies (or antigens) (JANEWAY et al., 2001). With reference to JANEWAY et al. (2001), Radioimmunoassays are commonly used to measure the levels of hormones in blood and tissue fluids and ELISA assays are frequently used in viral diagnostics. These methods work on the same principle where they have proved to measure the amount of antibodies quantitatively but the means of detecting specific binding is different (BEUVERY et al., 1984; JANEWAY et al., 2001). Radioimmunoassays measure scintillation due to decay of radioactive tracer while ELISA measures a colour change due to an enzyme tracer. Enzyme-linked Immunosorbent assay is the preferred method for most direct-binding assays because this technique avoids the hazards of radioactivity and data collection from the reaction tray is very easy (JANEWAY et al., 2001).

4.4 Materials and methods

4.4.1 Anti-Histamine Assay

The assay was conducted using a histamine ELISA kit (Genway Biotech Inc., San Diego) with a modification to test the competitive binding potentials of the ethanolic crude extracts (Section 2.4.2.1) to the receptors. The ethanolic extracts were evaluated because they would represent both the polar and non-polar extracts. In each Eppendorf tube containing 40 µl of crude plant extract (final concentrations of 400 µg/ml and 800 µg/ml), 40 µl of histamine (14 ng/ml) in plasma was added. The tubes were incubated for an hour at room temperature. Following the incubation period, 80 µl of indicator buffer and 16 µl of acrylation reagent were added to the tubes. The tubes were mixed on a vortex and incubated for 30 minutes at room temperature. Six hundred microlitres of the assay buffer were added to each tube and the tubes were mixed on a vortex machine. The resulting solution was the
acrylated solution, of which 50 µl of each was transferred to Mononuclear Phagocyte (MP) coated microplate wells. Fifty microlitres of the enzyme conjugate and 50 µl of histamine antiserum were added to the microplate. The microplate was covered with foil and incubated for 3 hours at room temperature on an orbital shaker (500 rpm). After incubation, the foil was removed and the solutions were discarded. The microplate was washed four times with 200 µl of wash buffer. One hundred microlitres of TMB solution was added to each well in the microplate, which was then incubated for 40 min at room temperature on an orbital shaker. The reaction was stopped by adding 100 µl TMB stop solution. The absorbance was measured at 450 nm using a reference of 600 nm within 15 min after adding the stop solution. The assay was repeated twice.

4.5 Results and Discussion

![Histamine receptor binding graph]

Figure 4.3 Determination of the level of histamine receptor binding to the medicinal plants used for the treatment of asthma and related conditions

Samples 1) Catha edulis leaves, 2) Zantedeschia aethiopica leaves, 3) Siphonochilus aethiopicus leaves, 4) Catha edulis stems, 5) Siphonochilus aethiopicus rhizomes, 6) Siphonochilus aethiopus stems, 7) Zantedeschia aethiopica rhizomes, 8) Zantedeschia aethiopica stems, 9) Zantedeschia aethiopica roots, 10) Datura stramonium leaves, 11) Datura stramonium Flowers and Fruits, 12) Datura
The objective of the immunological assay was to determine the level of competitive binding to the receptors for the treatment of asthma and related conditions. Histamine is an inflammatory mediator intimately associated with the pathology of allergies. Four levels of activity were defined for this assay: 0-20%- insignificant activity, 21-40%- low activity, 41-60%- moderate activity, 61-80%- high activity and 81-100% excellent activity. According to the results in Figure 4.3, most plants showed good immuno-biological activity. The *Ballota africana* leaves and stems, *Datura stramonium* flowers and fruits, roots and stems as well as *Zantedeschia aethiopica* leaves experienced excellent affinity with histamine. Therefore histamine receptor binding of 81-100% means *Ballota africana*, *Datura stramonium* and *Zantedeschia aethiopica* could potentially serve as an effective antihistamine compared to the currently available pharmaceuticals.

*Datura stramonium* belongs to the Solanaceae. It has been reported that the *Datura stramonium* produces steroid and tropane alkaloids, tannins, saponins, flavonoids, coumarins and anthocyanins (BANSO and ADEYEMO, 2006; DE LUCA and ST PIERRE, 2000; FACCINI, 2001; SONI et al., 2012; STONE, 2000). As shown in Figure 4.3 *Datura stramonium* flowers and fruits extracts showed histamine receptor binding of approximately 97% at both concentrations (400 and 800 μg/ml), the alkaloids contained in *Datura stramonium* could exert antihistaminic properties as did Loratadine (positive control) that showed 100% inhibition. Flavonoids are deduced as powerful antioxidants and anti-allergic nutrients that inhibit the release of chemical mediators (TANAKA and TAKAHASHI, 2013) and the presence of these compounds in *Datura stramonium* may provide an explanation for the anti-inflammatory and bronchodilator effects of *Datura stramonium*. *Datura stramonium* leaves showed a non-significant difference where the histamine receptor binding was moderate at lower concentration of 400 μg/ml as compared to excellent inhibition at higher concentration of 800 μg/ml. It showed dose-dependent increase in activity.
The higher the dose, the more intense the response of this plant to provide symptomatic relief of allergic symptoms induced by occupation of histamine receptors. Contrary to this effect, the propane alkaloids within *Datura stramonium* are fatally toxic in slightly higher amounts than the medicinal dosage. From the pharmacological kinetics of antihistamines, most antihistamines show good absorption when administered through an oral route (VAN SCHOOR, 2008). The risks associated with intoxication of *Datura stramonium* may outweigh the antihistaminic benefits that the plant may offer as a remedy for asthma and related conditions. Because some drugs have a higher rate of absorption, it may be advisable to prescribe smaller doses as may be the case with regards to *Datura stramonium* as a potential antihistamine. Incorrect dosage of *Datura stramonium* can lead to death.

As the family Lamiaceae is largely characterized with terpenoids, *Ballota africana* may contain terpenoids that induce a good response as the antihistaminic. Hispanolone is found in *Ballota africana* (GRAY et al., 2003). Ballotenol has been reported as an active ingredient of *Ballota africana* against respiratory problems (www.carecentre.org.za/medicinal.htm). Diterpenes have been isolated from other *Ballota* species (SAVONA et al., 1977; SEIDEL et al., 1999). Hispanolone and Ballotenol are terpenoids. The bronchospasmolytic effect of terpenoids and the pharmacological role of these compounds in the management of asthma and related allergic conditions has been established (EZIKE et al., 2008). The terpenoids in *Ballota africana* may account for the antihistaminic effect in Figure 4.3 as both leaves and stems showed excellent antihistaminic activity of approximately 90% at 400 µg/ml and 97% at 800 µg/ml.

*Zantedeschia aethiopica* belongs to the family of Araceae. Cycloartane triterpenes and phenylpropanoids are present in *Zantedeschia aethiopica* (GRECA et al., 1998). Triterpenes and phenylpropanoids have been identified as some of the major active constituents that play an important role in curing various diseases and inflammatory conditions (NISAR et al., 2012). The excellent antihistaminic activity of *Zantedeschia aethiopica* leaves in Figure 4.3 may be due to the phenolic content and terpenoids present in this plant. *Acorus calamus* Linn. and *Scindapsus officinalis* [Roxb.] Schott belong to the same family as *Zantedeschia aethiopica*. *Acorus calamus* has been
shown to possess antihistaminic constituents which are thought to provide the pharmacological basis to its traditional use in bronchial airway disorders (VIJAYAPANDI et al., 2013). Scindapsus officinalis is used in asthma (VELRAJ et al., 2013) and has been reported to have antihistaminic activity (SHRIVASTAVA et al., 2012). Zantedeschia aethiopica has been used to treat asthma and related conditions (VAN WYK et al., 1997). The Zantedeschia aethiopica leaves showed excellent histamine receptor binding of approximately 88% at both concentrations (400 and 800 µg/ml) as shown in Figure 4.3, proving that this plant has antihistaminic activity.

The Catha edulis leaves and stems, Pelargonium sidoides leaves, Siphonochilus aethiopicus leaves, rhizomes and stems as well as Zantedeschia aethiopica rhizomes and stems showed high affinity with histamine. Alkaloids have been isolated from Catha edulis and Datura stramonium (BRUNETON, 1993; DANIEL; 2006). Cathine and cathionine are alkaloids from Catha edulis. Siphonochilus aethiopicus has been reported to contain terpenoids (HOLZAPFEL et al., 2002; VILJOEN et al., 2002). A review by KOLODZIEJ (2007) has showed flavonoids, coumarin glycosides, coumarin sulphates, phenolic acids and phenylpropanoid derivatives as the chemical components of Pelargonium sidoides. The combination of phenolic compounds and numerous coumargins has been linked to the immunomodulatory effect of Pelargonium sidoides (BRENDLER and VAN WYK, 2008). A study by VASCONCELOS et al. (2009) investigated a coumarin compound in a mouse model of bronchial asthma and deduced that the mechanisms of its action may contribute towards the development of new drugs for the treatment of asthma. The coumarin derivatives have been reported to combine potent H1-antihistaminic properties with those of mast cell stabilization (BUCKLE et al., 1984) and this complements coumarins as potential anti-asthmatic agents (VASCONCELOS et al., 2009). Saponins have showed to be anti-allergic and their anti-asthma affect has been described (AKAGI et al., 1997). Moreover in a study by TIPSUWAN (1992), saponins were found to possess bronchodilator effects when tested on histamine-and methacholine induced bronchoconstriction of guinea pig tracheal muscle both in in vitro and in vivo experiments. The phytochemistry of these medicinal plants may possibly confirm their potential antihistaminic properties.
4.6 Conclusions

Over the years, medicinal plants have successfully served as essential components of traditional medicine and have demonstrated their use as potential remedies for respiratory tract infections. The immunological activity may be attributed to the various phytochemical constituents in the crude extracts. Histamine is a well-characterized and most potent vasoactive mediator in acute bronchoconstriction and the histaminic effect of the tested plant extracts may be linked to the various phytochemical constituents. Medicinal plants could serve as an upcoming generation of antihistamines that will be effective for the treatment of asthma, atopic dermatitis, rhinitis and other conditions. Asthma sufferers will greatly benefit from traditional medicine, as it has over the years been thought to treat conditions better than western medicine. In formulating a better antihistaminic treatment from medicinal plants, pharmacodynamics and pharmacokinetics would have to be considered.
5. General Conclusions

Asthma is a costly disease because the disease results in loss of productivity caused by absenteeism from work and school (GREEN et al., 1998). Asthma affects approximately 235 million people worldwide and the prevalence is increasing despite modern drug treatment. Currently available bronchodilators and anti-inflammatory treatment requires a lifetime of therapy although a subset of patients remain symptomatic, and can have side effects such as headaches, heart palpitations, sinus tachycardia, anxiety, tremors and increased blood pressure (BERTRAND, 2000; PATEL and GWILT, 2008). The incidence of side effects of the treatment is profound and the use of antibiotics is often not recommended for asthma. The increase of resistance to antibiotics has led to an urgent need for the development of new and innovative antimicrobial agents (DJEUSSI et al., 2013). Moreover, COS et al. (2006) anticipated that natural products provide unlimited opportunities for new drug discoveries. ESSAWI and SROUR (2000) emphasized that the side effects and resistance that pathogenic organisms develop against the antibiotic agents encourage the development of new and effective pharmaceuticals. Similarly, due to the adverse effects of the currently available bronchodilators and anti-inflammatory treatment, mankind has turned to ethnopharmacognosy (SASIDHARAN et al., 2011). Medicinal plants continue to be the pillars of traditional medicine and South Africa has a large repository of medicinal plants. Traditional medicine has become the alternative pathway to health in South Africa due to affordability, accessibility and cultural importance. The rich cultural and biological diversity of South Africa has contributed to successful and sustained ethnopharmacology research over the years.

Ethnobotanical studies have successfully served as one of the major methods for selecting plants for pharmacological evaluation (OGBOLE et al., 2010). Eight plants (Adansonia digitata, Ballota africana, Catha edulis, Datura stramonium, Pelargonium sidoides, Siphonochilus aethiopicus, Xerophyta retinervis and Zantedeschia aethiopica) were selected based on their anti-asthmatic potential in South Africa. Four solvents [Petroleum Ether (PE), Dichloromethane (DCM), 80% EtOH and water] were used for extraction in this study because of their wide polarity range.
The highest percentage yield of extracts was obtained from the water extract of *Datura stramonium* flowers and fruits (27.60%) and the lowest yield was obtained from the PE extract of *Pelargonium sidoides* roots (0.05%). Screening plants for their biological activity is important to establish the ethnobotanical knowledge for a particular disease and disorder in humans (JEYASEELAN et al., 2012; SHAHEEN et al., 2009). All plants responded significantly to the Gram-positive bacteria used in the current study. However, only 75% of the screened plants exhibited good antibacterial activity against all key bacteria; *Staphylococcus aureus* (ATCC 12600), *Klebsiella pneumonia* (ATCC13883), *Streptococcus pyogenes* (ATCC 12202) and *Haemophilus parainfluenzae* (ATCC 7901) as well as the fungus *Candida albicans* (ATCC 10231). The PE extract of *Pelargonium sidoides* leaves and DCM extract of *Zantedeschia aethiopica* stems yielded high antibacterial activity of 0.098 mg/ml and 0.195 mg/ml respectively against *Streptococcus pyogenes*. The results are noteworthy and indicate that all the medicinal plants screened for antibacterial activity in this study could be of considerable interest in the development of new drugs.

Medicinal plants are often considered to be intrinsically harmless due to their natural state (ATERE and AJAO, 2009; BATEMAN et al., 1998; FENNELL et al., 2004; STICKEL et al., 2000). It is important to assess the possible toxicity and mutagenic properties of the medicinal plants. The Ames test was performed using *Salmonella typhimurium* strains TA98 and TA100 without S9 metabolic activation. The objective of the test was to evaluate genotoxicity of plant extracts that have exhibited antibacterial activity equivalent or less than 0.5 mg/ml. The results revealed that most extracts of the selected medicinal plants were non-mutagenic in the absence of metabolic activation except for the *Siphonochilus aethiopicus* roots which showed a dose dependent increase. *Siphonochilus aethiopicus* was found to cause DNA damage and the genotoxic properties of this plant have been confirmed (STEENKAMP et al., 2005; TAYLOR et al., 2003). This raises a concern around the use of this plant. The tested extracts that were negative means that they were devoid of any mutagenic properties under the tested conditions. *Datura stramonium* is known to cause anticholinergic poisoning (PRETORIUS and MARX, 2006). Since this plant is reported to be cytotoxic (AHMAD et al., 2009; MERZA et al., 2010), then all the plants that showed good antibacterial activity (Table 3.1) should also be
tested for cytotoxicity. It is important to test in the presence of S9 metabolizing enzymes and other genotoxicity assays using screening methods both in vivo and in vitro to complement the mutagenic results of the current study.

Patients with asthma and related conditions experience overactive immune responses that can cause coughing, wheezing, shortness of breath and tightness of the chest. In light of asthma being an immunological disorder, the ethanolic crude extracts were screened in an immunological assay. This was conducted using a histamine ELISA kit with the aim to test the binding potential of the extracts of the selected plants to the receptors. Histamine, an inflammatory mediator, is intimately associated with allergies. *Datura stramonium* flowers and fruits showed histamine receptor binding of approximately 97% at both concentrations (400 and 800 µg/ml) and the alkaloids contained in *Datura stramonium* could exert similar antihistaminic properties as Loratadine (positive control) that showed 100% inhibition. Medicinal plants are viewed as a “reservoir of important biologically active compounds” and traditional medicines are the mainstay of drug discovery (Moshi, 2005; Oke et al., 2012), and therefore the immunological activity may be attributed to the various phytochemical constituents in the crude extracts. Since the *Ballota africana* leaves and stems, *Datura stramonium* flowers and fruits, roots and stems as well as *Zantedeschia aethiopica* leaves showed excellent affinity with histamine ranging between 88 and 97%, these medicinal plants could potentially serve as new effective antihistamines when compared to the currently available pharmaceuticals.

Over the years, medicinal plants have successfully served as essential components of traditional medicine and have demonstrated their use as potential remedies for respiratory tract infections. In vitro screening is important for the validation of traditional use of medicinal plants and provides leads in the search for new active principles. The noteworthy antimicrobial and immunological activities from in vitro testing may not directly confirm that the tested plant extracts are effective medicines but surely does provide an elementary understanding of the efficacy of these medicinal plants in traditional medicine and their potential use as a source of asthmatic therapy. Asthma sufferers will greatly benefit from traditional medicine as it has over the years been thought to treat conditions better than western medicine. Anti-inflammatory assays such as cyclooxygenase (COX) and in vivo investigations of the active plant extracts would be highly recommended for future studies.
According to DJUKANOVIĆ et al. (1990) and PIN et al. (1992), airway inflammation is considered to be important in asthma. Cyclooxygenase enzymes are the most important enzymes involved in the inflammatory pathway of asthma. Screening medicinal plants may therefore provide potential sources of anti-inflammatory agents against asthma. The plant extracts that would warrant further in vivo investigations are those that showed good antimicrobial activity (Table 2.3), are not mutagenic (Table 3.1), and exhibited best antihistaminic activity between 81-100% (Figure 4.3).
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