Antimicrobial and immunomodulatory properties of extracts of *Asparagus setaceous* Kunth and *Caesalpinia volkensii* Harm

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A Thesis submitted in partial fulfillment for the Degree of Doctor of Philosophy in Molecular Medicine in the Jomo Kenyatta University of Agriculture and Technology

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my late brother Bob Henry Ochieng' Ogila who passed away in 2008.

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LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS

AASA	Aqueous *Asparagus setaceous* aerial part extracts
AASR	Aqueous *Asparagus setaceous* root extracts
ACVL	Aqueous *Caesalpinia volkensii* leaf extracts
ACVS	Aqueous *Caesalpinia volkensii* stem extracts
ACVR	Aqueous *Caesalpinia volkensii* root extracts
AIDS	Acquired Immunodeficiency Syndrome
APCs	Antigen Presenting Cells
BPI	Bactericidal Permeability Increasing Proteins
C	Celsius
Ca^{2+}	Calcium ions
Camp	Cyclic Adenosine Monophosphate
CD	Cluster Designation
Con A	Concanavalin A
CP	Cyclophosphamide
CSF	Colony Stimulating Factor
DASA	Dichloromethane *Asparagus setaceous* aerial part extracts
DASR	Dichloromethane *Asparagus setaceous* root extracts
DCVL	Dichloromethane *Caesalpinia volkensii* leaf extracts
DCVS	Dichloromethane *Caesalpinia volkensii* stem extracts
DCVR	Dichloromethane *Caesalpinia volkensii* root extracts

DNA	Deoxyribonucleic Acid
EASA	Ethanol *Asparagus setaceous* aerial part extracts
EASR	Ethanol *Asparagus setaceous* root extracts
ECVL	Ethanol *Caesalpinia volkensii* leaf extracts
ECVS	Ethanol *Caesalpinia volkensii* stem extracts
ECVR	Ethanol *Caesalpinia volkensii* root extracts
FACS	Fluorescence Activated Cell Sorting
g	gram
GALT	Gut Associated Lymphoid Tissues
G- CSF	Granulocyte Colony Stimulating Factor
h	Hour
HASA	Hexane *Asparagus setaceous* aerial part extracts
HASR	Hexane *Asparagus setaceous* root extracts
HCVL	Hexane *Caesalpinia volkensii* leaf extracts
HCVS	Hexane *Caesalpinia volkensii* stem extracts
HCVR	Hexane *Caesalpinia volkensii* root extracts
HIV	Human Immunodeficiency Virus
H_2O_2	Hydrogen peroxide
IFN	Interferon
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IL	Interleukin
IL-1	Interleukin 1
IL-1β	Interleukin 1β
IL-2	Interleukin 2
IL-2R	Interleukin 2 Receptor
IL-3	Interleukin 3
IL-4	Interleukin4
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-6R	Interleukin 6 Receptor
IL-8	Interleukin 8
IL-9	Interleukin 9
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-18	Interleukin 18
LTB4	Leukotrine B4
Kg	Kilogram
M- CSF	Macrophage Colony Stimulating Factor
mAbs	Monoclonal Antibodies
MASA	Methanol *Asparagus setaceous* aerial part extracts
MASR	Methanol *Asparagus setaceous* root extracts

MCVL	Methanol *Caesalpinia volkensii* leaf extracts
MCVS	Methanol *Caesalpinia volkensii* stem extracts
MCVR	Methanol *Caesalpinia volkensii* root extracts
mg	Milligram
MM^3	Cubic millimetre
MHC	Major Histocompatibility Complex
MPO	Myeloperoxidase
MRSA	Methicilin Resistant *Staphylococcus aureus*
NADPH	Reduced form of nicotinamide adenine dinucleotide
NBT	Nitroblue tetrazolium
NH^+_4	Ammonium
NH_2CL	Monochloramine
NK	Natural Killer
Nm	Nanometre
NO	Nitric oxide
O_2	Oxygen
O_2	Superoxide
OD	Optical density
OH	Hydroxyl radical
PCV	Packed Cell Volume
PI	Phagocytic index
PHA	Phytohemagluttinin

PKA	Protein Kinase A
PKC	Protein Kinase C
PMNs	Polymorphonuclear Neutrophils
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
®	Registered trademark
SRBC	Sheep Red Blood Cells
$\mathbf{T_c}$	T-cytotoxic
$\mathbf{T_h}$	T-helper
$\mathbf{T_s}$	T- suppressor
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alpha
TNF-β	Tumor necrosis factor beta
µl	Microlitre
VRE	Vancomycin Resistant *Enterococci*
WBC	White Blood Cell
WHO	World Health Organization
α	alpha
β	Beta
γ	gamma

ABSTRACT

Infections due to antibiotic resistant microorganisms have become widespread in recent years, and resistance rates among key pathogens have continued to grow at an alarming rate worldwide and the search for novel antimicrobial agents to combat such pathogens have become crucial. It is now an accepted that majority of the antibiotics used in the treatment of these infectious diseases have failed and the ever-increasing resistance to current anti-infective drugs has become a major concern to the medical community. The problem of microbial resistance is still growing and the continued use of antimicrobial drugs in the future remains uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, and to continue to develop new novel drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period, plants have been a valuable source of natural product for maintaining human health and an impressive number of modern drugs have been isolated from them, many based on their use in traditional medicine. Currently, it is estimated that over 50 percent of all modern clinical drugs are of natural products origin.

This study investigated the mode of action of two Kenyan plants namely *Asparagus setaceous* Kunth and *Caesalpinia volkensii* Harm. *Asparagus setaceous* belong to the family Liliaceae while *Caesalpinia volkensii* belongs to Caesalpinacea. Many medicinal uses of the various parts of plants from these two families have been reported in traditional folklore medicines. These have reportedly been used in the

treatment of inflammatory diseases, bronchitis, pneumonia, syphilis and other venereal diseases, malaria and antihelminthic. However, their therapeutic mechanisms remain largely unclear. It is not clear how extracts of these two plants exert their beneficial and therapeutic effects. It is believed that their effects could be direct on the pathogens thus killing them, or to alter and enhance the functioning and activities of immune cells. There was need to investigate and clearly elucidate the mechanisms of actions of these two plants.

The aerial part and leaf of *A. setaceous* and leaf, stem and root of *C. volkensii* were extracted using polar and non polar solvents. The dichloromethane, hexane, methanol, ethanol and aqueous extracts of *A. setaceous* root and aerial part and *C .volkensii* leaf, stem and root were evaluated for their antibacterial and antifungal properties against gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*) and gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and the fungus *Candida albicans*. Antibacterial and antifungal activity was tested using the test disc diffusion method. Minimum inhibitory concentration (MIC) of the extracts was also determined. Extracts found to be active were also tested on their ability to affect the growth kinetics of selected pathogens.

An assessment to evaluate the ability of the crude extract to affect functioning and activities of immune cells was also carried out. Extracts were administered orally and then the crude extracts were evaluated on their effect on total and differential white

blood cell counts, and ability to reverse cyclophosphamide induced leucopenia in albino rats. The crude extracts were also evaluated on their ability to activate and enhance the function of neutrophils and macrophages. This was done through the neutrophil adherence, *Candida* and sheep red blood cell phagocytosis and nitroblue tetrazolium reduction tests (NBT). They were further assessed on their capacity to stimulate cellular mediated immunity through delayed type hypersensitivity (DTH) test. Dose dependent relationship tests were also carried out in order to establish the most active dosage. Data obtained from this study was analyzed using ANOVA and student t-test with the level of significance fixed at $p<0.05$.

The organic extracts of *A. setaceous* and *C. volkensii* were demonstrated to possess activity against some of the bacteria and fungus. The aqueous extracts were largely inactive. The extracts showed minimum inhibitory concentration (MIC) activity against some of the bacteria and the fungus with MIC ranging from 6.25mg-50mg/kg. The extracts were also able to affect the growth kinetics of the selected pathogens when compared to pathogens growing in presence of media alone. In immunomodulatory tests, the methanol, ethanol and aqueous extracts were found to be active. They were able to significantly increase total white blood cells count as well as neutrophil numbers in albino rats ($p<0.05$). They were found to significantly increase WBCs counts in rats 9 days after cyclophosphamide treatment ($p=0.000$). Rats pretreated with the extracts were found to be protected from the myelosuppressive effect of cyclophosphamide. The extracts significantly enhanced

percent neutrophil adhesion, *candida* phagocytosis and increased nitroblue tetrazolium reduction (p= 0.000) Sheep red blood phagocytosis by macrophage as determined spectrophotometrically was found to be enhanced. The methanol, ethanol and aqueous extracts were also found to significantly (p=0.000) enhance DTH response in rats to variable degree. The activities of the extracts were also found to be dose dependent with the dose of 500mg/kg and 1000mg/kg being the most active. In most cases, the two higher doses used had similar and comparable activity. The extracts were also demonstrated to increase the percent survival rates of rats exposed to S. aureus. Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, sterols, saponins and tannins.

This study goes along way in confirming and scientifically verifying and validating claims by traditional medical practitioners that these two plants have therapeutic values in managing infectious diseases. The study indicates that the main mechanism of action of the extracts of these plants is immunomodulation although further research is required to make definite conclusion. This will accelerate the integration of these medicinal plants into our health care system and of their use in the management of infectious diseases. Again the importance of demonstrating presence of immunostimulants from these plants portends a great conquest for medicine especially in this era of HIV/AIDS.

CHAPTER ONE

1. 1 INTRODUCTION

Infectious diseases remain the leading cause of death and account for one-quarter of all deaths in the world (WHO, 1999). Chemotherapy is one of the many options available for control of these infectious diseases and for many years, the use of antibiotics has been used for the treatment of pathogens. Antimicrobial agents are commonly used therapeutically and prophylactically to treat infectious diseases (Lee *et al*., 2006). Infections due to antibiotic resistant microorganisms have become widespread in recent years, and resistance rates among key pathogens continue to rise at an alarming rate in distinct geographic regions worldwide (Bell *et al*., 1998; Pfaller *et al*., 1998).

The search for novel antimicrobial agents to combat such pathogens has become crucial. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, the ever increasing resistance to current antiinfective drugs has become a major concern to the medical community (Wijkmans and Beckett, 2002). The problem of microbial resistance is still growing and the continued use of antimicrobial drugs in the future remains uncertain. Therefore, actions must be taken to reduce this problem, for example, through reduced use of antibiotics, and continued development of new novel drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs. For a long period, plants have been a valuable source of natural

products for maintaining human health and an impressive number of modern drugs have been isolated from them, many based on their use in traditional medicine (Nascimento *et al*., 2000; Nair *et al*., 2005). Currently, it is estimated that over 50 percent of all modern clinical drugs are of natural products origin (Cordell, 2002; Newman *et al*., 2003). Although herbal preparations have been used all over the world to treat various ailments, especially those that have defied conventional medicine, they are still being used as a measure of last resort. The main reason for this is because these "cures" are viewed with suspicion by both patients and western trained medical doctors and personnel. The main cause of this suspicion is because of unavailability of data on efficacy, mode of action and toxicology of these herbal preparations.

This study investigated the mode of action of two Kenyan plants namely *Asparagus setaceous* kunth and *Caesalpinia volkensii* harm. *Asparagus setaceous* belongs to the family Liliaceae while *Caesalpinia volkensii* belongs to Caesalpinacea. *A. setaceous* is distributed throughout Kenya while *C. volkensii* is found in five provinces in Kenya namely Rift Valley, Central, Nairobi, Western and Coast (Beentje, 1994). Medicinal uses of the various parts of plants from these two families have been reported in traditional folklore medicine. The most frequently cited uses are antibacterial, antimalarial and antihelminthic (Kokwaro, 1976). Examples of such plants are *Bauhinia acuminata, B. esculenta, B. rufescens, Brachstegia eurycoma, Cassia hirsuta* and *C. occidentalis* from the family Caesalpinia whose stem, bark, roots,

seeds and leaves have been used in the treatment of malaria, coughs, extrusion of guinea worms and as antihelminthic (Kokwaro, 1976; Iwu, 2000). From the family Liliaceae, some of the plants cited are *Crinum macowanii*, *C. portifolium*, *Gloriosa superba*, *Asparagus africanus*, *A. racemosus* and *A. setaceous*. These have reportedly been used in the treatment of inflammatory diseases, bronchitis, pneumonia, syphilis and other venereal diseases, malaria and antihelminthic (Kokwaro, 1976; Iwu, 2000; Njoroge and Bussmann, 2006a). However, their therapeutic mechanisms remain largely unclear. Several possible mechanisms of action of plant extracts used in ethno medicine have been postulated. It is believed that extracts and compounds from plants could be acting directly on pathogens thereby destroying them. It has also been reported that extracts of plants could be acting by modulating the immune system which would in turn clear these pathogens. It is also possible that plant extracts and compounds could be employing both modes of action, controlling and preventing disease establishment (Bray *et al*., 1990; Valigra, 1994; Chhabra and Uiso, 1995).

In a study conducted by Chhabra and Uiso (1995), crude methanolic extracts of some members of the genus Caesalpinia were investigated and demonstrated to lack antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhea* as well as *Klebsiella pneumoniae In vitro*. In the same studies, crude methanolic extracts of *A. africanus* and *A. racemosus* were shown to be only active against *Escherichia coli* but not against the other microbes. This was very interesting given that extracts from these plants have been employed in the traditional

methods of treatment to combat infections caused by some of these microbes mentioned above (Kokwaro, 1976; Iwu, 2000). Patients utilizing these extracts have indeed claimed beneficial effects yet studies by Chhabra and Uiso (1995) clearly demonstrated that the beneficial effects claimed by these individuals is not due to the ability of these extracts to act directly on the pathogens. Other studies have indicated that hydroalcoholic extracts from *A. africanus* inhibit *Plasmodium berghei* parasitaemia in Swiss albino mice (Dikasso *et al*., 2006) while fractions from *Caesalpinia paraguariensis* are active against *S. aureus* and *Enterococcus faecium* demonstrating a direct effect on certain pathogens (Woldemichael *et al*., 2002).

It has also been suggested that most extracts from plants could be acting by modulating the function of the immune system, boosting the body defense mechanisms against infectious pathogens (Valigra, 1994). Bray *et al*. (1990) suggested that most plant extracts do not exert their activities via direct action on pathogens but by modulation of immune functions. Dhuley (1997) reported that *A. racemosus* significantly inhibited ochratoxin induced suppression of chemotactic activity of macrophages and also enhanced production of the cytokine interleukin -1 (IL-1) and tumor necrosis factor alpha (TNF-α) by macrophages in mice. These molecules are known to be immunoregulators (Roit, 1998; Curtis *et al*., 1990). A review of immunomodulatory plants by Sagrawat and Khan (2007) reported that oral administration of a powdered root of *A. racemosus* in mice produced leucocytosis in addition to enhancing phagocytic activity of the macrophages and polymorphonuclear

cells. Alcoholic extract of *A. racemosus* was also found to enhance both humoral and cell mediated immunity of albino mice injected with sheep red blood cells as particulate antigen (Muruganadan *et al*., 2000). Thatte *et al*. (1987) demonstrated the protective effect of *A. racemosus* against cyclophosphamide induced leucopenia in mice. Extracts from African medicinal plants have been shown to enhance phagocytic and tumoricidal activity of macrophages in addition to stimulation of the oxidative burst of macrophage and granulocyte (Ottendorfer *et al*., 1994). A few extracts have been demonstrated to enhance the proliferative rates of lymphocytes (Ottendorfer *et al*., 1994). Pure and partially purified compounds from plants have been isolated and shown to possess immunomodulatory activities. These range from polysaccharides (Vollmar *et al*., 1986; Varljen *et al*., 1989) saponins (Wagner *et al*., 1987) to alkaloids (Peigen and Keji, 1988). These have been shown to enhance phagocytosis of macrophages and granulocytes, increase the weight and cell number of mouse spleen and promote production of specific antibody in spleen cells (Wagner *et al*., 1987; Peigen and Keji, 1988).

1.2 PROBLEM STATEMENT

Asparagus setaceous and *Caesalpinia volkensii* are two medicinal plants used in the ethnomedicinal practices of some communities in Kenya to treat and manage a number of infectious diseases. It is not clear how the extracts of the two plants are able to exert their therapeutical and beneficial effects and their mode of action

remains a mystery. It is therefore important to try and establish if the extracts exert their activities through antimicrobial or immunomodulatory mechanisms.

1.3 JUSTIFICATION

Plants in Kenya are becoming increasingly important as source of medicine and drugs in treatment of infectious diseases and other ailments afflicting mankind. Majority of people rely on traditional medical practitioners to treat them because of their easier accessibility and affordability. Most of these plants are also being destroyed and facing extinction even before they are surveyed and screened for biologically active ingredients. The use and miss- use of most drugs to prevent and treat infectious diseases has led to widespread appearance of resistant strains of microbes. There is need to develop new drugs with higher efficacy, and traditional medicines afford us this opportunity.

A. setaceous and *C. volkensii* have reportedly been used in ethno medicine practices of many communities in Kenya to treat a number of infectious diseases. It is not known how they exert their beneficial as well as therapeutic effects. There are no documented scientific investigations on *A. setaceous* and *C. volkensii* on either their antimicrobial or immunomodulatory properties. Indeed these two plants have not received any serious scientific attention inspite of there wider utilization. Lack of scientific data on many aspects of plants used in ethno medicine has greatly contributed to them being viewed with suspicion. There are no concrete and comprehensive data on their efficacy, mode of action and toxicology. Scientific

experiments and research are required to generate information that will shed more light on the mechanism of actions of plants and on the way they are able to exert their therapeutic effects. The importance of demonstrating the presence of antimicrobial and immunostimulating activities from these plants portends a great conquest for medicine, more so in this era of HIV/AIDS. Such compounds would find immediate use in management of AIDS and burn patients in fighting opportunistic infections associated with these conditions.

1.4 HYPOTHESIS

1.4.1 Null hypothesis

Extracts of *A. setaceous* and *C. volkensii* do not inhibit growth of selected microbes exposed to them nor do they have any effect on the functions and activities of immune cells of treated albino rats

1.5 OBJECTIVES

1.5.1 General Objectives

To investigate the antimicrobial and immunomodulatory properties of the crude extracts of *A. setaceous* and *C. volkensii*.

1.5.2 Specific Objectives

1. To determine the antimicrobial effects of the crude extracts of *A. setaceous* and *C. volkensii* on selected pathogens.

2. To investigate the immunomodulatory effect of the crude extracts of *A. setaceous* and *C. volkensii* by:-

a) Establishing total white blood cells and differential white blood cells in normal albino rats treated with crude extracts of *A. setaceous* and *C. volkensii*.

b) Investigating the effects of crude extracts of *A. setaceous* and *C. volkensii* in enhancing recovery of total WBC counts in cyclophosphamide treated rats.

c) Determining in vivo and *In vitro* effects of the crude extracts of *A. setaceous* and *C. volkensii* on the activities and functions of neutrophils and macrophages.

d) Determining the effect of crude extracts of *A. setaceous* and *C. volkensii* on cellular mediated immunity through evaluating delayed type hypersensitivity response in rats antigenically challenged with SRBCs.

3. To determine and establish time kinetics and dose response relationships of the extracts found to be active in antimicrobial and immunomodulatory tests.

4. To screen the crude extracts of *A. setaceous* and *C. volkensii* on their phytochemical components

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1An Overview of Infectious Diseases

Today, infectious diseases are a secondary, yet important cause of death in highly developed countries and the leading cause of death in many lesser developed countries (Iwu, 1999). Our environment contains a large variety of infectious pathogens but not all of these cause diseases. There are five major types of infectious agents: bacteria, protozoa, fungi, viruses and helminthes. In addition, a new class of infectious agents, the prions, has recently been recognized (http://www.i-sis.org.uk/antibiotic_resistance.shtml).

Majority of the diseases in human beings are as result of bacterial infections. The bacterial agents causing diseases include *Pneumococcus* spp, *Staphylococcus* spp, *Mycobacterium* spp, *Vibrio cholerae*, *Neisseria gonorrhoea*, *Treponema pallidum*. These are responsible for diseases such as pneumonia, coughs, venereal diseases, tuberculosis, cholera and typhoid (Roger *et al*., 1986). Bacteria are unicellular prokaryotic organisms and are frequently classified based on their cell wall structures, which influences their Gram stain reaction. Gram negative bacteria appear pink after the staining procedure. Familiar pathogenic Gram negative organisms are *Salmonella* and *Yersinia pestis*. Gram positive bacteria appear purple after the Gram stain procedures and examples include *Staphylococcus aureus* and *Clostridium tetani* (Monica, 2000).

Viruses have been found to infect all organisms from plants and animals to fungi and bacteria. Viruses however are not organisms themselves because apart from host cell, they have no metabolism and cannot reproduce (Karp, 1999; De Clercq, 2001). Viruses cause disease by disrupting normal cell function. This they do in a variety of ways, some viruses make repressor proteins that stop the synthesis of the host cell's proteins, RNA and DNA. Viral activity may also weaken cell and lysosomal membranes, leading to cell autolysis. Some viral proteins are toxic to cells, and the body's immune defenses may also kill virus infected cells (De Clercq, 2001). Viruses are classified on basis of shape, size and type of genome. Among the DNA viruses are the herpes virus that causes chicken pox, cold sores and painful genital lesions and the poxvirus that causes small pox. RNA viruses that cause human diseases include rhinoviruses that cause common colds; myxoviruses and paramyxoviruses that cause influenza, measles and mumps; rotaviruses that cause gastroenteritis; and retroviruses that cause AIDS and several types of cancer (Karp, 1999; De Clercq, 2001 and De Clercq, 2002).

Fungi are eukaryotic, heterotrophic organisms that have rigid cellulose – or chitin based cell walls and reproduce primarily by forming spores. Most are multicellular. Many fungi infect animals and life threatening fungal infections has been on the rise especially in immunocompromised patients during the last decade (Rex *et al.*, 1997). Examples of diseases caused by fungi are ringworm and histoplasmosis. Yeasts of the

Candida genus are opportunistic pathogens that may cause diseases such as vaginal yeast infections and thrush among people who are immunocompromised or undergoing antibiotic therapy (Runyoro *et al*., 2006).

Protozoa are unicellular, heterotrophic eukaryotes that include Amoeba and Paramecium. Protozoa can be acquired through contaminated food or water or by the bite of an infected arthropod such as mosquito (Krettli *et al*., 2001). Malaria is an infectious disease caused by the *Plasmodia* and four identified species of this parasite exist, which cause different types of malaria, namely *Plasmodium vivax*, *P. falciparum*, *P. ovale* and *P. malariae* (Krettli *et al*., 2001; Dikasso *et al*., 2006; Njoroge and Bussman, 2006b). *P. falciparum* is the most virulent human malaria parasite and is responsible for more deaths in Africa than any parasitic disease. The incidence of malaria is still increasing, largely because of the development of resistance to available drugs and insecticides by parasites and mosquitoes, respectively (Geary *et al*., 1983). The present global situation indicates a recent resurgence in the severity of the disease and that malaria could still be described as one of the most important communicable diseases, with an annual incidence of 300-500 million clinically manifest cases and a death toll of 1-2 million people (Dikasso *et al*., 2006) Thus, there is a great need for new anti-malarial agents and insecticides, ideally with different modes of action and chemical structures than currently used compounds.

2.1.2 Treatment of Infectious Diseases

Major improvements in the early recognition and treatment of infectious diseases have been done in the last 60 years. This has resulted in a significant reduction in the morbidity and mortality associated with these diseases. Unfortunately, most infectious agents have developed resistance to all classes of different drugs currently in the market (Alanis, 2005). The use and misuse of drugs has led to an increasing prevalence of multi drug resistant strains and there is now an urgent need to develop new effective antimicrobial agents (Cantrell *et al*., 2001). The incidence of serious microbial infections is increasing also due to the increasing number of immunocompromised patients due to HIV/AIDS (Espinel-Ingroff *et al*., 1998). Many infectious diseases, which were thought to have been eradicated from Western countries, might once again become serious health problem in the 21st century. There is thus urgent need for compounds that act on novel molecular targets that circumvent the established resistance mechanisms.

While literary meaning “destroyer of life’’ the term “antibiotic” has become the most commonly used word to refer to a chemical substance used to treat bacterial infections. The term “antimicrobial” has a somewhat broader connotation, generally referring to anything that inhibits the growth of microbes. Antimicrobials can either be micro- biostatic or microbicidal (Iwu, 1999). Since bacteria are prokaryotes, it is relatively easy to find and develop antibacterial drugs that have minimal side effects.

These drugs target structural features and metabolic characteristics of prokaryotes that are significantly different from those in eukaryotic cells.

Drugs used to treat bacterial diseases can be grouped into categories based on their modes of action. In general, these drugs inhibit the synthesis of the bacterial cell wall, protein, nucleic acid and folic acid or disorganizing membranes in addition to other mechanisms (Madigani *et al*., 2000). Penicillins and cephalosporins derived both from the fungal species *Penicillium chrysogenum* and *Cephalosporium* spp inhibit the synthesis of the bacterial cell wall by binding to the transpeptidase enzyme thus inhibiting the formation of peptidoglycan. Gram negative bacteria are in general more resistant than Gram positive bacteria to the actions of antibiotics since they contain an outer membrane with a lipopolysaccharide layer which renders them impermeable to certain antibiotics and bactericidal compounds (Nikaido, 1996). A second class of drugs, including chloramphenicol, tetracycline and erythromycin inhibit protein synthesis by binding to either 30s or the 50s subunits of the ribosome (Madigani *et al*., 2000). Rifampicin inhibits prokaryotic RNA synthesis while DNA synthesis in prokaryotes may be inhibited by fluoroquinolones. In contrast, the sulfonamides stop bacterial infection by inhibiting other enzymes (Madigani *et al*., 2000).

A decade ago, there were just five drugs licensed for treatment of viral infections. Since then, greater understanding of viral life cycles, prompted in particular by the need to combat human immunodeficiency virus, has resulted in the discovery and validation of several targets for therapeutic intervention. Consequently, the current

antiviral repertoire now includes more than 30 drugs (Milroy and Featherstone, 2002). Still, effective therapies for several viral infections is still lacking and established treatments are not always effective or well tolerated, thus highlighting the need for further refinement of antiviral drug design and development (De Clercq, 2001). One of the strategies that have been used to combat viral infections has been vaccination which has led to elimination of important viral pathogens, such as small pox, polio, and control of measles, mumps and rubella. (De Clercq, 2002). But other viral diseases particularly HIV and hepatitis C virus (HCV), have so far proved to be intractable to vaccine approach. The need for effective antiviral drugs is further emphasized by the lack of vaccines for most respiratory tract infections. Although vaccines have been developed for hepatitis B virus (HBV) and influenza types A and B, their use has not eliminated the need for effective chemotherapeutic agents (De Clercq, 2001; 2002). Antiviral drugs that are used typically target virus specific enzymes involved in viral nucleic acid synthesis (De Clercq, 2001).

Invasive fungal infections have emerged as major causes of morbidity and mortality. During the last decades, the frequency of life threatening infections has increased drastically along with the number of potentially invasive species (Nguyen *et al.*, 1996; Abi-Said *et al.*, 1997; Berrovne *et al.*, 1999). For many years the treatment of fungal infections was essentially limited to amphotericin B. Other drugs include fluconazole and itraconazole introduced in late 1980s. Today, the azole derivatives

are the most widely used antifungals. During the 1990s there has been major expansion in antifungal drug research (Groll *et al.*, 1998).

The antifungal drugs available can be classified into five major classes based upon their molecular mechanisms of action. a) Inhibition of DNA or RNA synthesis (flucytosine), b) Impairment of membrane function (e.g. amphotericin), c) Inhibition of 1, 3 – β D-glucan synthase (echinocandin derivatives), (d) Inhibition of fungal mitosis (griseofulvin), (e) Inhibition of synthesis of ergosterp; (azole derivatives, allylemines). Antifungal agents act on different genera of fungi, being active against certain but excluding others (Bossche *et al.*, 1994).

The development of drugs to treat protozoan and helminthic disease is challenging because agents that kill or inhibit the growth of these eukaryotic organisms are also highly toxic to mammalian cells. Malaria, a protozoan disease has successfully been treated for many years with chloroquine. In earlier times the common oral treatment for uncomplicated falciparum was limited to chloroquine, in chloroquine sensitive cases of malaria, and to quinine sulfate or mefloquine, tetracycline or doxycline, sulfadoxine/ pyrimethamine in chloroquine resistant cases (Dikasso *et al.*, 2006)

2.1.3 Resistance to antimicrobial agents

One of the ongoing problems scientists and medical workers face in the fight against infectious diseases is the development of resistance to the agents used to control them. Many pathogens have simultaneously acquired resistance to multiple drugs

(Alanis, 2005). Drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria, fungi, parasites and viruses. In particular, resistance of bacterial pathogens to current antibiotics has emerged as a major health problem. This is especially true in hospitals and chronic care facilities, which provide strong selection pressure for the emergence of resistance because of the large quantities and the variety of antibiotics used in these environments (Valigra, 1994). As a result, infections such as pneumonia, meningitis and tuberculosis that would once have been easily treated with antibiotics are no longer so readily treated. Hospital-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) are especially difficult to treat (Wijkmans and Beckett, 2002). At present, all widely used antibiotics, including some of the newer agents, such as streptogramins and the new-generation fluoroquinolones, are subject to bacterial resistance (Alanis, 2005). Antibiotic resistance appears as a result of changes in genes or the acquisition of genes that allow the pathogen to evade the action of antimicrobial drugs. Resistance mechanisms include structural changes in or around the target molecule that inhibit the drugs ability to bind to it; reduce permeability of the cell membrane to drug, actively pumping the drug out of the cell after it has entered and the production of enzymes that inactivate the antibiotic after it has been taken up by the cell (Wijkmans and Beckett, 2002; Alanis, 2005).

Bacteria have many strategies and mechanisms for developing resistance against antibiotics. Usually antibiotic resistance initially arises as mutation of existing genes; however, many bacteria acquire these genes; rather than experience the mutation themselves. Resistance genes are transferred to other members of the same species and across species by a variety of bacterial genetic exchange mechanisms. Many gram-negative bacteria including *E. coli* and *Salmonella* species can transfer extra chromosomal genetic material called plasmids via the process of conjugation. One large class of plasmids is called resistance plasmid because they carry genes that confer antibiotic resistance. Many of these resistance plasmids carry genes for resistance to multiple antibiotics (Suzuki *et al*., 1986; Karp, 1999).

Some species of bacteria are capable of taking up DNA from their environments in a process known as bacterial transformation. If they take up a DNA fragment containing an antibiotic resistance gene, they may become resistant to that antibiotic. Another mechanism of genetic exchange in bacteria is transduction, which is a process of bacterial recombination mediated by bacteriophages. Finally, transposons which are sequences of DNA that are capable of inserting themselves randomly into genomes may carry antibiotic resistance gene (Karp, 1999).

Other pathogens have more limited options for drug resistance. Strains of pathogens develop that are naturally less susceptible to a particular drug due to a normally occurring mutation. In the face of continuing drug use, this strain rapidly grows out of

the population being spread through the usual transmission process. Malaria, a protozoan disease, was successfully treated for many years with chloroquine, a drug that was widely available over the counter in regions where malaria was a problem. In recent decades, *Plasmodium* strains that are resistant to this drug have appeared and spread throughout Africa, South America and South East Asia (Njoroge and Bussman, 2006b). The development of drug resistance strains of pathogen has brought forth the urgent need of finding new, safer drugs with novel mechanisms of action. Traditional medicine is one avenue that offers the way forward in the search for safer and more efficacious drugs.

Traditional Medicine in Africa

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity, and there are marked differences between different regions of this continent when it comes to healing practices (Gurib-Farkim, 2006). Medicinal and poisonous plants, including a diverse array of woody plants, have always played an important role in African life. Most of the African cultures have a verbal tradition, and therefore written information on cultural features in the past are not so readily available from Africa as from many other parts of the world (Hedberg and Staugard, 1989). The traditions of collecting plants as well as processing herbal remedies and applying them have been handed down from generation to generation (Von Maydell, 1996). It is in recent times that documentations on African traditional medicine begun. An extensive review on African traditional medicine and on the use of plants

for medicine has been written by Maurice Iwu, a Nigerian pharmacognosist and ethnopharmacologist (Iwu, 1993). A number of traditional national pharmacopeias have appeared, starting with Madagascar in 1957, and research in the field of ethnobotany and ethnopharmacology has developed rapidly in many African countries (Hedberg and Staugard, 1989). The African Pharmacopoeia, covering traditional medicine of many African countries, has been published by the Scientific Technical Research Commission of the Organization of African Unity, starting with Volume 1 in 1985 (African Pharmacopoeia, Vol. 1, 1985).

Importance of Traditional Medicine in different African Countries

Traditional medicine is an important part of the health-care system in most of the African countries. People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. According to the WHO, about 80% of the people in developing countries rely primarily on medicinal plants for their primary health care (Wood-Sheldon *et al*., 1997; Hostettman *et al*., 2000; Gamboe and Chavez, 2008). In many remote areas in African countries people consult the traditional healer of the village in case of illness. In Sudan, traditional medicine plays an important role in health care, since access to hospitals and other medical facilities is limited and a high percentage of the population are nomads (Elegami *et al*., 2002). In Tanzania, over 60% of the health seeking population has a traditional healer as their first point of contact (Hedberg *et al*., 1982). In spite of an extensive programme to create health centers and to train Rural Medical Aids and Medical Assistants, the traditional healer is still the only

medical practitioner available, within reasonable distance, to many Tanzanians living in the rural parts of the country (Hedberg *et al*., 1982). The number of registered traditional healers in Tanzania has been estimated at about 30,000 – 60,000 (Mhame, 2000) in comparison with about 600 Western trained doctors. In Mozambique, traditional practitioner to patient ratio in 1994 was estimated at 1:200 while Western trained doctor to patient ratio was astonishing 1:50,000 (Marshall, 1998; Morgan, 1998). In Kenya, WHO estimated the same ratios as 1:500 and 1:40,000 respectively (Addea- Mensah, 1989).

In South Africa it is estimated that about 27 million people depend on traditional herbal medicines for their primary health care (Meyer *et al*., 1996; Mander, 1998). In Nigeria traditional medicine is well acknowledged and established as a viable profession (Kafaru, 1994), and almost all plants seem to have some kind of application in traditional medicine (Babayi *et al*., 2004). Traditional medicine seems to have certain advances over imported systems of medicine because it is an integral part of the people's culture and is particularly effective in solving certain cultural health problems (von Maydell, 1996).

The rising cost of Western medicine means that the people in African countries are increasingly turning to traditional medicine as an affordable alternative. The current policy in many African countries has been to incorporate traditional medicine in to the formal health care sectors (Tsey, 1997). In some African countries it is thought

that traditional medicine should be taught and practiced as part of formal health care sectors (Tamakloe, 1995). Gradually, traditional medicinal practitioners (TMP) are being officially accepted as part of African health services and their medical knowledge is finding its place in hospitals and clinics (Neuwinger, 2000). In many countries in Africa, traditional methods are now being used for the treatment of HIV infection (Morris, 2002) and malaria (Njoroge and Bussman, 2006a and b), including the use of medicinal plants that help alleviate the symptoms of these diseases.

Plants as Medicines

It has been estimated that less than 1 – 10% of the large diversity of 250000 – 500000 plant species on the earth have been studied chemically and pharmacologically for their medicinal properties (Farnsworth and Soejarto, 1991; Verpoorte, 2000). This is especially true for the tropical flora, as at date only 1% of the species in these habitats have been studied for their pharmaceutical potential (Gurib – Fakim, 2006). Tropical forests and many other tropical ecosystems are rich sources of a diversity of plant derived chemical compounds both because of the high species diversity but also because of the "eternal summer" which forces the plant species to the constant production of chemical defense compounds against herbivores and pathogens as well as against other plant species. For this reason a greater proportion of the tropical plant species contains secondary compounds, potentially useful as models for and as medicines (Wood-sheldon *et al.*, 1997). Plant derived compounds have been, and are still, important as such or as models for medicine. 50% of the prescription products in

various countries are either natural products or natural product derivates (Cordell, 2002; Newman *et al.*, 2003). Plants continue to be a potent source of lead compounds and examples of successful medicines derived from natural product leads include most antibiotics, the acetylcholine esterase (ACE) inhibitors, many anticancer agents, cyclosporine and rapamycin and the antiparasitic avermectins (Harvey and Waterman, 1998).

Higher plants are still poorly explored as sources of new drugs (Hostettman and Terreaux, 2000). There are several ways in selecting plant materials when searching for new medicinal plants or active compounds. Ethno pharmacological information on medicinal plants is often of substantial importance for the finding of new potential medicinal plants or new ways of using an already known plant. It has been estimated that 74% of the pharmacologically active, plant derived components were discovered after the ethno medicinal uses of the plants started to be investigated (Farnsworth and Soejarto, 1991; Wood-Sheldon *et al.*, 1997). Another important way of discovering new medicinal plants and lead compounds is the phylogenetic approach in which a number of closely related species of plants, assumed to contain related chemical compounds are screened for their biological effects (Cotton, 1996; Vuorela *et al.*, 2004). Random sampling collection of plants from certain habitats with high species diversity can be beneficial for finding novel chemical entities, but is somewhat time consuming and requires hard work (Vuorela *et al.*, 2004). This kind of sampling is likely to be used for evaluating plants for bioactive compounds.

Plant derived antimicrobials

The search for antimicrobial agents has mainly been concentrated on lower plants, fungi and bacterial sources. Much less research has been conducted on antimicrobials from higher plants (Iwu, *et al*., 1999). Since the advent of antibiotics, in the 1950s, the use of plant derived antimicrobials has been virtually nonexistent. The interest in using plant extracts for treatment of microbial infections has increased in the late 1990s as conventional antibiotics have become or are becoming ineffective (Cowan, 1999). For example, none of the conventional antifungal drugs used to date seems to be ideal in efficacy, safety and antifungal spectrum (Ablordeppey *et al*., 1991; Di Domenico, 1998).

In addition, many of the antimicrobial drugs in use have undesirable effects or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance (White *et al*., 1998). Although some new drugs have emerged for the treatment of obstinate fungal infections, such as allylamines and caspofungine (Vicente *et al*., 2003), and combination therapy is sometimes used to make the treatment more effective, there is a real need for a next generation of safer and more potent antifungal drugs (Bartoli *et al*., 1998). Also, it is becoming increasingly difficult to deliver new antibacterial leads by modifying known antibacterial compounds. Therefore, the focus on much antibacterial research has moved to the identification of new chemical classes (Barker, 2006), and many pharmaceutical companies have taken up this challenge (Boggs and Miller, 2004).

Antimicrobial compounds from plants may inhibit bacteria, fungi through different mechanisms than conventional antibiotics, and could therefore be of clinical value in the treatment of resistant microbes (Ellof, 1998).

Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including opportunistic AIDS infections (Iwu *et al.*, 1999). For example, plants containing protoberberines and related alkaloids, picralima type indole alkaloids and garcinia biflavanones used in African traditional medicines have been found to be active against a wide variety of micro organisms such as *Staphyloccocus* and *Streptoccocus* species (Iwu *et al.*, 1999). Investigations on plants used in traditional medicine for skin afflictions might provide new topical antiseptics urgently needed in the Third World countries (Taylor *et al.*, 2001). Rapid extinction of some habitats and plant species due to deforestation, especially in the tropical parts of the world has led to loss of valuable antimicrobial chemicals (Lewis and Elwin – Lewis, 1995). Thus, many pharmaceutical companies and research scientists are now intensifying their screening programs on medicinal plants.

Defense chemicals produced by plants

Higher plants produce a great diversity of chemicals that have antimicrobial and other biological activities beneficial to mankind (Balandrin *et al.*, 1985). Most of these defense molecules belong to a category of molecules known as secondary metabolites (Balandrin *et al.*, 1985). There are two broad categories of plant produced antimicrobials: Phytoalexins which are chemically diverse and include simple

phenylpropanoid derivatives, flavonoids, isoflavonoids, terpenes and polketides (Barley and Mansfield 1982; Dixon, 1986; Greayer and Harborne, 1994). These are low molecular compounds produced in response to microbial, herbivorous or environmental stimuli (Van Etten *et al.*, 1994). Flavonoids are constitutive compounds but are also synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). Nearly half of the 200 phytoalexins characterized belong to the flavonoids (Harborne, 1988). Flavonoids have been found to show *In vitro* antimicrobial activity against a wide range of Gram positive and negative bacteria. Their activity has been attributed to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996). The low toxic potential of flavonoids makes them ideal as antimicrobial medicines (Cowan, 1999).

Terpenes are a large group of compounds responsible for the fragrance of plants and comprise the so called essential oil fraction. Terpenes occur as monoterpenes, diterpenes, triterpenes, tetraterpenes, hemiterpenes and sesquiterpenes. When additional elements, such as oxygen, are added, they are called terpenoids (Cowan, 1999). Terpenes and terpenoids have been found to possess antimicrobial activity (Mendoza *et al.*, 1997; Amaral *et al.*, 1998). The mechanism of action of terpenes on microbes is not yet fully understood, but it is speculated to involve membrane disruption by the lipophilic compounds (Mendoza *et al.*, 1997). Cantrell *et al.* (2001)

investigated a series of terpenoids for their antimicrobial effects and found out that the more lipophilic compounds were significantly more antibacterial than their more polar analogues.

Phytoanticipins are also low molecular compounds that are present in plants before the challenge by microorganisms or are produced from pre-existing constituents after infection (Van Etten *et al*., 1994). Examples include phenolic, glycosides, glucosinolates and saponins. Simple phenolics, such as caffeic acid and cinnamic acids are known to possess antimicrobial effects (Brantner *et al*., 1996). Phenolic compounds are thought to inhibit microbial enzymes possibly through reaction with sulfhydryl groups (the oxidized phenols) or through nonspecific interactions with the proteins (Mason and Wasserman, 1987). Tannins are a large group of polyphenolic compounds which have received attention in recent years due to their claimed ability to cure variety of diseases (Serafinin *et al*., 1994). Tannins are subdivided into two groups: hydrolysable tannins and proantocyanidins (condensed tannins) (Haslam, 1989). A wide range of anti-infective actions have been assigned to tannins (Haslam, 1986). Tannins have the ability to complex with protein through nonspecific forces such as hydrogen bonding and hydrophobic effects and also through covalent bonding (Stern *et al*., 1996). The antimicrobial mode of action for tannin may thus be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins (Cowan, 1999). There is also evidence that tannins may directly inactivate microorganisms due to their ability to bind proteins and metals. Tannins also inhibit

the growth of microorganisms through substrate and metal ion deprivation (Brownlee *et al*., 1989). Hydrolysable and condensed tannins have been found to possess similar antifungal and antibacterial potency (Cowan, 1999).

Quinone is other group of chemicals found in plants and whose antimicrobial effects have been reported. Probable targets for quinone in the microbial cells are the surface-exposed adhesins, cell wall polypeptides and enzymes bound to the membranes. Quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al*., 1996) thus leading to inactivation of the protein and loss of its function. It is also possible that quinones render substrates unavailable to microorganisms (Cowan, 1999). Anthraquinones, the largest group of quinones (Harborne et al., 1999), have been found to possess antibacterial effects by inhibiting nucleic acid synthesis, at least in Bacillus subtilis (Leven *et al*., 1988).

Antimicrobial effects of plant extracts

In traditional and alternative medicine it is common to use crude extracts without isolating the active ingredients from them. Using crude extracts might be a more important way to use medicinal plants than has been realized in western medicine, since plants contain numerous secondary metabolites, and pathogens in nature interact with many chemicals simultaneously (Izhaki, 2002). Traditional plant remedies or phytomedicines include crude vegetable drugs (herbs) as well as gallenical preparations (extracts, fluids, infusions) prepared from them. Although a number of studies of the antimicrobial effects of plant extracts have been performed,

many plants used in different traditional medicinal systems have never been evaluated for their antimicrobial effects. For example, in Africa, over 5000 plants are known to be used for medicinal purposes, but only a small percentage have been described or studied, and different combinations of plant species used in traditional medicines have been studied even to a lesser extent (Taylor *et al.*, 2001). The major problem in investigations on the biological activities of plant extracts and phytomedicines lies in the fact that a variety of plants may be used in a single traditional medicine preparation, and in the possibility of synergistic effects resulting from the interactions of the compounds in the extract. This can even result in a loss of activity as the extract is purified (Couzinier and Mamatas, 1986).

Antibacterial activities of the alcohol ethyl acetate, acetone and chloroform extract of 5 plant species were studied and demonstrated to be active against a range of selected microbes (Ates and Edrogrul, 2003). A number of plant extracts have been subjected to antimicrobial tests and shown to possess antimicrobial activity (Chhabra and Uiso, 1995; Hammer *et al.*, 1999; Astal *et al.*, 2005; Adebolu and Oladimeji, 2005; Banso and Adeyemo, 2006). It would appear that extracts isolated using different solvent systems exhibit different activity. In a study of antimicrobial activity of extracts of *Terminalia brownie* roots and stem, the aqueous extracts exhibited the strongest activity against both bacteria and fungi. Petroleum ether extracts were inactive against all the tested organisms whereas methanolic extracts showed activity against some of the tested organisms (Mbwambo *et al.*, 2007).

It is known that plant extracts are able to affect a great number of physiological and pharmacological aspects in the human body and therefore screening for biological activities should be diversified to increase the chances of detecting a variety of useful and meaningful biological activities which would otherwise go undetected. Common plants such as *Carica papaya*, the pawpaw (Clagget *et al*., 1974; Balandrin *et al*., 1985) and *Allium sativum*, the garlic, have been reported to possess broad therapeutic properties ranging from anticancer, antibacterial, and antidiabetic to anticoagulant (Venugopal and Venugopal, 1995). Extracts of *Melia azederach* has been demonstrated to possess antiviral activity against a number of viruses (Wachsman *et al*, 1983). Andrei *et al*. (1988) further demonstrated that the antiviral effect was due to the induction of refractory state to viral infection in mammalian cells by a mechanism independent of interferon. Other plant extracts have been reported to possess antifungal, antibacterial and antimalarial effects (Nascimento *et al*., 2000; Dikasso *et al*., 2006).

In addition to antimicrobial effects of extracts, a number of them have been demonstrated to possess immunomodulatory activities. Alternanthera tenella aqueous extracts were shown to enhance production of antibodies (Guerra *et al*., 2003). Echinaceae purpurea has been reported to stimulate alveolar macrophage functions in rats http://www.columbiaphytotechnology.com/echimmune.htm. A number of compounds from Chinese medicinal herbs have been isolated and demonstrated to have immunomodulatory activities (Peigen and Keji, 1988). Wagner (1990) has given

a review of some plants with immunostimulatory activity. It seems that the mode of action of plant extract is varied. In recent times, a number of plants are increasingly being scrutinized for their immunomodulatory properties. There has been realization that immunomodulants may offer a better strategy to fight off infectious diseases that are proving to be refractory to available drugs.

Immunomodulants

In the last 25 years, the frequency of life threatening infections has increased dramatically among cancer patients, transplant recipients, AIDS patients and those receiving broad spectrum antibiotics, corticosteroids, and cytotoxic drugs (Anaissie *et al*., 2003). Numerous risk factors have been identified that can impair host defense, predisposing to serious diseases (Anaissie *et al*., 2003). To further complicate matters, there has been an upsurge in the number of strains of infectious agents that no longer succumb to antibiotics in the last two decades (Valigra 1994). It is apparently clear that, antibiotics have lost their magic touch after decades of incautious prescription, improper use and inevitable spread of bacterial genes that confer drug resistance (Njoroge and Bussmann, 2006b). In the old days, scientists concentrated on looking for antimicrobials mainly in organisms from soil but today researchers have broadened their search. In many laboratories, the break with tradition has even been greater. Instead of focusing on drugs that block bacterial growth, researchers have been trying to develop carbohydrate or protein molecules that can boost people's natural defenses against microbes (Valigra, 1994). This

immunological approach has been postulated as the most probable way to deal with small pockets of resistant bacteria that survive antibiotic treatment.

Widespread efforts have been made to identify immunomodulatory agents to combat infections as prophylactic or therapeutic regimens, or to enhance host immune mechanisms. Given that human and animals are repeatedly exposed to different risk factors such as pathogenic agents and mycotoxins which impair immune function, immunomodulators are recommended to boost the immune system (Shokri *et al*., 2006). Immunomodulators have become very popular worldwide as people realize the importance of a healthy immune system in the maintenance of health and the prevention and recovery from disease. An immunomodulator is any substance that helps to regulate the immune system. This regulation is said to be normalization process, so that an immunomodulator helps to optimize immune response (Wagner, 1999). How immunomodulators work in the body is still largely a mystery. Part of their benefits would appear to be their ability to naturally affect function of certain immune cells and increase the body's production of certain messenger molecules such as cytokines that mediate and regulate the immune system (Kendall, 1990; Shokri *et al*., 2006).

A number of compounds with immunomodulatory activity have been isolated especially from bacteria (Lederer, 1980) and shown to affect the function of white blood cells. Betafectin, a carbohydrate polymer based on glucan, a substance found in

yeast cell wall is one such compound that stimulates the body's natural defense against infection by enhancing the efficiency of white blood cells called macrophages and neutrophils (Valigra, 1994). These cells are usually the body's first line of defense against invading bacteria and other pathogens (Suigiura *et al.*, 2001). The job of these cells is to destroy the invading organisms before the expression of acquired immunity. Valigra (1994) has provided an insight of how betafectin might function and it is thought to work by mimicking bacterial attack. Usually when a bacterium comes into contact with phagocytic cell it stimulates an antenna-like molecule found on the cells' surface known as the β-□ glucan receptor. This in turn triggers a series of biochemical message which increase the antibacterial activity of the white cells, at the site of infection as well as attracting more macrophages and neutrophils to the area and in some instances, increasing the number of leucocytes in the blood. The overall result is an improved immunity.

2.1.5.1 Immunomodulation

This is a very broad term which refers to any changes in the immune response and may involve induction, expression, amplification or inhibition of any part or phase in the immune response. It may involve strengthening or suppression of the indicators of cellular and humoral immunity and nonspecific defense factors. The essence of immunomodulation is that a pharmacological agent acting under various dose and time regimens displays an immunomodulating effect. The extreme manifestations of

immunomodulation action of biologically active substances are immunosuppression or immunostimulation of the immune reaction (Sagrawat and Khan, 2007). Modulation may be specific and limited to a given antigen/agent or non specific, with a general effect on immune response. Stimulation of the immune response is desired for certain people such as immunocompromised patient, whereas suppression of the immune response is thought for others such as transplant recipient or patient with autoimmune or inflammatory diseases (Sell, 1987; Wagner, 1999).

2.1.5.2 Immunostimulation

Stimulation of nonspecific defense mechanisms in man has a long tradition in medicine. Injection of the body's own blood or inactivated microorganisms, animal organ or plant extracts, as well as inflammatory agents such as mustard oil or turpentine were considered to influence chronic inflammatory, allergic, response. Certain minerals and some plant fatty oils are also used as adjuvants in immunotherapy, since they increase the production of antibodies triggered off by antigens (Goodman, 1994). Immunostimulation constitutes an attractive alternative to conventional chemotherapy and prophylaxis of infections, especially when the host defense mechanisms have to be activated under conditions of impaired immune responsiveness (Wagner *et al*., 1985a). Immunostimulants or immunopotentiators are compounds leading predominantly to a nonspecific stimulation of the immunological defense system. They do not affect immunological memory cells. Therefore the terms

immunomodulation or immunoregulation, denotes any effect on or change of immune responsiveness, very often seen to be more appropriate (Wagner, 1999).

2.1.5.3 Mechanisms of Immunostimulation

Immunological defense is a complicated interplay between nonspecific and specific, cellular and humoral immune responses, stimulation and suppression of immunocompetent cells, and the influence of endocrine and other mechanisms upon the immune system. Primary targets of the immunostimulants are T or B lymphocytes or the complement system, while an increase in phagocytosis by macrophages and granulocytes also plays a central role in immunostimulation (Kuby, 1994). Activation of macrophages is probably important for the stimulating agents to remain in contact with the reactive cell. The second most important role is the stimulation of T lymphocytes, which can be achieved either directly or indirectly via macrophages (Wagner *et al*., 1985c; Wagner, 1999).

2.1.6 Classification of immunostimulating Compounds

Natural compounds with potential immunostimulating activity studied can be classified as high and low molecular weight compounds. These compounds belong to different classes of substances. Terpenoids, phenolic compounds, and alkaloids dominate among low molecular weight compounds, polysaccharides dominate among the high molecular weight compounds (Wagner, 1999). A classification according to their mechanism of action is feasible only in a few cases, because detailed

immunological studies are lacking and pharmacological efficacy for human use cannot be predicted for sure in cases where these compounds have only been tested *In vitro.*

2.1.6.1 Alkaloids

Amongst the chemical classes of compounds present in medicinal plant species, alkaloids stand as a class of major importance in the development of new drugs because they possess a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal plants. Alkaloids have been known to enhance immune response and more recently a large number of alkaloids are being investigated for their immunostimulating properties. Oxindole alkaloids found in the bark and roots of Uncaria tomentosa (cat’s claw) have been documented to stimulate the immune system. Six of these alkaloids have been reported to increase immune function by up to 50 % in relatively small amounts (Wagner, 1999). This has led to its use around the world as an adjunctive treatment for cancer and AIDS, as well as other diseases that negatively impact on the immunological system. The oxindole alkaloids have also been shown to significantly enhance the ability of phagocytes to attack, engulf and digest harmful microbes or foreign bodies (Wagner *et al*., 1985b). Juliflorine, an antimicrobial isolated from Prosopis juliflora was investigated and shown to possess some immunostimulating activity especially that of promoting production of antibodies (Wagner *et al*., 1985b).

2.1.6.2 Terpenoids

Several terpenoids are reported to possess antiarthritic or antiphlogistic activity and their biological activities appear to be mediated by immunological processes (Hall *et al.*, 1979). Effect of these compounds on immune system appears to be two fold; first, to enhance antibody production and second, to suppress T-cell response. These activities are suggested to be due to the presence of the sesquiterpene lactones, helenalin, dihydrohelenalin and its esters.

2.1.6.3 Lectins

Lectins are sugar-binding, carbohydrate-specific proteins or glycoproteins, which agglutinate cells or precipitate glycoconjugates. Lectins were first discovered in plants and were therefore called phytohemagglutinins. Later, detected in fungi, bacteria, invertebrates, lower vertebrates, and at last they were also found in higher animals and human organs. Lectins bind predominantly to lymphocytes where they induce mitosis (Geppert, 1998). As compared with bacterial polysaccharides, the number of polysaccharides isolated from fungi and higher plants that have been studied chemically and pharmacologically in detail is relatively small. Lentinan, a yeast polysaccharide, isolated from *Lentinus edodes*, is one of the best studied polysaccharides and anti-tumor activity of this compound is mainly the result of T-lymphocyte activation (Chihara *et al.*, 1970). Schizophyllan from *Schizophyllum commune* (Komatsu *et al*, 1976; Tabata *et al.*, 1981) and pachymaran from Poria cocos display good immunomodulatory activity (Narui *et al.*, 1980; Okawa *et al.*,

1982). Vesiculogen, from *Pezisa vesiculosa*, was demonstrated to be an adjuvant and mitogen, mainly activating B cells. Studies of phagocytosis activation revealed that its mechanism of action was mediated by the reticuloendothelial system (Suzuki *et al.*, 1982).

2.1.6.4 Phenols, Quinones and Lipids

Among the aromatic or phenolic compounds, the lignan compound cleistanthin derived from *Cleistanthus collinus* was found to increase the number of granulocytes in rats, cats, and monkeys at a dose of 0.75 mg/kg (Rao and Nair, 1970). A stimulation of granulocytes and enhancement of the carbon clearance are observed with catechins and protocatechuic acid (Wagner and Kreutzkamp, 1985). Other group of compounds that stimulate the immune system is constituted by ubiquinones (Block *et al.*, 1978). Ubiquinones Q 7 and Q 8 are especially capable of significantly increasing the phagocytosis of mouse macrophages at concentrations of 100µg/ml. The carbon clearance test revealed that ubiquinone Q7 at 30 mg/kg increased the clearance rate by a factor of 2. In animals pretreated with cyclophosphamide, ubiquinone Q7 induced the secretion of colony-stimulating factor (CSF) and increased the number of peripheral granulocytes (Wagner *et al.*, 1985b).

Synthetic Immunomodulation agents

Several laboratories are working in developing new drugs that effectively modulate the host's natural defenses and restore impaired immune functions. A number of novel immunomodulating agents are currently being studied. They all offer potential

new approaches for the treatment of various conditions such as infections, organ transplantation, cancer, Down syndrome, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus and other autoimmune diseases and, more recently, the acquired immune deficiency syndrome (Sany, 1987; Bartlett *et al.*, 1991; Gonsette, 1996). The knowledge of the intricate relations and multiple levels of interactions between various components of the immune system have been of paramount importance in the understanding of how the immune system provides protection against foreign intruders and against a number of pathological conditions. This has led to a major effort to develop drugs that might modulate the host's natural defense mechanisms and restore impaired immune function (Georgiev, 1987).

The aliphatic sodium diethyldithiocarbamate has been shown to increase the production of interleukins 1 and 2 (IL-1, IL-2) by macrophages and lymphocytes respectively, and normalizes the white blood cell count altered by the inflammatory process (Georgiev, 1987). The antirheumatic drug lobenzarit has been found to prevent the development of autoimmune lupus nephritis in mice, abrogates the secretion of IL-1 by LPS-stimulated human monocytes and induces the production of γ–interferon. It also increases the production of IgM and IgG without affecting the secretion of IL-2 and the lymphocyte mitogenic-response to *Staphylococcus aureus* (Fujimoto *et al.*, 1986).

2-Cyanaziridine compounds (azimexone in particular) were found to increase in a dose-dependant manner, the serum level of colony stimulating factor (CSF) in male BALB/c mice, as well as the CSF secretion by mononuclear bone marrow cells and macrophages (Schlick *et al*., 1985). Azimexone also increases the number of monocytes in both normal and cyclophosphamide-suppressed mice without inducing any detectable tumoricidal activity. The observed immunomodulating activity of the 2-cyanaziridines offers the potential of selectively augmenting the growth and differentiation of myelomonocytic cells in normal and bone marrow-suppressed mice without significantly affecting their immunological status. This in turn, may have clinical importance in the development of cancer chemoimmunotherapy which acts by normalizing the suppressed levels of myelopoietic functions and restoring natural immunity (Schlick *et al*., 1985). Studies on the immunological profile of ciamexone, another 2-cyanaziridine derivative, have shown that it selectively influenced the balance between T-helper lymphocytes and T lymphocytes responsible for the delayed-type hypersensitivity reaction in mice (Bicker and Pahike, 1985).

LS-2616 (a quinolin-2-one-3-carboxamide analogue) was shown to enhance the activity of natural killer cells in mice leading to an increase in spontaneous cytotoxicity against cancerous cells (Georgiev, 1987). It is particularly interesting that a combined treatment with cyclophosphamide and LS-2616 markedly diminished the number of lung cancer colonies in mice after two weeks of treatment (Georgiev, 1987).

Oxamisole (PR 879-317A), an imidazo [1, 2-alpyridine] derivative, is an effective, orally active T-cell-selective immunorestorative agent (Radov et al., 1988). It had little or no effect on the 1gM or 1gG responses to sheep erythrocytes. The humoral immunocompetence of both normal and immunosuppressed animals sensitized with trinitro-phenyllipopolysaccharide (a T – cell independent antigen) was un-affected by oxamisole. The drug enhanced T-cell in-vitro proliferative response, macrophage chemo tactic, but not phagocytic function, indicating a possible role in stimulation of the reticuloendothelial system (Radov *et al*., 1988).

Imidazol [2, 1-b] benothiazole-2-carboxamides was found to exhibit immunomodulating activity. The carboxamide analogue at a 0.2μgml showed a 5.2 fold potentiation of the immune response in the anti-sheep erythrocyte plaque forming cell assay. A number of acridine derivatives have immunomodulating properties which may especially be useful in the treatment of cancer (Georgiev, 1987).

Immunosuppression

The goal of immunosuppression in organ transplantation is to blunt the immune response of the patient to the allograft, while maintaining sufficient resistance to avoid opportunistic infections and malignancy. Rejection processes remain an important cause of morbidity and graft loss. Initial successful efforts with chemical

immunosuppression began in 1960 utilizing the nucleoside antagonist 6-meraptopurine. This strategy replaced the use of irradiation (Murray *et al*., 1963). During the past decades a variety of chemical antiproliferative agents have been tested including the alkylating agent's cyclophosphamide, nitrogen mustard, chlorambucil and actinomycin-D (Alexandre *et al*., 1963; Robertshaw *et al*., 1967) and more potent antiproliferative agents such as mycophenolate mofetil have been recently introduced.

The second strategy that has been exploited for immunosuppression is T-cell depletion. Initially, polyclonal antilymphocytic globulins and, subsequently, anti-T cell monoclonal antibodies (mAbs) (Woodruff and Anderson, 1963; Starzl *et al*., 1967; Najarian *et al*., 1969; Cosimi *et al*., 1981) were introduced to deplete or modulate alloresistance. The present era of immunosuppression was heralded by the discovery of the immunosuppressive activity of cyclosporine and its application in the clinical arena (Borel *et al*., 1976).

2.1.8.1 The immunosuppressive agents and their mechanism

Immunosuppressive drugs are given in order to prevent the rejection of transplanted organs, or to treat disease like Crohn's disease, ulcerative colitis, neutropenia, rheumatoid arthritis and connective tissue disorders. They have many different mechanisms, through which they exert their suppressive activity. Some drugs with different mechanism of action are discussed below.

Immunocytotoxic agents include the antimetabolites, such as purine and pyrimidine analogs and folic acid antagonist (methotexate), which interfere with DNA synthesis. Azathioprine and Mycophenolate mofetil are given below as an example of the most commonly used drug. Other immunocytoxoxic drugs that are used clinically include: Dactinomycin, leflunomide, cyclophosphomide, vincristine, vinblastine and methotrexate (Douglas *et al*., 2001). Azathioprine, an imidazole derivative of 6-mercaptopurine, acts as an antimetabolite to inhibit nucleic acid synthesis via both de novo and salvage pathways. Rapidly dividing cells, including not only T- and B- cell lymphocytes but also, for example, gut endothelium and bone marrow elements, are most susceptible to the drug's antiproliferative effects. In addition, it has been suggested that the imidazole residue of Azathioprine alkylates thiol groups on T-cell surface membranes block antigen recognition (Chan *et al*., 2007). Myelosuppression, particularly leucopenia, is the most common and serious side effect of Azathioprine therapy (Rossi *et al*., 1993). Other toxicities relate to its antimitotic effects, including alopecia, oral ulcers, nausea, vomiting, diarrhea, anorexia and esophagitis. The increased incidence of malignancies with azathioprine tends to be less than that observed with other immunosuppressants, probably owing to its modest relative potency (Blohme and Bryinger, 1985; Rossi *et al*., 1993).

Mycophenolate mofetil is a synthetic analog of mycophenolic acid, a compound that is produced by several Penicillium species. Mycophenolic acid is a selective, reversible, noncompetitive antagonist of inosine monophosphate dehydrogenase

activity (Platz *et al*., 1991; Allison and Eugui, 1993). T and B lymphocytes are dependent upon de novo nucleic acid synthesis and have been claimed to be especially susceptible to mycophenolic acid. However, one of the primary toxicities of mycophenolate mofetil therapy is bone marrow depression, particularly leucopenia, suggesting that the drug is not an exclusive inhibitor of T and B cells (Sollinger *et al*., 1992).

Alkylating agents, such as cyclophosphamide, is one example of lymphocytotoxic agents. They interfere with cell division by altering guanine so that base pairing errors occur. They also can cross-link the two strands, thus blocking DNA replication.

Lympholytic agents as general reduce the lymphoid content or size of spleen and lymph node. They don't exert any toxic effect on stem cell of the bone marrow. One example of lympholytic agent is corticosteroidal or cortisone drugs which are considered immunosuppressive as well as anti-inflammatory. They are used in transplant immunosuppression but also remain a cornerstone of most regimens despite their many side effects (Vineyard *et al*., 1974; Antal, *et al*., 1993). In lymphocytes, steroids seem to dampen the generation of activation protein-I and inhibitory kinase kappa, thereby down regulating expression of genes encoding proinflammatory cytokines, including the co-stimulatory factors IL-1 and IL-6, and the proinflammatory molecules platelet activating factor, prostaglandins, leukotrienes and tumor necrosis factor-α (Kelso, 1984). Four corticosteroid compounds are most

often used in clinical transplantation, hydrocortisone, prednisone, prednisolone and methylprednisolone, because they display a high degree of anti-inflammatory potency. The diverse side effects of corticosteroids may be both serious and life threatening. Although the adverse reactions usually subside with dose reduction during maintenance therapy (Fryer *et al*., 1994; Ponticelli and Opelz, 1995), many effects have long-term, dose-independent consequences. The most serious complication is interference with APC as well as monocytes; macrophage and granulocyte function, leading to paresis of nonspecific host resistance and potential emergence of life threatening bacterial, viral or fungal infections.

Antibiotics, such as cyclosporine, exert an inhibitory effect on interleukin-2 action, thus blocking the expansion of the helper/inducer T-cell population. It is particularly effective in suppressing graft rejection reactions. Cyclosporine, a cyclic fungal endocapeptide acts primarily on T cells and to a lesser extent on B cells by blunting T-cell help (Kupiec-Weglinski *et al*., 1984). Cyclosporine-cyclophilin complexes bind to catalytic units of calcium, calmodulin and calcineurins A and B, thereby blocking calcineurin phosphatase activity toward a variety of substrates, all of which regulate the expression of T-cell growth promoting genes. In lymphocytes, cyclosporine blocks gene transcription and generation of mRNA for certain cytokines (IL-2, IL-3, IL-6, IL-7, and IFN-γ) (Elliot, 1984; Shevach, 1985).

2.1.8.2 Plant derived immunomodulants

Investigation of compounds with immunomodulatory activities has been going on for sometimes now. Most of the search has been concentrated on lower organisms. A number of compounds have been isolated from bacterial cell wall and shown to possess immunomodulatory activities (Lederer, 1980). The hot water extract of Pleurotus ostreatus mycelium has been shown to possess immunostimulatory effects on cyclophosphamide treated mice (Morris *et al*., 2003), while the extract of the edible mushroom Agaricus blazei has been demonstrated to significantly increase serum 1gG levels, T-cell populations and polymorphonuclear neutrophils leucocytes phagocytic activity in mice (Chan *et al*., 2007).

In recent times, many researchers have been turning to higher plants as a source of immunomodulators. Many traditional herbal plants have been used since ancient times because of their antiallergenic, anti-inflammatory, and antipruritic activities. Among 250,000 known species, only a small percentage have been investigated phytochemically and even a smaller portion have been submitted to biological and pharmaceutical screening. Unfortunately, the effects and mechanisms of the activities of plant derived drugs on the immune system have not been well defined (Basaran *et al*., 1997). It is only in the most recent times that scientific literature is continuously reporting plant drugs having immunomodulatory activity. Most of the leads for this activity are from traditional medicines from different parts of the world (Basaran *et al*., 1997; Guerra *et al*., 2003; Shokri *et al*., 2006; Thakur *et al*., 2006). In the search

for plant constituents with immunomodulatory potency one can select those plant drugs which so far have been used in traditional medicine. In the old literature, one will barely find the term immunostimulation. One can assume, however, that many plants described for their antibacterial, antiviral, antifungal or antitumoral activities are good candidates for screening (Wagner, 1990). Another criterion that can be applied is when quantities of drugs with claimed antiinfectious or antitumor activities are so small that a direct antimicrobial or antitumor effect can be excluded and an immune induced effect can be assumed (Wagner, 1990).

A number of plant extracts have been investigated for their immunomodulatory properties and their mechanisms of action elucidated. These studies reveal that plant extracts are able to affect diverse functions of the immune system and cells. Echinacea preparations are commonly used as nonspecific immunomodulatory agents (Zhai *et al.,* 2007). Echinaceae species have been widely studied and several major constituents of Echinaceae have been isolated, identified and reported to be biologically active. These include echinacoside, cynarin, chicoric acid, alkamides and polysaccharides which have been reported to act on the immune system to boost its effect against pathogens http://www.columbiaphytotechnology.com/echimmune.htm). These active constituents of Echinaceae may strengthen or stimulate the immune response by interacting with various cells of the immune system. Echinaceae preparations have been demonstrated to activate macrophage, natural killer cells, and

polymorphonuclear leucocytes in addition to enhancing production of cytokine molecules (Wagner, 1999; Zhaietal.,2007;
http://www.columbiaphytotechnology.com/echimmune.htm).

Ethanolic and sapogenin root extracts of *Chlorophytum borivilianum* have been demonstrated to be effective immunostimulatory principles that not only potentiate non specific immune response, but are also effective in improving humoral as well as cell mediated immunity (Thakur *et al*., 2006). Immunomodulatory activities of some Turkish plants were investigated and shown to vary (Basaran *et al*., 1997). Immunomodulatory activity of the Indian plant *Triphala* on neutrophil functions was investigated and shown to be significantly enhanced (Srikumar *et al*., 2005). Some Chinese plants have also been investigated and active ingredients isolated from them and reported to treat leucopenia while other compounds were reported to have the ability to increase the weight of spleen and thymus of mice and promote the production of specific antibody in spleen cells, together with macrophatosis of peritoneal macrophages (Peigen and Keji, 1988). Shokri et al. (2006) showed that the Iranian herb *Zataria multiflora* affects function of the innate immunity in mice by increasing phagocytosis and TNF –α secretion. A great number of compounds with potential immunomodulatory activity have been described and these include saponins, alkaloids, terpenoids, quinones, phenolics, polysaccharides, peptides, glycoproteins and nucleotides (Wagner, 1985b).

Immunomodulation encompasses immunostimulation as well as Immunosuppression (Wagner, 1990) and several plant secondary metabolites have been found to also suppress the immune functions. Ethanolic stem bark extract of *Mammea africana* has been found to significantly decrease white blood cells counts in extract treated rats (Antia *et al*., 2006). Antia *et al*. (2006) investigated several plants from the genus *Euphorbia* and found that some were able to cause leucopenia while others caused leucocytosis. In another study, Antia *et al*. (2006b) showed that extracts of four plants *A. cordifolia*, *C. acontifolium*, *P. amarus* and *P. muellerianus* reduced the number of white blood cell counts in rats. Administration of ethanolic leaf extract of *Croton zambesicus* to rats has been demonstrated to produce a reduction in packed cell volume (PCV) and WBC (Okokon *et al*., 2004) and it was postulated that the drop in WBC could be possibly due to suppression of their production in the bone marrow.

Plant species such as *Viscum album*, *Panax ginseng* and *Tripterygium wilfordii* have been known to present both immunostimulatory and immunosuppressive activity and have been subjected to detailed studies to elucidate the active components and their mechanisms of action (Peigen and Keji, 1988; Souza-Fagundes *et al*., 2002). Tao *et al*.(1991) demonstrated the immunosuppressive effect of *T. Wilfordii* alcoholic extract and the immunosuppressive effect was reported to be due to the inhibition of T-cell receptor signaling pathway (Souza-Fagundes *et al*., 2002). Several plants from Brazil were demonstrated to have antiproliferative effect on human peripheral blood mononuclear cells (Souza-Fagundes, *et al*., 2002).

From the foregoing, it would appear that modulatory effect of plants and compounds derived from plants are able to affect the immune system in a variety of ways, altering their functions by either enhancing or depressing them. It would also seem that very little studies have been carried out in Africa and Kenya in particular regarding the immunomodulatory properties of plants.

2.1.8.3 Methods for Detection of Immunomodulating Compounds

A number of assays, both *In vitro* and in vivo have been developed to test for immunomodulatory activities of plant extracts and other compounds (Wagner, 1999). Studies of how plant substances affect immune response employ mechanistic bioassay methodologies. Basic research on natural substances with immunomodulating properties is performed by assays primarily carried out on the stimulation of non specific immunity of the innate responses, such as the efficiency of granulocytes, macrophages, complement, and natural killer cells, and their effects on phagocytosis, lymphocyte proliferation, and T-lymphocyte migration. More recent research on immunomodulating substances includes studies on cytokine production by macrophages such as IL-1, 1L-6 and TNF-α (Williams, 2001).

Simple tests used to assay for immunomodulation includes establishment of total WBCs and differential WBCs (Srikumar *et al*., 2005). These cells usually include the eosinophils, basophils, monocytes, neutrophils and lymphocytes. An increase in their activity or numbers would be a good indicator of enhanced immunity (Valigra, 1994).

The chemotactic activities of the immunomodulators can also be evaluated by the Boyden migration chamber method. It involves using millipore cellulose acetate membranes. The neutrophil suspension is placed in the top compartment of the chamber while the lower well is filled either with serum activated with each plant extract separately or zymosan activated serum (ZAS) as a chemotactic stimulus for positive control. The distance of cell migration is analyzed by a light microscope and the chemotactic index calculated by the ratio of the distance traveled by stimulated leukocytes in the membrane towards the lower chamber to those of non stimulated leukocytes (Basaran *et al.*, 1997).

To evaluate neutrophils for their enhanced activity, neutrophil adhesion test is used. It is analyzed by getting an initial count of total WBCs and differential WBCs from a blood sample. The blood sample is then incubated in sterile nylon fiber column packed in a silicanazed Pasteur pipette. After a brief incubation period, the blood sample is again analyzed for total WBCs and differential WBCs. The product of total WBCs and differential WBCs gives the neutrophil index of the blood sample. Percentage of neutrophil adherence is then calculated from this (Srikumar *et al.*, 2005; Thakur *et al.*, 2006).

The activity of neutrophils or even macrophages can further be evaluated by utilizing the nitroblue tetrazolium reduction test (Suigiura *et al.*, 2001). This test relies on the generation of bactericidal enzymes (like NADPH-oxidase) in neutrophils during

intracellular killing. These enzymes are necessary for normal intracellular killing against foreign antigens (Srikumar *et al*., 2005). During intracellular killing, the cellular oxygen consumption increases and glucose metabolism reduces the colorless NBT to blue formazan (Suigiura *et al*., 2001; Srikumar *et al*., 2005).

Other assays that have been used to monitor *In vitro*, the activity of neutrophils and macrophages includes Candida, SRBC and bacterial phagocytosis (Ottendorfer *et al*., 1985; Suigiura *et al*., 2001; Srikumar *et al*., 2005; Bao *et al*., 2006). The most important test systems among the variety of immunological assays available at present are those that allow determination of the functional state and the efficiency of the mononuclear phagocyte system.

In vivo, neutrophils and phagocytic activity can be evaluated by intraperitoneal injection of SRBCs in laboratory rodents. Intraperitoneal cells are then later washed out with saline and cells fixed onto slides and stained with Giemsa's solution. The percentage of macrophages that ingest SRBC (phagocytic ratio) and the number of SRBC in the macrophage (phagocytic index) are calculated by counting 100 macrophages under a microscope (Morris *et al*., 2003). Alternatively, carbon clearance test can be used. The study of phagocytosis *In vivo* has depended on measuring the rate of clearance from the blood of foreign or altered autologous particulate substances. It involves injecting standard dose of colloidal carbon, liposome's, denatured albumin or Indian black ink into the tail vein of laboratory

rodents. Blood is then drawn out at different time intervals and the concentration of residual particles determined spectrophotometrically (Van Etten, 1998; Nestor *et al*., 2001; Morris *et al*., 2003).

Assay of cytokines has also been used and involves measuring the concentration of plasma soluble IL-2 receptor and the concentration of tumor necrosis factor (TNF), IL- 6, IL-10 and interferon (IFN) by ELISA technique (Chan *et al*., 2007). Assays of cytokines production gives an indication to the extent to which macrophages and to an extent, lymphocytes are activated.

Flow cytometric determination of various cell types can be determined and utilizing this, the percentages of T-cell-, B-cell- can be ascertained (Bao *et al*., 2006; Chan *et al*., 2007; Zhai *et al*., 2007). In addition sheep erythrocyte rosettes to determine the proportion of T cells in a given population of lymphocytes can be used to determine subpopulation of T- lymphocytes. T-lymphocytes have a receptor for sheep red blood cells. When incubated with 1% sheep erythrocytes, T cells will bind or 'clumps' red blood cells around them in the form of a rosette. This test has now largely been superseded by assays using antibodies to the sheep red blood cell receptor (CD2) (Hudson and Hay, 1989).

Lymphocyte proliferation assay (LPA) is a test that measures the influence of compounds on T-lymphocyte cell population. Lymphocyte Transformation Test (LTT) reflects the degree of blastogenesis of T lymphocytes after having contacted

with mitogen. Stimulation rate can be measured by [^{3}H] thymidine incorporation in T lymphocytes, using phytohemagglutinin (PHA) or Concanavalin A (con-A) as control mitogen. Using monoclonal antibodies and fluorescence activated cell sorting (FACS), it is even possible to monitor T-lymphocyte subpopulations (Olson and Bicker, 1982).These measures allow prediction of whether a substance displays predominantly either stimulating or suppressing activity (Weir *et al*., 1991).

Antigen specific T cell proliferation is a major technique for assessing the functional capacity of CD4$^+$ lymphocytes to respond to various stimuli. In many of the LPA, peripheral blood mononuclear cells (PBMCs) are isolated, placed in a 96-well plate with or without various stimuli and the cells allowed to proliferate for several hours at 37^{0}C in a CO_2 incubator (Ottendorfer *et al*., 1985; Tao *et al*., 1991). The amount of proliferation is detected by adding radioactive [^{3}H] thymidine for a few hours. This is incorporated into newly synthesized DNA of the dividing cells (Tao *et al*., 1991). The amount of radioactive [^{3}H] thymidine incorporated into DNA in each well is measured in a scintillation counter and is proportional to the number of proliferating cells, which in turn is a function of the number of lymphocytes that were stimulated by a given antigen to enter proliferative response (Ottendorfer *et al*., 1985; Tao *et al*., 1991; Souza-Fagundes *et al*., 2002).

Assays that measure production of antibodies and other functions of specifically the B cell have been developed and are also in use.

2.1.8.4 Effect of plant extracts on the specialized cells of the immune system

In mammals, the immune system plays a vital role as the main line of defense against infections. Its integrity and efficiency is important during chemotherapeutic intervention for the treatment of many diseases (Souza – Fagundes *et al*., 2002). The immune system is a remarkably sophisticated adaptive defense system within vertebrates, which protects them from invading pathogens and cancer. It is able to generate varieties of cells and molecules capable of recognizing and eliminating limitless varieties of foreign invaders. Functionally the immune system is divided into two interrelated activities, recognition and response. Immune system is able to recognize foreign substances, and discriminate foreign molecules from own cells and proteins (Roit *et al*., 1998; Karp, 1999). Once foreign molecule or organism is recognized, the immune system responds to eliminate or neutralize these by utilizing the initial recognition information. The same response could be repeated if the immune system encounters the same antigen again (Kuby, 1994).

The immune system is a network of specialized tissues, organs, cells, and chemical mediators. Two types of immunity protect the body: innate and adaptive (Chan *et al*., 2007). Innate immunity is present at birth and provides the first barriers to keep unwanted agents away from more vulnerable tissues. Adaptive immunity is the second barrier to infection and it is acquired after first exposure. The adaptive immune system retains a memory of all the invaders it has encountered and becomes activated after attack from the same invader (Vitetta *et al*., 1991). There are five

distinctly different kinds of white blood cells: neutrophils, monocytes, lymphocytes, eosinophils and basophils. Some have the ability to change with needs and situations in the body.

2.1.8.4.1 Leucocytes

Leucocytes (white blood cells) are the main cells of the immune system that provide either innate or specific adaptive immunity. They are motile with specialized functions. The number of leucocytes in normal blood ranges between 4,500 and 11,000 per cubic millimeter. They are further classified into lymphocytes, granulocytes, monocytes, and natural killer cells (Mossman and Coffman, 1989). There are three types of granulocytes: neutrophils (50 to 70%), eosinophils (1 – 4%), and basophils (0.5%) (Kuby, 1994).

Plant extracts and plant derived constituents have been known to affect leucocytes by increasing or reducing their numbers resulting in leucocytosis or leucopenia. Aqueous extract of *Fadogia agretis* stem was found to significantly increase WBC (Yakubu *et al.*, 2008). Aqueous root extract of *Harungana madagascarensis* was found to significantly increase total leukocyte among other hematological parameters in acetaminophen injured rats (Adeneye *et al.*, 2008). This effect of *Harungana* was believed to protect the rats against acetaminophen nephrotoxicity. Nwinuka *et al.* (2008) demonstrated that aqueous extract of Mangifera indica was able to increase leukocyte levels in normal albino rats. The mechanism by which the plant extracts cause an increase in WBC remains largely unclear. It may be that they stimulate

production of more WBC in the bone marrow or they enhance production of soluble mediators that results in leucocytosis. Other plant extracts have been shown to decrease WBC in experimental animals. Ethanolic stem bark extract of *Mammea africana* has been found to significantly decrease white blood cell counts in extract treated rats (Antia *et al*., 2006). Antia *et al*. (2006) investigated several plants from the genus Euphorbia and found that some were able to cause leucopenia while others caused leucocytosis. In another study, Antia *et al*. (2006) showed the extracts of four plants *A. cordifolia*, *C. acontifolium*, *P. amarus* and *P. Muellerianus* reduced the number of white blood cell counts in rats. Administration of ethanolic leaf extract of *Croton zambesicus* to rats has been demonstrated to produce a reduction in packed cell volume (PCV) and WBC (Okokon *et al*., 2004) and it was postulated that the drop in WBC could be possible due to suppression of their production in the bone marrow.

2.1.8.4.2 Neutrophils

These are one of the major types of cells that are recruited to ingest, kill and digest pathogens. Neutrophils are important components in the surveillance and protection systems for a broad spectrum of host defenses. They play the main role as effectors or killer cells for many types of antigenic challenges especially for infections (Srikumar *et al*., 2005). The primary functions of the neutrophils in host resistance are the migration towards the challenge which is called chemotaxis and the intracellular killing of microorganisms by the formation of oxygen radicals (Basaran *et al*., 1997). Phagocytosis by neutrophils constitutes an essential arm of the host to defense against

foreign antigens. Neutrophils have receptors for fragment crystallizable and complement component (C3b) which are involved in the uptake of foreign antigens Srikumar et al., 2005). Neutrophils engulf and digest bacteria and other microorganism and microscopic particles.

Plant extracts and plant derived compounds have been demonstrated to have diverse effect on the activity of neutrophils. In some cases they are known to increase the number of neutrophils resulting in neutrophilia while in other cases increases the chemotactic, neutrophils adherence and phagocytic capacity of neutrophils (Basaran *et al*., 1997; Srikumar *et al*., 2005). In some other instances, plant extracts seem not to have an effect on neutrophils. Aqueous and ethanol extracts of *Aeginetia indica* Roxb had no effect on the number and activity of neutrophils following oral administration in female B6C3F1 mice (Saraphanchotiwitthaya *et al*., 2008). Myeloperoxidase (MPO) is one of the antimicrobial enzymes produced by neutrophils and changes in the MPO activity indicate the functional alteration of neutrophils. Exposure to the extracts of *Aeginetia* for 28 days did not result in an increased MPO levels (Auttachoat *et al*., 2004)

2.1.8.4.3 Eosinophils and Basophils

These are also motile and phagocytic, and migrate into the tissues. They are particularly important in the defense against parasites and participate in hypersensitivity and inflammatory reactions. Their cytotoxicity is mediated by cytoplasmic granules (Karp, 1999). Basophils also migrate into the tissue and have

many large cytoplasmic granules, which contain heparin and histamine. When aggravated, they release histamine and other mediators that are involved in allergic reactions. Basophils display high affinity surface membrane receptors for IgE antibodies (Roit *et al.*, 1998). It is not known how plant extracts affect the number and activity of these types of granulocytes given their importance in parasitic infections and inflammatory reactions. There is scarcity of information and in most cases; differential leukocyte count in animals reveals that the numbers of these granulocytes remain unchanged following administration of plant extracts.

2.1.8.4.4 Monocytes/ Macrophages

These are also produced in the bone marrow. They constitute up to 10% of the blood leucocytes. However, most of them leave the blood after a few hours and migrate into almost all tissues, where they develop into macrophages. Both monocytes and macrophages are highly adherent, motile and phagocytic. They marshal and regulate other cells of the immune system. They also serve as antigen processing-presenting cells and act as cytotoxic cells when armed with specific IgG antibodies (Shah *et al.*, 2002). Macrophages play an important role in host defense by phagocytizing foreign invaders, undergo oxidative burst, present antigen, and secrete cytokines such as tumor necrosis factor-α and interleukin-1 (Dhuley, 1997; Shah *et al.*, 2002; Napolitano *et al.*, 2005). Macrophages are activated in inflammatory sites and can also be activated by bacterial lipopolysaccharide, interferon, IL-12, IL-18, ultraviolet radiation and ozone. The activated or angry macrophages as they are sometimes called can efficiently destroy microbes, parasites, and tumor cells (Shah *et al.*, 2002).

Through TNF-α and IL-1 macrophages control the proliferation, differentiation, and effector functions of lymphocytes (Suigiura *et al*., 2001).

It would seem that this is one of the immune cells that are highly affected by plant extracts both in terms of their numbers and activities. Benny and Vanitha (2004) reported that polysaccharides isolated from *Astragalus membraneus* increased macrophage count as well as enhancing their activity. A polyphenol extract from *Geranium sanguineum* L. induced a rise of the number and migration of alveolar macrophages in both influenza virus infected mice and healthy mice (Toshkova *et al*., 2006). Methanolic extracts of *Pouteria cambodiana* was demonstrated to enhance lysosomal enzyme activity in macrophage indicating stimulation effect (Manosroi *et al*., 2005). The inhibitory activity on nitric oxide (NO) production by the extracts of Chinese medicinal plants in LPS activated macrophages was demonstrated by Ryu *et al* (2001).The anti-inflammatory activity of these Chinese medicinal plants can be linked to this inhibition of NO production. It is known that at low concentrations NO can dilate the blood vessels and improve the circulation, but at high concentrations it can cause circulatory shock and induce cell death. Thus, inflammatory diseases can arise in the presence of the large amount of the physiological concentrations of NO (Iwalewa *et al*., 2009). Peritoneal macrophages from male BALB/c mice orally treated with 500-1500mg/kg of *Anoectochilus formosanus* were shown to exhibit high phagocytic activity (Lin and Hsieh, 2005). Extracts of *Echinaceae* and *Terminalia*

bellerica were demonstrated to have a stimulatory effect on macrophage phagocytic activity (Berman, 2003; Saraphanchotiwitthaya *et al*., 2008).

2.1.8.4.5 Natural killer (NK) cells

These cells resemble macrophages and are also called large granular lymphocytes. They are also produced in the bone arrow and constitute less than 10% of blood leucocytes. They have little phagocytic function and no specialized lymphoid functions. They kill many virus infected cells and cancer cells, with the help of T lymphocytes (Kendall, 1990). Echinaceae extracts have been reported to enhance natural killer cell activity. In in-vitro assays utilizing PBMCs from three groups of subjects (Healthy, with AIDS, or with chronic fatigue syndrome), *E. purpurea* whole plant extract enhanced natural killer cell function in all groups (Berman, 2003). Phytosterols a standardized plant extract has also been shown to enhance natural killer cells. It was found to increase the amount of ^{51}Cr released in the supernatant by the cancer cell line K562 incubated with NK pretreated with phytosterols (Bouic *et al*., 1996). Polysaccharides from *Ganoderma lucidum* have been reported to increase natural killer cell count (Benny and Vanitha, 2004).

2.1.8.4.6 Lymphocytes

Lymphocytes are small white cells found in lymphoid organs and in the blood. Lymphocytes constitute about 25-50% of the blood leucocytes (Roit *et al*., 1998). They are non-motile and enter the circulation through lymphatic channels. They are found in large numbers in the secondary lymphoid organs. Lymphocytes are divided

into two main classes according to their origin and differentiation. T- Lymphocytes which are produced in the bone marrow pass through thymus and mature there. The other class is the B-lymphocytes; they do not pass through thymus but mature in bone marrow (Mossman and Coffman, 1989; Kendall, 1990; Nestor, 2001).

2.1.8.4.7 T-lymphocytes

There are two major classes of T-lymphocytes: T helper (Th) and T-cytotoxic lymphocytes (Tc). T helper (Th) lymphocytes regulate the antibody-forming function of B-lymphocytes and participate in rejection of transplants. They possess CD4 surface molecules (also called CD4 cells). T helper cells are functionally further subdivided into at least two types, Th-1 and Th-2 (Kendall, 1990; Karp, 1999). The other T lymphocytes are those also involved in the defenses against viral infections, and include T-cytotoxic lymphocytes (Tc) and T-suppressor (Ts).

Cytotoxic T (Tc) cells are capable of destroying a target cell, that is infected with virus or that expresses some form of foreign antigen. These cells are the major immune effectors of the cellular immune response. They express CD8 molecules (Levine *et al*., 1998). T-suppressor (Ts) cells act to diminish helper T-cell activity; they directly kill virus infected or cancer cells when the battle is over. They express CD8 molecules. In contrast to helper T-cells, Ts cells down-modulate immune responses. Thus, the combination of helper and suppressor cells determines the level of the immune response to any specific antigen (Levine *et al*., 1998).

T-lymphocytes are known to be affected by a number of compounds including plant products which cause an increase in their number through proliferation or by increasing their capacity to produce cytokines. Usually specific binding between a lymphocyte surface receptor and ligands initiate intracellular signals that include membrane cytoskeleton interaction, calcium influx, activation of phospholipase C, inositol triphosphate induced calcium release and gene activation. This membrane signaling process ultimately causes a change in the behavior of lymphocytes such as receptor distribution, secretion of lymphokines and antibodies, cell motility, cell-cell recognition, or initiation of cell division (Bourguignon and Bourguignon, 1984; Kendall, 1990). There are a vast number of ligands that can initiate cell division. This includes plant mitogens PHA, Con-A and, bacterial products such as LPS and *S. aureus* Cowan I, recall antigen (*C. albicans*, tetanus toxoid, etc), allogenic lymphocytes, cytokines and monoclonal antibodies to cell surface receptors such as CD3 or CD2 (Fletcher *et al*., 1992; Karp, 1999).

Methanolic seed extracts of *Citrullus colocynthis* was shown to be able to increase lymphocyte numbers in rabbits at a dose of 100mg/kg (Shafaei *et al*., 2007). *Ocimum basilicum* aqueous and methanolic extracts were found to induce high lymphoproliferative response as were extracts of *P. Americana*, *P. virginica* and *Rosa* spp (Gomez-Flores *et al*., 2008). In this investigation, the presence of flavonoids, terpenes, tannins and saccharides was detected and it is not clear whether lymphoproliferative activity seen can be attributed to these compounds. Methanolic

extract of *Euphorbia cheiradenia* was demonstrated to have a significant stimulatory effect on T lymphocytes by increasing IL-2 secretion in cultures treated with the extract. This was an indication of the ability of the extract to enhance activation of T cells and release IL-2 (Amirghofran *et al*., 2008). An increase of approximately five-fold in ^{3}H-thymidine incorporation above suboptimal PHA stimulation was found when T cells were incubated with beta-sitosterol indicating enhancement in the proliferation of T cells (Bouic *et al*., 1996). In a study conducted by Saraphanchotiwitthaya *et al*. (2007) extract of *B. superba* moderately activated T and B lymphocyte proliferation while *P. indica* fully activated T lymphocytes. Methanolic extracts of *Justica gendarussa*, *Plumbago indica*, *Aloe vera* and *Aegle marmelos* inhibited lymphocyte proliferation (Arokiyaraj *et al*., 2007).

2.1.8.4.8 B Lymphocytes

These have a relatively short life span compared to T-cells. As B-cells mature, they turn into antibody-producing plasma cells found in lymph nodes and in the spleen. Once the B-cells have created a specific antibody to attack a specific pathogen, their primitive intelligence remembers this information and will know it later should it encounter it (Roit *et al*., 1998; Karp, 1999). Two major functions of B cells are to secrete immunoglobulins and to bind, process and present antigen to T cells. Each B cell produces immunoglobulins with a single specific antigen binding site. By virtue of having immunoglobulin on their surface, B cells bind specific antigens which then deliver a signal to these cells to proliferate and clonally expand into memory cells or to differentiate into Ig-secreting cells. Since only one in 10^{7} – 10^{8} normal B cells has

specificity for a particular antigen, the number of potential antigen-binding B cells are too few in number to provide an appropriate model to study the mechanisms of cell activation (Kendall, 1990; Levine *et al*., 1997).

Studies by Amirghofran *et al*. (2008) demonstrated stimulatory effects of E. cheiradenia methanolic extracts on the humoral immune response. Immunized mice showed a significant increase in anti-SRBC hemagglutinine titre. A Japanese plant, Cistanche Salsa extract was shown to enhance antibody production in human lymph node lymphocytes especially those of IgM and IgG (Maruyama *et al*., 2008). The extract was also demonstrated to induce interleukin-6 receptor (IL-6R) on surface immunoglobin positive B cells (Maruyama *et al*., 2008). Antibody response to SRBC was demonstrated to be increased by extracts of three *Echinaceae* spp (Zhai *et al*., 2007). Anoctochilus formosanus fraction was found to significantly increase the interferon gamma (Th1-type) production but not IL-4(Th2-type). It also did not affect the serum levels of IgG and IgM. The extract of *Terminalia bellerica* has been shown to suppress lymphocyte proliferation at low concentration while the extracts at high concentration led to activation of both T and B cells. These investigations revealed that the extract affected B cell proliferation through T cell- independent and T-cell-dependent mechanisms (Saraphanchotiwitthaya *et al*., 2008). T2, an ethanolic extract of *Tripterygium wilfordii* hook was shown to inhibit IgM, IgG and IgA production by B cells (Tao *et al*., 1991).

Role of Plant Extracts on the Immune System Defense Activity

Individuals depend on the harmony of phagocytosis, cell-mediated and humoral immunities to develop resistance to infectious diseases and these three facets of the immune system are interactive and important in general disease resistance (Nestor *et al.*, 2001). One of the most crucial parts of the host defense is the phagocytosis process against the invading microorganisms. Cells involved in phagocytosis are polymorphonuclear leukocytes and mononuclear cells (professional phagocytes) (Rabinovitch, 1995). These cells ingest other cells, microbes, and foreign particles. PMNs are released into the circulation in vast numbers in a fully differentiated state. After invasion of the tissues by microorganisms, PMNs become activated. This process is triggered by bacterial cell wall products (lipolysaccharides, peptidoglycan), cytokines and many other molecules. Activated PMN or macrophages adhere to endothelial cells and move through the endothelial barrier to the site of the infection. This process of migration is called chemotaxis (Roit *et al.*, 1998; Srikumar *et al.*, 2005).

2.1.9.1 Chemotaxis

This is the first stage of phagocytosis and is defined as cell movement in one direction in response to an agent. Neutrophils chemotaxis requires the binding of chemoattractants to specific membrane receptors and these include bacterial products, activated complement factors (C5a), arachidonic acid metabolites, and cytokines such as IL-1, IL-8 (Brandolini *et al.*, 1997). These bind to G proteins of PMNs, leading to signal transmission to the interior of the cell which in turn leads to activation of the

PMN and thus expression of adhesion molecules. The first event during chemotaxis is margination of the circulating leukocytes within the venule where the white cells are loosely adhered to the vessel wall and roll along the surface of the endothelium. After rolling, many neutrophils firmly adhere to the endothelial cell surface and become activated, changing from a spherical configuration to a flattened shape. Adhesion molecules called integrins are responsible for this firm adhesion of leukocytes to endothelium. Activation of the integrins stops the flow of leukocytes. Many of the activators are produced by the endothelial cells themselves, and by monocytes or bacteria (Srikumar *et al*., 2005). Basaran *et al*. (1997) reported a number of plants extract that enhanced chemotactic capacity of neutrophils and macrophages. These plant extracts included *Viscum album*, *Arctium minus*, *Momordica charantia* and *Urtica dioica*.

2.1.9.2 Opsonization

Microorganisms are phagocytosed after they have been opsonized. Opsonins are substances that facilitate the phagocytosis process. There are two main classes of opsonin IgG and IgA antibodies and the third component of complement (Roit *et al*., 1998). Opsonin binds to the surface of the phagocytosed particle with one end and, via specific receptors to the other end. There are two main classes of receptors, Fc receptors specific for the Fc region of IgG, IgA and IgE antibodies; and complement receptors specific for C3b fragments (Klein and Horejsi, 1997). Upon binding of immune complexes containing the antibodies of appropriate isotypes these receptors induce phagocytosis and other activating reactions in phagocytosis.

In the early stage of infection, phagocytosis process does not seem to depend on opsonins but it seems to rely on other set of alternative mechanisms. One of that is by direct recognition of the organism, via surface lectins. Some other microorganism activates the alternative pathway of complement in the absence of antibodies. Immediate opsonization can also occur by natural antibodies present in the plasma. Attachment of those antibodies to microbial surface initiates activation of the complement classical pathway (Klein and Horejsi, 1997). Investigations on the role of plant extracts and plant derived compounds on how it affects the process of opsonization is lacking and there is need to initiate more research into this area. It is possible to hypothesize that some plant extracts could by themselves act directly as opsonin, or increase the levels of IgG secretion, activating the third complement factor, thereby enhancing opsonization.

Phagocytic cell membranes contain various other receptors. After the attachment of microorganism or the foreign particle they are engulfed. The process of phagocytosis is known to be enhanced by a number of factors such as exercise, microbial and plant products. Peritoneal macrophages from male BALB/c mice orally treated with 500-1500mg/kg of *Anoectochilus formosanus* were shown to exhibit high phagocytic activity (Lin and Hsieh, 2005). Extracts of *Echinaceae* and *Terminalia bellerica* were demonstrated to have a stimulatory effect on macrophage phagocytic activity (Berman, 2003; Saraphanchotiwitthaya *et al*., 2008). Three Peruvian plants, *Pistia*

startiotes, *Piper peltatum* and *Uncaria tomentosa* were demonstrated to enhance phagocytosis. Shokri *et al* (2006) showed that the Iranian herb *Zataria multiflora* affects function of the innate immunity in mice by increasing phagocytosis and TNF –α secretion. Pycnogenol was demonstrated to enhance phagocytosis of fluorescein-conjugated Escherichia coli particles by J774 cells (Shah *et al*., 2002). The mechanisms involved in killing after phagocytosis and ingestion are discussed below.

2.1.9.3 Respiratory Burst (Killing)

The most important mechanism contributing to the killing of the phagocytosed microorganism is the respiratory burst in which the oxygen metabolites are produced. Resting phagocytes consume little oxygen but upon stimulation their oxygen uptake markedly increases. The increase is more pronounced for neutrophils than for other phagocytes. It occurs rapidly and is accompanied by other metabolic changes usually associated with respiration hence it's called respiratory burst (Dinauer, 1993; Klein and Horejsi, 1997; Srikumar *et al*., 2005). *Z. multiflora* extract was demonstrated to increase respiratory burst by neutrophils in BALB/c mice following intraperitoneal injection (Shah *et al*., 2002). Pycnogenol, a procyanidins extracted from the bark of French maritime pine has been reported to inhibit macrophage oxidative burst (Shah *et al*., 2002).

PMNs are able to kill microorganisms by two distinct mechanisms, one of which is oxygen dependent, while the other is oxygen-independent.

2.1.9.4 Oxygen-dependent killing mechanism

The oxygen-dependent antimicrobial mechanisms are initiated when PMNs undergo a 'respiratory burst'. How the bacterial interaction lead to a respiratory burst is not yet clear. It is believed that G protein serves as intermediate between cell surface receptors and effector enzymes responsible for generating second messengers (Karp, 1999). The interaction with G protein leads to changes in configuration of the G-protein and activation of adenylcyclase and or phospholipase. This process, results in the activation of second messengers such as cAMP, inositol triphosphate, Ca_2+ and, arachidonic acid, etc. High cAMP and Ca_2+ levels lead to activation of protein kinase A (PKA) and protein kinase C (PKC) respectively which play a role in the activation of NADPH oxidase and other bactericidal enzymes in the cell membrane (Karp, 1999; Srikumar *et al.*, 2005). This results in the reduction of 0_2 to superoxide. Reduction of 0_2 is the first step in a series of reactions that produce toxic oxygen species during the respiratory burst. Superoxide can react with hydrogen peroxide to produce hydroxyl radical (OH^-), one of the most reactive molecules in the living cells. Hydroxyl radical can cause the peroxidation of membrane lipids, breakage of DNA strands, and inactivation of enzymes in cells (Chen and Pan, 1996).

H_2O_2 and O_2 can also give rise to more potent oxidants in the presence of ferric ion or Fe^{3+} chelates. HOCI is one such potent microbicidal agent and it reacts rapidly with ammonium (NH_4^+) to yield monochloramine. Because the concentration of nitrogen compounds is very high, HOCL has little opportunity to react directly with target cells. There is some (still controversial) evidence that human neutrophils produce

NO, although the effectiveness of this radical in killing microorganism is unclear (Verhoef, 1998; Shah *et al*., 2002). Macrophages are known to produce NO which in high concentration, acts as inflammatory mediator (Napolitano *et al*., 2005; Toshkova *et al*., 2006).

Polyphenol extract from Geranium sanguineum was shown to modulate the excessive production of oxygen radicals, hydrogen peroxide and NO in mice infected with influenza virus thereby alleviating disease symptoms and reducing mortality (Toshkova *et al*., 2006). Several crude extracts from Brazilian plants down modulated nitric oxide production in murine macrophages validating their use as anti-inflammatory agents (Napolitano *et al*., 2005).

2.1.9.5 Oxygen-independent killing mechanism

PMN granules contain additional antimicrobial agents that are released into phagolysosomes and do not require the production of oxidants for activity. These agents include proteases, other hydrolytic enzymes, such as phospholipases, glycosidases and lysozymes, and other proteins and peptides that disrupt microbial functions or structural components. These agents must bind to certain microbial cell surface for their antimicrobial activity. Three well-characterized granule components, bactericidal permeability increasing protein (BPI) and defensins, have shown microbicidal activity *In vitro* (Cullor *et al*., 1990). BPI is 58kDa protein of the primary (azurophil) granules and contributes to killing mainly gram-negative bacteria (Von Der Mohlen *et al*., 1996). Plant extracts and plant derived compounds have also been demonstrated to have an effect on the oxygen independent killing mechanisms

by enhancing the release of certain enzymes such as lysosomal and myeloperoxidase. Most of the microorganisms are digested after being killed and the numerous granule associated enzymes rapidly degrade their components. The degree of digestion of the engulfed organism depends on the structure of the bacterial cell wall and the digestive enzymes in the phagocyte (Verhoef, 1998). Certain anti-inflammatory and pro-inflammatory cytokines are also released in the presence of plant extracts and participate in the oxygen independent killing mechanism.

Cytokines

Cytokines are a group of low molecular weight regulatory proteins secreted by leucocytes and a variety of other cells, in response to a number of inducing stimuli. Cytokines in general act as immune "messenger molecules" that modulate, educate, stimulate, and regulate various aspects of the immune response by acting on cells. Cytokines bind to specific receptors on the surface of target cells. Cytokines that are secreted from lymphocytes are termed lymphokines, whereas those secreted by monocytes or macrophages are termed monokines. Many of the lymphokines are also known as interleukins (ILs), since they are not only secreted by leukocytes but also have an affect on leukocytes. Cytokines include interferons, colony stimulating factors and tumor necrosis factor (Kendall, 1990; Feldmann, 1992; Kuby, 1994). There is good evidence to suggest that the systemic immune response is at least partially controlled by a group of cytokines that allow the cellular component of this defense mechanism to communicate with each other and which are also capable of directing the nature of the response. Some of these molecules such as TNF-α is

predominantly synthesized and secreted by activated macrophages. On the other hand, IL-2, IL-4, IL-5, IL-10 and IFN-γ are produced by activated T-lymphocytes. These 2nd group of cytokines have now been classified into 2 subsets (Th1-type and Th2-type) based on the types of cells that produce them. Th1 cells secrete mostly IL-2 and IFN-γ, and Th2 cells secrete IL-3, IL-4, IL-5, IL-6 and IL-10 (Lyu and Park, 2005). Th1 cells are responsible for cell mediated immunity, phagocyte-dependent host response, cytotoxicity, and macrophage activation. IL-2 has effect on the several other immune cells, including natural killer cells, B-cell monocytes/macrophages and neutrophils (Lyu and Park 2005).

The major producers of the cytokines are the activated T-Helper ((Th-1 and Th-2) cells and macrophages. These cells are important in the development of cellular and humoral immune responses, induction of inflammatory reactions, and regulation of hematopoiesis (Feldmann, 1992; Kishimoto, 1992). IFN-gamma is one of the cytokine produced by these cells and whose main biological activity is immunomodulation. Nearly all cells express receptors for IFN-γ and respond to IFN-γ binding by increasing the surface expression of class II MHC proteins, thereby promoting the presentation of antigen to T-helper ($CD4^+$) cells (Heath *et al*., 1991; Hillman *et al*., 1994). IFN-γ has very important role in regulation of the phenotype of subsequent antigen-specific T-cell response (Heath *et al*., 1991). It enhances T-helper cell activity (Frasca et al., 1985), and it is one of the Th1-specific cytokines, which

promote Th1 responses and inhibit Th2 responses (Gajewski *et al*., 1988, Gajewski *et al*., 1989).

Interleukin-4 (IL-4) was first described as stimulating or growth factor of B cell (Coffman *et al*., 1986; Paul *et al*., 1987). In addition to regulation of B cell growth it also affects T cells. Their major roles include Th2 cell differentiation, immunoglobulin E (IgE) secretion and eosinophil recruitment (Smily and Grusby, 1998). In addition to Th lymphocytes, IL-4 may be derived from cytotoxic T cells, eosinophils, mast cells, and basophils (Moqbel *et al*., 1995). IL-4 is generally considered to favor humoral immunity (Mosmann and Coffman, 1989).

TNF-alpha is a potent cytokine produced by monocytes and macrophages as well as by other cells including T and B lymphocytes and fibroblasts in response to inflammation, infection and injury (Klein and Horejsi, 1997). It induces the expression of other autocrine growth factors, increases cellular responsiveness to growth factors and induces signaling pathways that lead to proliferation. Like other growth factors, TNF-α induces expression of a number of nuclear proto-oncogene as well as of several interleukins (Beutler and Cerani, 1989; Perez *et al*., 1990). Pro inflammatory cytokines such as TNF-α and IL-1β plays an important role in inflammatory diseases. Both TNF-α and IL-1β activate the inflammatory cell and induce the production of other inflammatory mediators that in turn modulate the important cellular events including gene expression, DNA damage and cellular

proliferation contributing to various inflammatory disorders. TNF- β (LT-α) is characterized by its ability to kill a number of different cell types. It enhances the phagocytic activity (Kuby, 1994).

Colony Stimulating Factors (CSFs) are cytokines that stimulate the proliferation of specific pluripotent stem cells of the bone marrow in adults. Granulocyte-CSF (G-CSF) is specific for proliferative effects on cells of the granulocyte lineage. Macrophage-CSF (M-CSF) is specific for cells of the macrophage lineage. Granulocyte-macrophage-CSF (GM-CSF) has proliferative effects on both classes of cells. Erythropoietin (EPO) is also considered a CSF as well as a growth factor, since it stimulates the proliferation of erythrocyte colony-forming units. IL-3 (secreted primarily from T-cells) and is also known as multi-CSF, since it stimulates stem cells to produce all forms of hematopoietic cells (Weisbart *et al.*, 1989). The well documented effects of GM-CSF on the immune response include, activation of macrophage (Gasson, 1991), increasing MHC class II antigen expression, enhancing dendritic cell maturation and migration (Fischer *et al.*, 1988). The immunostimulating function include inducing localized inflammation at the site of injection and exerting immunomodulatory effects by it's systemic impact on the hematopoietic cytokine net work (Lenz *et al.*, 1993).

Effect of some plant extracts on cytokine action

Bouic *et al*. (1996) demonstrated that beta sitosterol from Hypoxis spp caused a release of more growth factors specifically lL-2 and IFN- γ in their supernatants. Maruyama *et al*. (2008) showed that the expression of 1L-6R was increased in B cell surface following treatment with *C. salsa* extract. However, the cells treated with the extracts for a 7 day time period did not express up regulated IL-6R. These results suggested that the *C. salsa* extract induced activation is not as simple as mitogen induced activation which is mainly dependent on cell growth. *Eurphobia cheiradenia* significantly increased IL-2 secretion from lymphocyte (Amighofran *et al*., 2008). Saponins and acidic polysaccharides isolated from the roots of *Panax ginseng* were reported to stimulate the production of IL-1, IL-6, IL-12, TNF-α and TNF-β (Benny and Vanitha, 2004). *Ocimum basilicum* crude methanolic extract was shown to inhibit the key pro-inflammatory cytokines and mediators therefore accounting for its anti inflammatory effect (Selvakkumar *et al*., 2007). *Anoctochilus formosanus* fraction was found to significantly increase the IFN- γ (Th1-type) production but not IL-4(Th2-type). IFN-γ is known to be potent macrophage activator as well as immunomodulating agent (Lin and Hsieh, 2005). It was suggested that *A. formosanus* fraction activated macrophages by up regulating the synthesis and production of this cytokine. Pyconogenol isolated from the bark of French maritime pine was demonstrated to enhance secretion of TNF-α and IL-1 by macrophages (Shah *et al*., 2002). These two cytokines are known to provide stimulatory signals to enhance both the humoral and cellular immune response to promote host defense.

Investigations by Lyu and Park (2005) showed that some flavonoids repressed NO production and TNF-α secretion while others increased or decreased IL-2 secretion indicating the capacity of these flavonoids to modulate the immune response in addition to their anti inflammatory potential. Benny and Vanitha (2004) have reported that polysaccharides, saponins, flavonoids, peptides and glycoproteins are able to induce production of different types of cytokines.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

Male and female albino rats weighing between 140-210g used in the study were obtained from the animal house in the Department of Zoology, JKUAT. They were housed five per cage, maintained in an animal house under a 12:12-h light-dark cycle at a temperature of 25 ^{o}C and fed on rat pellet and tap water *ad libitum*.

3.2 Plant materials and their collection

Plant materials were collected from Gatundu [1°3‘0’S: 36°54‘0’E] located in Central province of Kenya during the month of January 2007. Gatundu is approximately 40km north of Nairobi. Aerial part and root of *Asparagus setaceous* (Plate 1) and the leaves, stem and root of *Caesalpinia volkensii* (Plate 2) were collected. The plants were identified in the herbarium, Department of Botany JKUAT, where voucher specimens were deposited. The plant materials were dried under shade at temperature below 30°C and pulverized in a hammer mill fitted with a sieve of 0.5mm pore

Plate 1: *Asparagus setaceous* plant

Plate 2: Stem and leaves of *Caesalpinia volkensii* Harm.

3.3 Preparation of Plant extracts

3.3.1 Preparation of organic extracts

The grounded plant material was extracted twice with organic solvents, hexane, dichloromethane, methanol and 90% ethanol at room temperature. Hundred grams of plant powder were extracted by mixing with 300 ml of the extracting solvent. The slurry of solvent and plant powder was stirred and left to stand for 48 hours, after which the supernatant was decanted. The decanted supernatants were filtered through Whatman® GF/C glass microfibre filter paper and the filtrate concentrated under vacuum at 40°C in Buchii rotary evaporator and dried in a freeze drier. The dry extracts were weighed and kept desiccated at 4°C until use.

3.3.2 Preparation of aqueous extracts

Plant powders weighing 300 grams were boiled for twenty minutes in 800 ml of distilled water. After cooling to room temperature, the supernatants were decanted, centrifuged at 5400× gravity for 10 minutes after which the supernatants were filtered through Whatman® GF/C glass microfibre filter paper, frozen at -15°C and then dried in a freeze- drier. The extracts were weighed and kept desiccated at 4°C.

The percentage yield of the extract was determined according to the expression provided by Banso and Adeyemo (2006).

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \times 100$$

3.4 Antimicrobial screening of *A. setaceous* and *C. volkensii* crude extracts on selected pathogens

Antibacterial and antifungal activities of the plant extracts of *A. setaceous* and *C. volkensii* were tested by disc-diffusion method. The minimal inhibition concentrations (MIC) of the extracts were determined. Five bacterial strains, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis* and the fungus *Candida albicans* were used in the study. The organisms were obtained from a culture collection maintained in the Department of Botany, JKUAT. The bacteria were tested for purity by culturing on nutrient agar and maintained on nutrient agar slants.

3.4.1 Preparation of inocula

Stock cultures were maintained on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller- Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi to reactivate them by culturing overnight at 37◦ C. Cultures were diluted with fresh MHB and SDB and compared with McFarland standard to achieve values corresponding to $2x10^6$ colony forming unit for bacteria, and $2x10^7$ spores/ ml for fungal strain.

3.4.2 Antibacterial activity of the extracts

Antibacterial activity of the plant extracts was tested by the disk diffusion method as described by Mbwambo *et al*. (2007). Five strains of bacteria were used, Gram negative *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus faecalis*, and Gram positive *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633). Filter paper discs (Whatman No.1) 6 mm diameter were impregnated with crude extracts (25mg/disc). Disks dipped into dimethylsulfoxide served as negative controls. All the bacteria were incubated at 30^0C for 24h by inoculation into nutrient broth. Sterilized petri dishes were inoculated with 0.01 ml of one of the above culture media (10^5 – 10^6 bacteria per ml). Mueller – Hinton agar sterilized in a flask and cooled to 45 – 50^0C was distributed by pipette (15ml) into each inoculated petri dish and swirled to distribute the medium homogenously. Disks injected with extracts were applied on the solid agar medium by pressing slightly. Standard antibiotic disks (ampicilin 25 µg, tetracycline (100 µg), nitrofurantoin (200 µg), nalidixic acid (30µg), streptomycin (25 µg), sulphamethoxale (200 µg), cotrimoxale (25 µg) and gentamicin (10 µg) were also included and tested for their antibacterial activity against the test micro organisms. The treated petri-dishes were placed at 4^0C for 1 – 2h and then incubated at 35^0C for 18 – 24h. The discs were tested in triplicate. At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

3.4.3 Screening of the extracts for anti-*Candida* activity

This was carried out as described by Runyoro *et al*. (2006) with modification. Sabouraud dextrose agar was used to prepare the culture medium according to the manufacturer's direction. Candida albicans (ATCC 90028) kept in the department of Botany, JKUAT, was aseptically inoculated on petri-dishes containing autoclaved, cooled and settled medium. The petri dishes were incubated at 31^0C for 48h to give white round colonies against a yellowish background. These were aseptically subcultured on SDA slants. *Candida albicans* colonies from SDA slants were suspended in sterilized 0.9% Sodium chloride solution (normal saline), which was compared with McFarland solution. The microbial suspension (1ml) in normal saline was added to 74ml of sterile medium, kept at 45^0C to give concentration of 2×10^7 cells/ml. Sterilized petri dishes (9cm diameter) were inoculated with 1ml of the above solution SDA sterilized in a flask and cooled. It was then distributed by pipette (15ml) into each inoculated petri dish and swirled to distribute the medium homogenously. Disks injected with extracts were applied on the solid Sabouraud dextrose agar medium by pressing slightly. The treated petri-dishes were placed at 4^0C for 1 – 2 h and then incubated for 48h at 37^0C. At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

3.4.4 Determination of the minimum inhibitory concentration (MIC)

Five concentrations of each extract (3.125, 6.25, 12.5, 25 and 50 mg/ml) were prepared. The antimicrobial effect of each concentration was measured. The various concentrations were loaded onto 6 mm disks which were then pressed onto already prepared Mueller-Hinton agar plates and SDA plates. The inoculated plates were incubated at 35°C for 48h. MICs were determined after 24h for the bacteria and after 48h for *C. albicans*. Zones of inhibition were measured at the end of the incubation period. The MICs were determined as the lowest concentrations of extracts inhibiting the visible growth of each organism on the agar plate.

3.4.5 Effects of selected plant extracts on the growth Kinetics of susceptible bacteria

In order to determine the bacteriostatic effect of selected plant extracts on the growth of bacteria, 1/10 of MICs of the active extracts were incorporated into the broth tubes containing selected susceptible bacteria culture adjusted with McFarland standard to give an OD reading of 0.05. The bacterial culture growing in presence or absence of extracts were incubated at 37°C. OD readings was taken at 0 minute, 30 minutes and then after each hour till 8 hours. Graph was plotted between OD percent values versus time.

3.4.6 Survival rate against *Staphylococcus aureus* exposure in rats following treatment with methanol, ethanol and aqueous extracts of *A. setaceous* and *C. volkensii*

Selected plant extracts were subjected to in vivo assay to see if they were able to protect rats exposed to *S. aureus*. Methanol, ethanol and aqueous extracts at a dose of 500mg/kg were orally administered to rats once daily for 3 days. Another group of rats was orally treated with gentamicin. On the third day, the rats were exposed to *Staphylococcus aureus* and the rate of mortality monitored for a month.

3.5 Assessment of immunomodulatory activity of the plant extracts

The ability of crude extracts of *A. setaceous* and *C. volkensii* to affect a number of immune functions was assessed in normal white albino rats. This included looking at the ability of extracts to affect total WBCs and differential WBCs, neutrophils and macrophage functions. The ability of extracts to reverse leucopenia induced by cyclophosphamide treatment in albino rats was also investigated.

3.5.1 Preparation of extracts for administration to rats

The dichloromethane, hexane, methanol, ethanol and aqueous extracts of *C. volkensii* leaf, stem and root, and *A. setaceous* aerial part and root were prepared by dissolving in dimethylsulfoxide. All the plant extracts were dissolved so that the final volume of the solutions did not exceed 1ml.

3.5.2 Experimental Groups and Treatment of the rats

Rats were divided into 27 groups of 5 individuals each with two groups serving as controls. The groups were categorized as follow: - Group 1 to 5 rats treated with

dichloromethane extracts of C. volkensii leaf, stem and root and A. setaceous aerial part and root respectively and coded as DCVL, DCVS, DCVR, DASA, and DASR. Groups 6 to 10 were treated with methanolic extracts and coded as MCVL, MCVS, MCVR, MASA and MASR while groups 11 to 15 were treated with ethanolic extracts of the two plant parts and coded as follow, ECVL, ECVS, ECVR, EASA and EASR. Groups 18 to 22 were treated with hexane extracts of the two plant parts (HCVL, HCVS, HCVR, HASA and HASR) whereas groups 23 to 27 were treated with aqueous extracts of C. volkensii and A. setaceous and coded as ACVL, ACVS, ACVR, AASA and AASR.

The 27 groups of experimental animals used in the study were first screened for immunomodulatory activity. Group 16 categorized as the dry control was not manipulated in any way while Group 17 which was taken as treated control was administered with the solvent used to dissolve the extracts, dimethylsulfoxide. All the other remaining groups were treated with the various crude extracts of *C. volkensii* and *A. setaceous*. The treated groups were dosed orally with the extracts at a dosage of 500mg/kg body weight for three consecutive days. The plant crude extracts were administered through intra gastric route using the stomach tube to ensure the safe ingestion of the extracts and the vehicle.

3.5.3 Bleeding of the rats

Blood was obtained from the tails of the rats. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails snipped with sterile scissors.

Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tip was sterilized again by swabbing with 70% ethanol.

3.5.4 Effect of crude extracts of the two plants on total and differential white blood cell counts in rats

Total white blood cell counts in tail blood of rats was determined by the method of Srikumar *et al*. (2005) using a Neubauer haemocytometer. Total white blood cell counts were conducted before, and after treatment of animals with the various crude extracts. Control groups in all cases were given physiological saline. The crude extracts were administered orally.

Differential WBC count was done as previously described by Monica (2000). Briefly, thin blood smears were made from rats before and after treatment of experimental animals with crude extract isolates of the two plants. The thin blood smears were fixed with absolute methanol, stained with Giemsa's stain and the stain flushed off with tap water and the slide left to dry. The cells were then observed under the compound microscope using oil immersion objective, 100 white blood cells were identified, counted and recorded. The results were given as mean of the percentage together with the standard errors.

3.5.5 Evaluation of the effect of the crude extracts on total WBC counts in cyclophosphamide treated rats

Animals were divided into the same groups as mentioned in section **3.5.2** above. Each group consisted of a minimum of 5 animals. Total WBC were conducted in these

groups of rats to establish their total WBC levels as previously described in section **3.5.4**. Leucopenia was then induced by treating the rats with 200 mg cyclophosphamide per kilogram body weight intra-peritoneally (Thatte et al., 1987). Once a drop in the total WBC level was ascertained, treatment with the crude extracts was conducted for a total of three days. Total WBC counts were then conducted to determine if there was reversal of the leucopenic effect of cyclophosphamide. Control groups in all cases received physiological saline.

3.6 Evaluation of the effect of crude extracts and fraction isolated from the plants on neutrophils and macrophages activity

3.6.1 Neutrophil Adherence

This was carried out as described by Thakur *et al*. (2006). Briefly, neutrophil adherence was analyzed by the initial count of TLC and DLC from the blood sample. After initial count, blood sample was incubated in sterile nylon fiber column (80mg/ml) packed in a silicanazed Pasteur pipette (column length 15 mm). After 30 minutes of incubation, blood sample was again analyzed for TLC and DLC. The product of TLC and percentage of neutrophil gave the neutrophil index (NI) of blood sample. Percentage of neutrophil adherence was calculated according to the formula described by Thakur *et al*. (2006).

(Neutrophil index of untreated blood samples) – (neutrophil index of treated blood sample) / (neutrophil index of untreated blood samples) x100 gave the percentage of neutrophil adherence.

3.6.2 Candida Phagocytosis

This was carried out as described by Srikumar *et al*. (2005). Briefly, the phagocytic ability of neutrophil was assessed by separating the buffy coat from the blood sample. To this, the incubating medium, 0.1 ml of inactivated fetal calf serum and 0.1ml of heat killed *Candida albicans* (2×10^8cell/ml) was added and incubated for 30 minutes at 37° C, followed by centrifugation. From the sediment, thin smears were made and stained with Leishman's stain. The number of neutrophils positive for *Candida* in 100 cells gave phagocytic index (PI).

3.6.3 Nitroblue Tetrazolium (NBT) reduction Test

The killing ability of the neutrophils was assessed by nitroblue tetrazolium reduction test (NBT) as described by Srikumar *et al*. (2005). Briefly, the blood sample was incubated for 30 minutes at 37° C in a clean glass slide. After incubation, the slide was gently washed with cold saline to remove other cell populations. To this, NBT medium and 0.2ml of inactivated fetal calf serum was added and incubated for 30 minutes at 37° C. After incubation, slide was washed with cold saline and stained with safranin. When NBT was phagosomed by the cells, intracellular dye was converted it into an insoluble blue crystalline form (formazon crystals). One hundred cells were observed and the positive cells with the formazon granules counted.

3.7 Time and Dose dependent relationship of the most active plant extracts of *A. setaceous* and *C. volkensii* with immunomodulatory activity

Further tests were conducted with plant extracts showing immunomodulatory activity. Aqueous, ethanolic and methanolic extracts aerial part and root of *A. setaceous* and leaf, stem and root of *C. volkensii* were chosen for experiments as they were showing activity as assessed by the various immunomodulatory tests. Different concentrations of the extracts (125, 250, 500 and 1000mg/kg) were administered orally in rats and the various immunomodulatory tests evaluated as previously described in sections **3.5.4., 3.5.5., 3.6.1., 3.6.2., 3.6.3**.

This involved evaluating the time taken for the aqueous, ethanolic and methanolic extracts of the plants to reverse experimentally induced leucopenia and establishing if the extracts protected pretreated rats from the leucopenic effect of cyclophosphamide treatment. Other tests carried out included investigating the effect of various doses of the plant extracts on nitroblue tetrazolium reduction test, phagocytic index, neutrophil adherence test following oral administration in white albino rats. In addition, phagocytic activity of elicited peritoneal macrophages and delayed type hypersensitivity tests were evaluated in rats to determine how the extracts affected functions of the macrophage as well as cellular immunity.

3.7.1. Relationship between Time and Activity of Aqueous, methanolic and ethanolic extracts on leucopenic rats

This was done as previously described in section **3.5.5**. WBC counts were first conducted in animals which were then placed into different groups each comprising of 5 animals. Animals were then injected with cyclophosphamide and left for three days and another WBCs count done. Different groups were then injected with 500mg/kg of the extracts. Further WBCs count was then performed at days 3, 6, 9, 12 and 15. To evaluate the protective effect of the plant extracts, rats were first pretreated with the plant extracts for 3 days after carrying out the initial WBCs counts. Following this, more WBCs counts were performed at days 3, 6, 9, 12. The rats were injected with 200mg cyclophosphamide per kilogram body weight intra-peritoneally on the fourth day following extracts treatment. In addition, the crude extracts were evaluated on how they compared with lithium carbonate.

3.7.2 Dose-dependent relationship of the aqueous, ethanol and methanol plant extracts in the neutrophil adhesion assay

Animals were put into fifteen groups, each of five animals and given various doses (125mg/kg, 250mg/kg, 500mg/kg, and 1000mg/kg) of the aqueous, ethanol and methanol plant extracts of aerial part and root of *A. setaceous* and the leaf, stem and root of *C. volkensii*. Neutrophil adhesion test was then carried out as previously described in section **3.6.1** above.

3.7.3 Dose-dependent relationship of the aqueous, ethanol and methanol plant extracts in the Phagocytic index

Animals were put into fifteen groups, each of five animals and given various doses (125mg/kg, 250mg/kg, 500mg/kg, and 1000mg/kg) of the aqueous, ethanol and methanol plant extracts of aerial part and root of *A. setaceous* and the leaf, stem and root of *C. volkensii*. Phagocytic test was then carried out as previously described in section **3.6.2** above.

3.7.4 Dose-dependent relationship of the aqueous, ethanol and methanol plant extracts in the NBT reduction test

Animals were put into fifteen groups each of five animals and given various doses (125mg/kg, 250mg/kg, 500mg/kg, and 1000mg/kg) of the aqueous, ethanol and methanol plant extracts of aerial part and root of *A. setaceous* and the leaf, stem and root of *C. volkensii*. NBT reduction test was then carried out as previously described in section **3.6.3** above.

3.7.5 Determination of phagocytic activity of peritoneal macrophages *In vitro*

Animals were put into various groups and then phagocytic activity was measured as described by Ottendorfer *et al*. (1994). Elicited peritoneal macrophages ($1x10^6$ml) were prepared by injecting the animals with 1ml sterile paraffin oil, intraperitoneally and leaving them for several hours. After harvesting, cells were allowed to adhere for

a few hours in fibronectin-coated flat bottomed microwell plates to minimize cell loss during washing procedure. They were then washed and incubated overnight with various doses (125mg/kg, 250mg/kg, 500mg/kg, and 1000mg/kg) of the aqueous, ethanol and methanol plant extracts of aerial part and root of *A. setaceous* and the leaf, stem and root of *C. volkensii*. Control cells received only complete cell culture medium. After 16 hours, the cells were washed and 10^7 sheep red blood cells (opsonized with anti-forsman-antibody, final dilution 1:500) were added for a short period of time. After vigorously washing, the residual sheep red blood cells were eliminated by short treatment (3 to 5 seconds) with erythrocyte lysis buffer. The number of ingested erythrocytes was determined by spectrophotometric determination of hemoglobin and readings were taken at 550nm.

3.7.6 Dose-dependent relationship of the aqueous, ethanol and methanol plant extracts in the delayed type hypersensitivity using SRBC as an antigen

This was done using the method of Thakur *et al*. (2006). Treatment of all groups with extracts began 3 days before challenge. Animals were put into fifteen groups and given various doses (125mg/kg, 250mg/kg, 500mg/kg, and 1000mg/kg) of the aqueous, ethanol and methanol plant extracts of aerial part and root of *A. setaceous* and the leaf, stem and root of *C. volkensii*. All the groups were immunized by injecting 20µl of 5×10^9 SRBC per ml subcutaneously into the right foot pad. After 7 days of treatment the thickness of left foot pad was measured using calipers. The mice were then challenged by injecting 20µl of 5×10^9 SRBC per ml intradermally on

the left hind foot pad (time zero). Foot thickness was measured before and after 48 h of challenge. The difference between the thickness of left foot just before and after challenge in mm was taken as a measure of immunogen- elicited delayed type hypersensitivity.

3.8 Phytochemical screening of extracts

3.8.1 Testing for presence of compounds present in plant extracts

The methods described by Akinyemi *et al*., (2005) and Banso and Adeyemo (2006) were used to test for the presence of alkaloids, flavonoids, sterols and steroids, saponins and tannins.

3.8.1.1 Determination of alkaloids

Extract of each plant sample was separately stirred with 1% hydrochloric acid (HCL) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drop of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

3.8.1.2 Determination of flavonoids

To 1 ml of each plant extract in a test tube was added a small piece of magnesium ribbon followed by drop wise addition of concentrated hydrochloric acid. Development of pink or magenta red colors indicated the presence of flavonoids.

3.8.1.3 Determination of sterols and steroids

One millitre of extract was put into a test tube in which 0.5 ml sulfuric acid, acetic anhydride and chloroform in similar amount was added. A red coloration indicated presence of sterols while a green color indicated presence of steroids.

3.8.1.4 Determination of saponins

One millilitre of each extract under test was put into a test tube and 50 ml of tap water added. The mixture was then shaken vigorously. Foaming which persisted on warming was taken as an evidence for the presence of saponins but this was subjected to further confirmatory test. This involved dissolving 1ml of the extract in carbon tetrachloride to which 4 drops of concentrated sulphuric acid was added to the mixture. A blue, green, or red color accompanied by a pink ring confirmed presence of saponins.

3.8.1.5 Determination of Tannins

Ethanolic extract of each sample was separately stirred with 10 ml of distilled water and then filtered. To the filtrate was added two drops of 5% iron III chloride ($FeCl_3$) reagent. Blue- Black or blue green coloration was taken as an indication of the presence of tannins.

3.9 Statistical Analysis

Data were statistically analyzed using one way ANOVA followed by Dunn/Bonferonni multiple comparison tests for those showing difference. t- Student test for paired samples was used to analyze data on differential WBC and total WBC counts following cyclophosphamide treatment. The level of significance was fixed at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Yields from the various plant parts of *A. setaceous* and *C. volkensii* extracted with non polar and polar solvents

The plants studied gave aqueous, methanolic, ethanolic, dichloromethane and hexane extracts with yields ranging from 0.8 to 11 % (Table 4.1). The highest yields were obtained with ethanol, methanol and aqueous extracts (4.5% - 11.6%). Lower yields were obtained with dichloromethane and hexane (0.8% - 4.2%).

Table 4.1: Percentage yields of extracts from aerial part and root of *Asparagus setaceous* and leaf, stem and root of *C. volkensii*

Plant Parts	Solvent of extraction				
	Dichloromethane	Hexane	Ethanol	Methanol	Aqueous
C. volkensii Leaf	4.2	2.1	5.8	9.1	6.4
C. volkensii stem	3.95	1.99	4.5	6.4	7.0
C. volkensii root	2.6	1.9	7.3	4.6	9.1
A. setaceous aerial part	3.3	1.7	11.6	5.2	10
A. setaceous root	1.4	0.8	4.8	7.5	11

4.2 The antimicrobial effects of *A. setaceous* and *C. volkensii* extracts

The results of the antimicrobial effects of the different parts of the two plant extracts against the six microorganisms tested are shown in table 4.2. All microbial strains tested were found to be affected by dichloromethane extracts from *C. volkensii* leaf as assessed by inhibition zones that ranged from 1.6 mm to 4 mm. The extract showed antibacterial activity against both Gram positive and negative bacteria and also demonstrated antifungal activity against *C. albicans* (inhibition zone 2.7 mm). Dichloromethane extracts from *C. volkensii* stem was equally active against Gram positive *S. aureus* and *B. subtilis* with inhibition zone of 3 mm. In addition the extract showed antibacterial activity against *S. faecalis* and *E. coli* (inhibition zone 4 mm and 2 mm respectively) but lacked activity against *P. aeruginosa*. Furthermore the extract did not show any antifungal activity against *C. albicans*. Dichloromethane extracts from *C. volkensii* root demonstrated antibacterial activity against both Gram positive and Gram negative bacteria exhibiting inhibition zones ranging from 2 mm to 5 mm being more active against *B. subtilis* and *S. aureus* (inhibition zone 5mm and 4mm respectively). Amongst all the extracts tested in this study dichloromethane extracts from *C. volkensii* root demonstrated the highest antifungal activity with an inhibition zone of 4.3mm. Dichloromethane extracts from *A. setaceous* aerial part showed slight antibacterial activity against B. subtilis, *S. faecalis* and *E. coli* (inhibition zone 0.67 mm to 2 mm) and antifungal activity against the fungus *C. albicans* (inhibition zone 2.3 mm). Dichloromethane extracts from *A. setaceous* root demonstrated antibacterial activity against *S. faecalis* (inhibition zone 5.67 mm), *B. subtilis* (inhibition zone 4.33

mm) and *E. coli* (inhibition zone 2 mm) but completely lacked activity against *S. aureus* and *P. aeruginosa*. Dichloromethane extracts from *A. setaceous* root showed minimal antifungal activity against *C. albicans* (inhibition zone 1.33 mm).

Methanolic extract from *C. volkensii* leaf was active against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *S. faecalis* with inhibition zones ranging from 2 mm to 4 mm. The extract was equally active against *B. subtilis*, *P. aeruginosa* and *S. faecalis* (inhibition zone 4 mm) but completely lacked activity against *E. coli* (inhibition zone 0 mm) while it showed minimal activity against *C. albicans* (inhibition zone 1.33 mm). Methanolic extract from *C. volkensii* stem was active against all the tested microorganisms with inhibition zones ranging from 1.33 mm to 4 mm. The extract was equally active against *B. subtilis* and *S. faecalis* (inhibition zone 4 mm), less active against *E. coli* (inhibition zone 2.33 mm) and showed minimal activity against *S. aureus* and *P. aeruginosa* (inhibition zone 1.33 mm). Methanolic extract from *C. volkensii* leaf also had antifungal activity against *C. albicans* (inhibition zone 2 mm). Methanolic extract from *C. volkensii* root also demonstrated antibacterial activity against all the test microbes used in this study with inhibition zones ranging from 1.3 mm to 5 mm. The highest antibacterial activity was observed with *B. subtilis* (inhibition zone 5 mm) while the lowest activity was seen with *S. aureus* and *P. aeruginosa* (inhibition zone 1.33 mm). The extract also demonstrated antifungal activity against *C. albicans* (inhibition zone 1.67 mm). Methanolic extract from *A. seta*ceous aerial part showed activity against all the test microbes (inhibition zones

1.3 mm to 3.3 mm). The highest antibacterial activity was seen with *P. aeruginosa* (inhibition zone 3.3 mm) and was equally active against E. *coli*, *B. subtilis* and *S. faecalis* (inhibition zone 2.7 mm) while being less active against *S. aureus* (inhibition zone 1.3 mm). Methanolic extract from *A. setaceous* aerial part had less antifungal activity against *C. albicans* (inhibition zone 1.3 mm). Methanolic extract of *A. setaceous* root demonstrated antimicrobial activity against the tested microbes with the exception of *E. coli*. Zones of inhibition ranged from 1.33 mm to 5.33 mm. The highest antibacterial activity of the extract was observed against *B. subtilis* (inhibition zone 5.33 mm) and *S. faecalis* (inhibition zone 4 mm), was less active against *S. aureus* (inhibition zone 2 mm) and *P. aeruginosa* (inhibition zone 1.67 mm) and showed less antifungal activity against *C. albicans* (inhibition zone 1.33 mm).

The ethanolic extracts from *C. volkensii* leaf demonstrated antimicrobial activity against all the test microbes used in this study with the exception of *P. aeruginosa*. Inhibition zones ranged from 1 mm to 3.3 mm. The highest antibacterial activity was observed against Gram negative *S. faecalis* (inhibition zone 3.3 mm) followed by *B. subtilis* (inhibition zone 2.67 mm) and *E. coli* (inhibition zone 2 mm). Minimal activity against *S. aureus* (inhibition zone 1 mm) and *C. albicans* (inhibition zone 1.67 mm) was observed. The ethanolic extract from *C. volkensii* stem was active against most microbes as indicated by formation of inhibition zones that ranged from 1.33 mm to 5.33 mm. Highest antibacterial activity was seen with *B. subtilis* and *S. faecalis* (inhibition zone 5.33 mm) while minimal activity against *E. coli*, *S. aureus*

and *C. albicans* (inhibition zones 1.67 mm, 1.33 mm and 1.33 mm respectively) was observed. The ethanolic extract from *C. volkensii* root demonstrated activity against all the test microbes with inhibition zones ranging from 0.67 mm to 5 mm. The extract was more active against *S. faecalis* (inhibition zone 5 mm) and *B. subtilis* (inhibition zone 4.33 mm) followed by *E. coli* (inhibition zone 2.67 mm). The extract showed minimal antibacterial activity against *P. aeruginosa* (inhibition zone 1.33 mm) and *S. aureus* (inhibition zone 0.67 mm). The extract also showed minimal antifungal activity against *C. albicans*. The ethanolic extract from *A. setaceous* aerial part was active against most of the test microbes except against *P. aeruginosa* with inhibition zones ranging from 1.33 mm to 6.33 mm. The extract had highest antibacterial activity against *E. coli* (inhibition zone 6.33 mm) followed by *S. faecalis* (inhibition zone 3 mm), had minimal activity against *S. aureus* (inhibition zone 1.67 mm) and *B. subtilis* (inhibition zone 1.33 mm), and also showed minimal activity against *C. albicans* (inhibition zone 2 mm). Ethanolic extract from *A. setaceous* root was equally active against most of the test microbes with the exception of *P. aeruginosa*. The highest antibacterial activity was observed against *B. subtilis* (inhibition zone 8.33 mm) and *S. faecalis* (inhibition zone 3.67 mm), showed minimal activity against *S. aureus* (inhibition zone 2.33 mm) and *E. coli* (inhibition zone 1.67 mm) and had minimal antifungal activity against *C. albicans* (1.33 mm).

The hexane extracts from *C. volkensii* and *A. setaceous* exhibited antibacterial and antifungal activity against all the test microbes with inhibition zones ranging from 1

mm to 6.67 mm. The hexane extracts from *C. volkensii* leaf was active against all the microbes tested with inhibition zones ranging from 1 mm to 4 mm. Highest antibacterial activity was observed against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. faecalis* with all showing an inhibition zone of 4 mm. The extract showed minimal antibacterial activity against *S. aureus* (inhibition zone 1 mm). The extract demonstrated antifungal activity against *C. albicans* (inhibition zone 3 mm). The hexane extracts of *C. volkensii* stem showed varying degree of antimicrobial activity with its highest antibacterial activity observed against *B. subtilis* (inhibition zone 5.33 mm) and *S. faecalis* (inhibition zone 4.33 mm). The hexane extracts from *C. volkensii* stem had less antibacterial activity against *E. coli* (inhibition zone 0.33 mm), *S. aureus* (inhibition zone 1.33 mm) and *P. aeruginosa* (inhibition zone 1.67 mm) and also had less antifungal activity against *C. albicans* (inhibition zone 1 mm). The hexane extracts from *C. volkensii* root was active against all the test microbes with inhibition zones ranging between 1.33 mm to 6 mm. The extract was more active against *B. subtilis* (inhibition zone 6 mm) and *S. faecalis* (inhibition zone 5.33 mm). It showed less antibacterial activity against *C. albicans* (inhibition zone 1.33 mm). The hexane extracts of *A. setaceous* aerial part demonstrated activity against all the test microbes with inhibition zones ranging from 1.33 mm to 3.6 mm. The extract was equally active against *B. subtilis* and *S. faecalis* (inhibition zones 3.33 mm and 3.6 mm respectively). It was less active against *P. aeruginosa* (inhibition zone 2.33 mm), *S. aureus* (inhibition zone 1.6 mm) and *E. coli* (inhibition zone 1.33 mm). It also had antifungal activity against *C. albicans* (inhibition zone 2 mm). The hexane extracts

from *A. setaceous* aerial root was active against all the tested microbial strains giving inhibition zones ranging between 1.33 mm to 6.67 mm. The highest antibacterial activity of the extract was observed against *B. subtilis* (inhibition zone 6.67 mm) and *S. faecalis* (inhibition zone 5.67 mm). It showed less antibacterial activity against *E. coli* (inhibition zone 2.33 mm), *S. aureus* (inhibition zone 1.33 mm) and *P. aeruginosa* (inhibition zone 1.33 mm). It also had less antifungal activity against *C. albicans* (inhibition zone 2 mm).

From the result of the antibacterial and antifungal activity of the plant extracts presented in table 4.2, the aqueous extracts were generally found to be inactive against most of the test microbes. All the aqueous extracts lacked antifungal activity against *C. albicans* and antibacterial activity against *S. faecalis*. The aqueous leaf extract of *C. volkensii* was only slightly active against *S. aureus* while aqueous stem extract showed slight activity against *S. aureus* and *B. subtilis* but were largely inactive against the rest. The aqueous root extracts of *C. volkensii* was slightly active against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa*. Aerial part of *A. setaceous* extract showed minimal activity against *S. aureus*, *B. subtilis* and *P. aeruginosa* while root of *A. setaceous* aqueous extract also showed slight activity against *S. aureus* and *B. subtilis* only. *S. aureus* was the most susceptible microorganism to aqueous extracts followed by *B. subtilis*. The most resistant microbes to aqueous extracts were *C. albicans*, *S. faecalis*, *P. aeruginosa* and *E. coli.*

Table 4. 2 Antimicrobial effects of the various parts of the plant extracted with different solvents. All values are expressed as mean inhibition zones (mm) ± SEM of three replicates

Group	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
DCVL	2.67±0.33	1.67±0.33	4±0.58	1.67±0.33	2±0.58	2.67±0.33
DCVS	2±0.58	3.33±0.88	3.33±0.33	-	4±0.58	-
DCVR	3.33±0.67	4±0.58	5±0.58	2.33±0.33	2±0.00	4.33±0.33
DASA	0.67±0.33	-	2±0.58	-	1.67±0.33	2.33±0.33
DASR	2±0.58	-	4.33±0.33	-	5.67±0.33	1.33±0.33
MCVL	-	2±0.58	4±0.58	4±0.58	4±0.58	1.33±0.33
MCVS	2.33±0.33	1.33±0.33	4±1.00	1.33±0.33	4.33±0.33	2±0.58
MCVR	2±0.58	1.33±0.33	5±0.58	1.33±0.33	3.33±0.67	1.67±0.33
MASA	2.7±0.58	1.3±0.58	2.7±0.58	3.3±0.58	2.7±0.33	1.3±0.33
MASR	-	2±1.15	5.33±0.33	1.67±0.67	4±0.58	1.33±0.33
ECVL	2±0.58	1±0.00	2.67±0.67	-	3.33±0.33	1.67±0.33
ECVS	1.67±0.33	1.33±0.33	5.33±0.67	-	5.33±0.33	1.33±0.33
ECVR	2.67±0.33	0.67±0.33	4.33±0.67	1.33±0.33	5±0.58	0.67±0.33
EASA	6.33±0.33	1.67±0.33	1.33±0.33	-	3±0.00	2±0.58
EASR	1.67±0.33	2.33±0.33	8.33±0.33	-	3.67±0.33	1.33±0.33
HCVL	4±0.58	1±0.00	4±0.58	4±0.58	4±0.00	3±0.58
HCVS	0.33±0.33	1.33±0.33	5.33±0.33	1.67±0.33	4.33±0.33	1±0.00
HCVR	1.67±0.33	1.67±0.33	6±0.58	2.33±0.33	5.33±0.33	1.33±0.33
HASA	1.33±0.33	1.6±0.33	3.33±0.33	2.33±0.33	3.6±0.67	2±0.58
HASR	2.33±0.33	1.33±0.33	6.67±0.33	1.33±0.33	5.67±0.33	2±0.58
ACVL	-	2.6±0.33	2.33±0.33	-	-	-
ACVS	-	1.67±0.33	-	-	-	-
ACVR	2.33±0.33	2±0.58	1.67±0.33	1.67±0.33	-	-
AASA	-	2.33±0.33	1.33±0.33	1±0.00	-	-
AASR	-	3.33±0.33	1.67±0.33	-	-	-

Footnote: - Sign indicate lack of antimicrobial activity by extract against test organism

4.2.1 The antimicrobial effects of standard antibiotic drugs

According to the data presented in table 4.3 most of the tested microbial strains were resistant to the effect of ampicilin. The antibiotic lacked any activity against *S. aureus*, *P. aeruginosa* and *C. albicans*. Ampicilin exhibited its highest antibacterial activity against *S. faecalis* (inhibition zone 7.7 mm) and *E. coli* (inhibition zone 6.3 mm) but had the lowest antibacterial activity against *B. subtilis* (inhibition zone 2.3 mm). Tetracycline was observed to be active against all the tested microbes showing its highest antibacterial activity against *B. subtilis* (inhibition zone 24mm), *S. faecalis* (inhibition zone 22 mm), *S. aureus* (inhibition zone 18.4 mm) and *E. coli* (inhibition zone 17.3 mm). Its lowest antibacterial effect was seen with *P. aeruginosa* which gave a zone of inhibition of 7mm. Nitrofurantoin displayed greater antibacterial activity against all bacterial strain but completely lacked antifungal activity against *C. albicans*. The highest antibacterial activity of nitrofurantoin was observed against *S. faecalis* (inhibition zone 14.4 mm), *E. coli* (inhibition zone 11.6 mm) and *B. subtilis* (inhibition zone 10.4 mm) and was almost equally effective against *S. aureus* and *P. aeruginosa* (inhibition zones of 5.37 mm and 5.93mm respectively). Nalidixic acid also demonstrated antibacterial effect against all the bacterial strains tested with inhibition zones ranging from 10.2 mm to 23 mm and was very active against *E. coli* (inhibition zone 23.1 mm), *S. aureus* (inhibition zone 21.4 mm) and *P. aeruginosa* (inhibition zone 22.4 mm). Nalidixic displayed very good antibacterial activity against *B. sub*tilis (inhibition zone 15.2 mm) and *S. faecalis* (inhibition zone 10.2

mm). The antibiotics completely lacked any form of antifungal activity against *C. albicans*.

Streptomycin showed antimicrobial activity against all the tested microbial strains. The highest antimicrobial activity was observed against *B. subtilis* (inhibition zone 16.3 mm) and *E. coli* (inhibition zone 12.9 mm). Streptomycin also showed activity against *P. aeruginosa* (inhibition zone 8.2 mm), *S. aureus* (inhibition zone 7.9 mm) and *S. faecalis* (inhibition zone 7.5 mm). It was one of the three standard antibiotics among the tested antibiotics that demonstrated antifungal activity against *C. albicans* giving an inhibition zone of 5.8 mm. Sulphamethoxale was observed to display antibacterial activity against *E. coli* (inhibition zone 15 mm) and *S. faecalis* (inhibition zone 4 mm) while lacked activity against the rest of the microbes. A similar pattern was observed with the antibiotic cotrimoxale which also had activity only against *E. coli* (inhibition zone 23 mm) and *S. faecalis* (inhibition zone 9.7 mm). Gentamicin was observed to possess antimicrobial activity against all the tested microbes and was active against most microbes with inhibition zones greater than 12 mm. The highest antibacterial activity of gentamicin was observed against *S. faecalis* (inhibition zone 23.9 mm) and the lowest with *P. aeruginosa* (inhibition zone 12.5 mm). Gentamicin had the greatest antifungal activity amongst the three antibiotics showing antifungal activity (inhibition zone 16 mm).

Tetracycline, streptomycin and gentamicin had broad spectrum activity acting against both the Gram positive and negative bacteria in addition to possessing antifungal activity against the fungus *C. albicans*. Nitrofurantoin and nalidixic also possessed broad spectrum activity against both the gram positive and gram negative bacteria but lacked antifungal activity against the fungus *C. albicans*. Sulphamethoxale and cotrimoxale were inactive against most microbes being only active against *E. coli* and *S. faecalis*.

Table 4. 3: Antimicrobial activity of standard antibiotics against test microbes. The results are expressed as mean inhibition zones ± SEM of triplicates

	E. coli	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***S. faecalis***	***C. albicans***
A	6.3±0.17	-	2.3±0.17	-	7.7±0.06	-
T	17.3±0.17	18.43±0.09	24±1.15	7.07±0.18	22±0.23	5.07±0.07
NF	11.6±0.21	5.37±0.12	10.47±0.23	5.93±0.07	14.4±0.21	-
NA	23.13±0.24	21.43±0.22	15.2±0.12	22.47±0.09	10.2±0.12	-
S	12.9±0.21	7.93±0.15	16.33±0.09	8.23±0.15	7.57±0.15	5.87±0.09
SX	15±0.12	-	-	-	4.03±0.32	-
Co	23.03±0.15	-	-	-	9.7±0.12	-
G	15.3±0.21	14.43±0.18	14.3±0.21	12.5±0.12	23.9±0.21	16.33±0.2

KEY: A= Ampicilin 25µg, T= Tetracycline (100µg), NF= Nitrofurantoin (200µg), NA= Nalidixic acid (30µg), S= Streptomycin (25µg), SX= Sulphamethoxale (200µg), Co= Cotrimoxale (25µg) and G=Gentamicin (10µg).

4.2.2 The minimum inhibitory concentrations of *C. volkensii* and *A. setaceous* extracts against test microbes

MIC was taken to be the lowest concentration of the extracts that completely inhibited the growth of the microorganism. Data are reported as mean inhibition zones (mm) ± SEM of triplicates. Dichloromethane leaf extracts of *C. volkensii* had a MIC value ranging from 12.5 mg/ml to 25 mg/ml. With *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans* it had a MIC of 12.5 mg/ml while with *P. aeruginosa* and *S. faecalis* had MIC of 25 mg/ml. The dichloromethane extracts of *C. volkensii* stem showed MIC ranging from 12.5mg/ml for *S. aureus* and *B. subtilis* to 25 mg/ml for *E. coli*, *S. faecalis* and *C. albicans*. The extracts were largely inactive against *P. aeruginosa* at all the concentrations tested. The dichloromethane extracts of *C. volkensii* root were active at most of the concentrations tested with MIC ranging from 3.2 mg/ml against *E. coli*, 6.25 mg/ml for *S. aureus* to 12.5 mg/ml against *B. subtilis*, *P. aeruginosa* and *C. albicans* to 25 mg/ml for *S. faecalis*. Dichloromethane extract of aerial part of *A. setaceous* had high MIC ranging from 25 mg/ml to 50 mg/ml with MIC of 25 mg/ml for *E. coli*, *B. subtilis*, *S. faecalis* and *C. albicans*. The extracts lacked activity against *S. aureus* even at the highest concentration whereas it was only active against *P. aeruginosa* at the highest concentration. Dichloromethane root extract of *A. setaceous* had MIC of between 6.25 mg/ml and 50 mg/ml. The MIC was 6.25 mg/ml for *E. coli* and *B. subtilis*, 12.5 mg/ml for *S. faecalis*, 25 mg/ml for *P. aeruginosa* and *C. albicans*. The MIC for *S. aureus* was 50 mg/ml (Table 4.4).

Table 4. 4: Minimum Inhibitory concentration of dichloromethane extracts of *C. volkensii* and *A. setaceous* against test microbes

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
DCVL	50	4.67±0.33	2.67±0.33	4.33±0.33	2.33±0.33	1.67±0.33	2.67±0.33
	25	3±0.58	3±0.00	2±0.58	1.67±0.33	1.33±0.33	2.33±0.33
	12.5	1.33±0.33	1.33±0.33	1.67±0.33	-	-	1.33±0.33
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
DCVS	50	4±0.58	3.33±0.33	4.33±0.33	-	4±0.58	2.33±0.33
	25	2±0.58	2.33±0.33	3.33±0.33	-	2±0.58	1.33±0.33
	12.5	-	1.33±0.33	1.33±0.33	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
DCVR	50	5.67±0.33	4.67±0.33	5.33±0.67	5.33±0.33	3.33±0.33	4.33±0.33
	25	3.67±0.33	3.67±67	5±0.58	3.33±0.33	1.67±0.33	2.33±0.33
	12.5	2.67	2.33±0.33	2±0.58	2.33±0.33	-	1.33±0.33
	6.25	1.33±0.33	1.33±0.33	-	-	-	-
	3.2	1±0.00	-	-	-	-	-
DASA	50	1.33±0.33	-	4±0.58	2±0.58	2.33±0.33	3.33±0.33
	25	1±0.00	-	2±0.58	-	1.33±0.00	2.33±0.33
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
DASR	50	2.67±0.33	1.33±0.58	7.33±0.33	3.33±0.33	5.67±0.33	1.33±0.33
	25	2±0.58	-	5±0.58	2.33±0.33	5±0.58	1±0.00
	12.5	1±0.00	-	2±0.58	-	2.33±0.33	-
	6.25	1±0.00	-	1.33±0.33	-	-	-
	3.2	-	-	-	-	-	-

For the methanolic extracts, the *C. volkensii* leaf had MIC ranging from 12.5 to 50 mg/ml with MIC of 12.5 mg/ml for *B. subtilis* and 25 mg/ml for *S. aureus*, *P. aeruginosa*, *S. faecalis* and *C. albicans*. The extract was only active against *E. coli* at the highest concentration tested (50 mg/ml). The MIC ranged from 6.25 to 25 mg/ml for the methanolic extract of *C. volkensii* stem. The extract had MIC of 6.25 mg/ml for *S. aureus* and *B. subtilis* and 25 mg/ml for *E. coli*, *P. aeruginosa*, *S. faecalis* and

C. albicans. *C. volkensii* root extracts MIC ranged from 6.25 to 25 mg/ml with low MIC for *B. subtilis* at 6.25 mg/ml, and 12.5 and 25 mg/ml for *S. aureus*, *P. aeruginosa* and *E. coli*, *S. faecalis* and *C. albicans* respectively. Methanolic extract of *A. setaceous* aerial parts had MIC of 6.25 mg/ml for *B. subtilis*, 12.5 mg/ml for *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*. For *S. faecalis*, it was 25 mg/ml. *A. setaceous* root extract; *B. subtilis* had a low MIC of 6.25 mg/ml followed by 12.5 mg/ml for *S. aureus* and 25 mg/ml for *P. aeruginosa*, *S. faecalis* and *C. albicans*. The root ethanolic extract lacked activity against *E. coli* even at the highest concentration tested (Table 4.5)

Table 4. 5: Minimum Inhibitory concentration of methanolic extracts of *C. volkensii* and *A. setaceous* against the test micobes. Values are reported as mean inhibition zones (mm) ± SEM of triplicates

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
MCVL	50	2±0.58	2.33±0.33	4±0.58	3.67±67	4±0.58	2.33±0.33
	25	-	1.33±0.33	2.67±0.33	2±0.58	1.67±0.33	1.33±0.33
	12.5	-	-	1.33±0.33	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
MCVS	50	5.33±0.33	3±0.58	4±1	3.33±0.33	4.33±0.33	2.33±0.33
	25	4.33±0.33	1.67±0.33	3±0.58	1.67±0.33	3.33±0.33	1.33±0.33
	12.5	-	1.33±0.33	1.67±0.33	-	-	-
	6.25	-	1.00±0.00	1.33±0.33	-	-	-
	3.2	-	-	-	-	-	-
MCVR	50	3.33±0.33	1.67±0.33	6±0.58	4.67±0.33	3.67±0.33	2.33±0.33
	25	2±0.58	1.33±0.33	4.33±0.33	3.67±0.33	3±0.58	1.67±0.33
	12.5	-	1±0.00	2.33± 0.33	2±0.58	1.33±0.33	-
	6.25	-	-	1.67±0.33	-	-	-
	3.2	-	-	-	-	-	-
MASA	50	5.33±0.33	3.33±0.33	3±0.58	4.33±0.33	3.33±0.33	2.67±0.33
	25	3.67±0.33	1.33±0.33	2.67±0.33	3.67±67	2.67±0.33	2.33±0.33
	12.5	2.33±0.33	1±0.00	1.67±0.33	1.67±0.33	-	1.67±0.33
	6.25	-	-	1±0.00	-	-	-
	3.2	-	-	-	-	-	-
MASR	50	-	3.67±0.33	5±0.58	4±0.58	3.67±0.33	2.00±0.58
	25	-	2.67±0.33	3±0.58	1.67±0.33	3±0.58	1.33±0.33
	12.5	-	1.33±0.33	2.00±0.58	-	-	-
	6.25	-	-	1.33± 0.33	-	-	-
	3.2	-	-	-	-	-	-

MIC for the ethanolic extracts of *C. volkensii* leaf were 6.25 mg/ml for *S. aureus*, 12.5 mg/ml for *B. subtilis* and 25 mg/ml for *E. coli*, *P. aeruginosa*, *S. faecalis* and *C. albicans*. For the ethanolic stem extracts of *C. volkensii*, the MIC was 6.25 mg/ml for *B. subtilis* and *S. faecalis* and 25 mg/ml for *E. coli*, *P. aeruginosa* and *C. albicans*.

With the root extracts of *C. volkensii*, MIC were 6.25 mg/ml for *S. aureus*, 12.5 mg/ml for *E. coli* and *B. subtilis* and 25 mg/ml for *P. aeruginosa*, *S. faecalis* and *C. albicans*.

The MIC for ethanolic extracts of *A. setaceous* aerial part ranged from 3.2mg/ml for *S. aureus*, 6.25 mg/ml for *E. coli* and 25 mg/ml for *B. subtilis*, *P. aeruginosa*, *S. faecalis* and *C. albicans* while for the ethanolic root extracts of the same plant, MIC ranged from 6.25 mg/ml for *S. aureus* and *B. subtilis* to 25 mg/ml for *E. coli*, *P. aeruginosa*, *S. faecalis* and *C. albicans* (Table 4.6)

Table 4. 6: Minimum Inhibitory concentration of ethanolic extracts of *C. volkensii* and *A. setaceous* against test microbes

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
ECVL	50	2.67±0.33	4±0.58	4.33±0.67	3.33±0.33	3.33±0.33	1.67±0.33
	25	2±0.58	2.33±0.33	2.33±0.33	3±0.58	2.67±0.33	1.33±0.33
	12.5	-	1.33±0.00	1.33±0.33	-	-	-
	6.25	-	1±0.00	-	-	-	-
	3.2	-	-	-	-	-	-
ECVS	50	2.33±0.33	4.33±0.33	4.67±0.67	1.33±0.33	5.33±0.33	2.33±0.33
	25	1.67±0.33	2±0.58	2.67±0.67	1.33±0.33	4±0.58	2±0.00
	12.5	-	2±0.33	1.33±0.33	-	1.67±0.33	-
	6.25	-	1±0.33	-	-	-	-
	3.2	-	-	-	-	-	-
ECVR	50	4.33±0.33	4.33±0.33	4.33±0.58	2±0.58	5.67±0.33	1.67±0.33
	25	2.67±0.33	2.33±0.33	4.33±0.33	1.33±0.33	3±0.58	1.33±0.33
	12.5	2±0.00	1.67±0.33	2.00±0.33	-	-	-
	6.25	-	1±0.00	-	-	-	-
	3.2	-	-	-	-	-	-
EASA	50	7.33±0.33	2.33±0.33	2.67±0.33	1.33±0.33	2.67±0.33	2±0.58
	25	6.67±0.33	2±0.00	1.33±0.00	1.33±0.33	1.67±0.33	1.33±0.33
	12.5	4.67±0.33	4.33±0.33	-	-	-	-
	6.25	1.67±0.33	3.00±0.33	-	-	-	-
	3.2	-	2.67±0.33	-	-	-	-
EASR	50	4.67±0.33	5.33±0.33	8.33±0.33	2.67±0.33	3.67±0.33	2.33±0.33
	25	2.67±0.33	3.33±0.33	6.33±0.67	2.33±0.33	2.00±0.33	1.33±0.33
	12.5	-	1.67±0.33	3±0.58	-	-	-
	6.25	-	1±0.00	1.67 0.33	-	-	-
	3.2	-	-	-	-	-	-

The hexane extracts of both *C. volkensii* and *A. setaceous* had low MIC ranging from 3.2 mg/ml to 25 mg/ml. For the hexane leaf *C. volkensii* extract, it was 3.2 mg/ml for *E. coli*, 6.25 mg/ml for *S. aureus* and *C. albicans*, 12.5 mg/ml for *B. subtilis* and 25 mg/ml for *P. aeruginosa* and *S. faecalis*. With the stem extracts of *C. volkensii*, MIC ranged from 12.5 mg/ml for *S. aureus* and *B. subtilis* to 25 mg/ml for *E. coli*, *P.*

aeruginosa and *S. faecalis*. The extract was only active against *C. albicans* at the highest concentration tested (50 mg/ml). For the hexane root extract of *C. volkensii*, MIC ranged from 12.5 mg/ml for *S. aureus*, *B. subtilis* and *C. albicans* and 25 mg/ml for *E. coli*, *P. aeruginosa* and *S. faecalis*. The MIC for hexane *A. setaceous* aerial parts were 12.5 mg/ml for *S. aureus* and *B. subtilis* and 25 mg/ml for *E. coli*, *P. aeruginosa*, *S. faecalis* and *C. albicans*, while the same values for hexane root extracts of *A. setaceous* were 6.25 mg/ml for *S. aureus*, *B. subtilis*, and 12.5 mg/ml for *S. faecalis* and 25 mg/ml for *E. coli*, *P. aeruginosa* and *C. albicans* (Table 4.7).

Table 4 7: Minimum Inhibitory concentration of hexane extracts of *C. volkensii* and *A. setaceous* against test microbes. Values are reported as mean inhibition zones (mm) ± SEM of triplicates

Plant Extract	Conc. Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
HCVL	50	6.33±0.33	3.33±0.33	4.33±0.33	4±0.58	4±0.00	5.33±0.33
	25	5.67±0.33	2.67±0.33	3.33±0.33	3.33±0.33	1.33±0.33	3.33±0.33
	12.5	3.67±0.33	1.67±0.33	1.67 ±0.33	-	-	2.67±0.33
	6.25	3.33±0.33	1.33±0.33	-	-	-	1.67±0.33
	3.2	1.67±0.33	-	-	-	-	-
HCVS	50	2.67±0.33	3.67±0.33	5±0.58	2.67±0.33	4±0.58	1.33±0.33
	25	1.33±0.33	2.67±0.33	4.67±0.67	2.33±0.33	2.67±0.33	-
	12.5	-	1.67±0.33	1.33±0.33	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
HCVR	50	2.33±0.33	3.33±0.33	5.33±0.88	2.67±0.33	4.33±0.33	2.33±0.33
	25	2.±0.58	3.33±0.33	3.67±0.33	1.67±0.33	3.67±0.33	1.67±0.33
	12.5	-	1.67±0.33	1.33±0.33	-	-	1.33±0.33
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
HASA	50	2.33±0.33	3.33±0.33	2.67±0.33	2.33±0.33	4.33±0.33	3.33±0.33
	25	1.33±0.33	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33	2.33±0.33
	12.5	-	1.33±0.33	1.33±0.33	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
HASR	50	4.67±0.33	3±0.58	7.33±0.33	4±0.58	5.33±0.67	3.67±0.33
	25	2.67±0.33	1.67±0.33	5.67±0.88	1.67±0.33	4.33±0.33	2±0.58
	12.5	-	1.33±0.33	2±0.58	-	2.33±0.33	-
	6.25	-	1±0.00	1.33±0.33	-	-	-
	3.2	-	-	-	-	-	-

Aqueous extracts were largely inactive at lower concentrations of 3.2, 6.25 and 12.5 mg/ml and those that were active demonstrated activity at concentration above 25 mg/ml. The aqueous extract of *C. volkensii* leaf was only active against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *S. faecalis* with a MIC of 25 mg/ml. The aqueous extract of *C. volkensii* stem had a MIC of 25 mg/ml against *S. aureus* and *S. faecalis*. The

MIC for aqueous extract of C. volkensii root were 25 mg/ml for *E. coli*, *S. aureus*, *P. aeruginosa* and S. faecalis but the extract lacked activity against *C. albicans* at all the tested concentrations. The aqueous extracts of *A. setaceous* aerial part and root were only active at concentration of 25 mg/ml and mostly against *S. aureus*, *B. subtilis* and *S. faecalis* (Table 4.8).

Table 4. 8: Minimum Inhibitory concentration of aqueous extracts of *C. volkensii* and *A. setaceous* against test microbes. Values are reported as mean inhibition zones (mm) ± SEM of three replicates

Plant Extract	Conc Mg/ml	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. faecalis*	*C. albicans*
ACVL	50	-	3.33±0.33	2.67±0.33	1.67±0.33	2.67±0.33	-
	25	-	2.33±0.33	1.67±0.33	1±0.00	2.33±0.33	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
ACVS	50	-	2.67±0.33	-	-	2±0.58	-
	25	-	1.67±0.33	-	-	1±0.00	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
ACVR	50	3.33±0.33	3.33±0.33	2.33±0.33	1.67±0.33	2±0.58	-
	25	3±0.58	2±0.58	1.33±0.33	2±0.00	1.33±0.33	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
AASA	50	-	3±0.58	2.33±0.33	-	2.33±0.33	-
	25	-	2.33±0.33	1.33±0.33	-	1.33±0.33	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-
AASR	50	-	3.67±0.33	3.33±0.33	-	-	-
	25	-	2.67±0.33	1.67±0.33	-	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	3.2	-	-	-	-	-	-

4.3 The effect of selected extracts on growth kinetics of *S. aureus*, *P. aeruginosa* and *E. coli*

The effect of selected plant extracts on the growth of *S. aureus* was also investigated. *S. aureus* experienced slow growth in the presence of dichloromethane leaf and root extracts of *C. volkensii* and ethanolic aerial part extract of *A. setaceous* in comparison to *S. aureus* growing in medium alone. For the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one hour period, the growth rate then rose exponentially for *S. aureus* growing in media alone but remained suppressed for *S. aureus* growing in the presence of extracts for the next 7 hours with percent OD values ranging from 20% to 25% compared to percent OD value of *S. aureus* growing in media alone that was almost 50% (Fig 4.1).

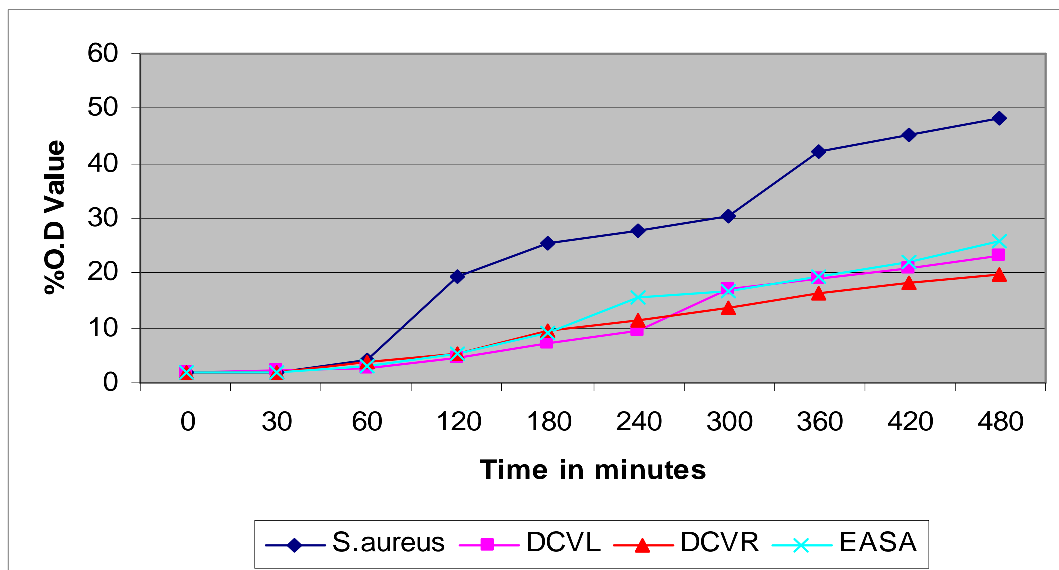

Figure 4.1: Effect of dichloromethane *C. volkensii* leaf, root and ethanolic *A. setaceous* aerial part extracts on the growth kinetics of *S. aureus*

The capacity of dichloromethane root extracts of *C. volkensii* leaf, methanolic extracts of *C. volkensii* stem and aerial part of *A. setaceous* to affect the growth rate of *P. aeruginosa* was also tested. For the first one hour, growth rate remained the same for all the groups of microorganisms growing in the presence or absence of the different extracts. After that the growth rate rose exponentially for most of the groups although growth rates for groups growing in the presence of extracts remained low when compared with the growth rate of *P. aeruginosa* growing in medium alone. The growth rate of *P. aeruginosa* growing in the presence of dichloromethane root extract of *C. volkensii* was the most affected with percent OD values increasing at a very slow rate (Fig 4.2).

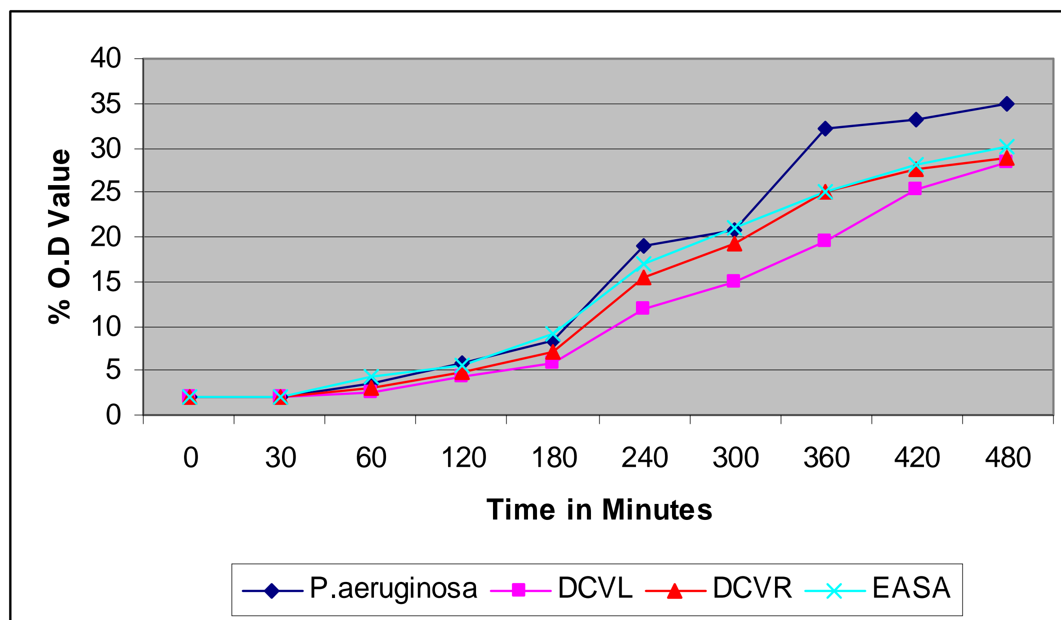

Figure 4. 2: Effect of dichloromethane root extracts of *C. volkensii* leaf, methanolic extracts of *C. volkensii* stem and aerial part of *A. setaceous* extracts on the growth kinetics of *Pseudomonas aeruginosa*

The effect of dichloromethane leaf and root extracts of *C. volkensii*, methanolic extracts of *C. volkensii* stem and aerial part of *A. setaceous*, and the ethanolic extracts of root of *C. volkensii*, aerial part and root of *A. setaceous* on the growth rate of *E. coli* were determined. For the first one hour, growth rate remained the same for all the groups growing in the presence or absence of the different extracts. After that the growth rate rose exponentially for all the groups although groups growing in the presence of extracts had slow growth when compared with the growth rate of *E. coli* growing in medium alone. The growth rate of *E. coli* growing in the presence of methanolic stem extracts of *C. volkensii* and the ethanolic extracts of *A. setaceous* root were the most suppressed. *E. coli* growing in presence of the other extracts had their growth rates also greatly inhibited (Fig 4.3).

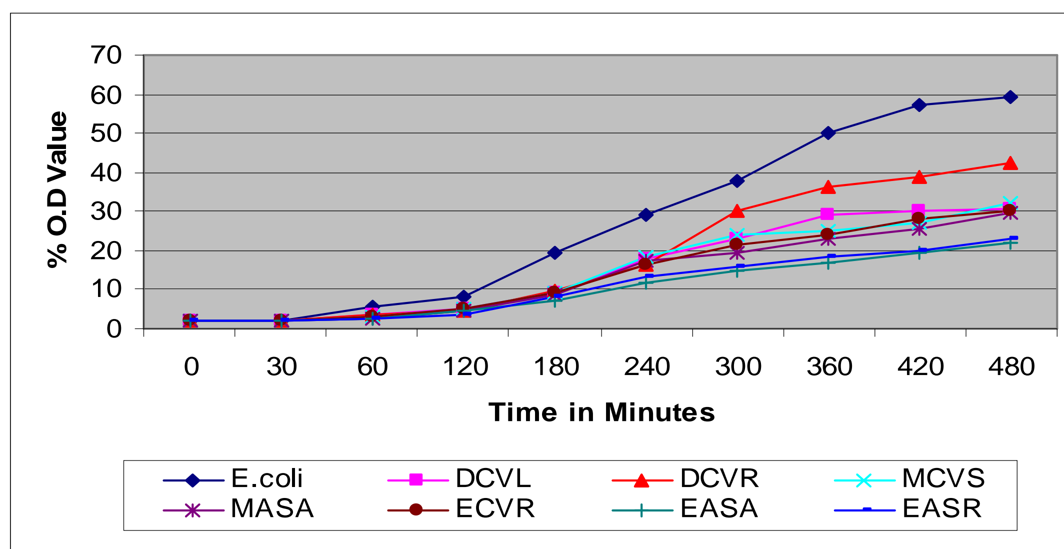

Figure 4. 3: Effect of dichloromethane leaf and root extracts of *C. volkensii*, methanolic extracts of *C. volkensii* stem and aerial part of *A. setaceous*, and the ethanolic extracts of root of *C. volkensii*, aerial part and root of *A. setaceous* on the growth kinetics of *E. coli*

4.4 The effect of ethanol, methanol and aqueous extracts of *C. volkensii* and *A. setaceous* on the survival rates of rats exposed to S. aureus

The survival rate of the treated animals was considerably enhanced after treatment with ethanol, methanol and aqueous extracts of *C. volkensii* and *A. setaceous*.

The ethanolic extracts of *C. volkensii* leaf, stem and root and ethanolic extracts of *A. setaceous* aerial part and root at a concentration of 500mg/kg were effective in prolonging the survival of rats exposed to *S. aureus*. The animals treated with the extracts survived for up to 4 weeks with percent survival ranging from 100% by the end of week 1, 80% to 100% by end of week 2, 66% to 80% at the end of week 3 and 66% to 80% at the end of week 4. Positive control (gentamicin treated group) exhibited percent survival of over 80% up to the end of week 4 (Fig 4.4 and Fig 4.5).

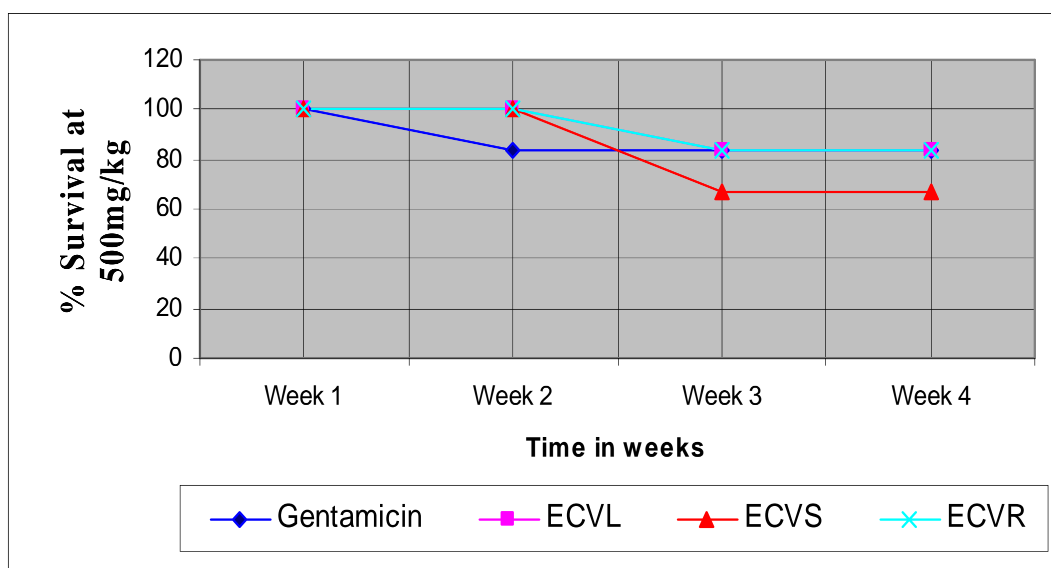

Figure 4. 4: Survival of rats treated with 500mg/kg ethanolic extracts of *C. volkensii* leaf, stem and root and exposed to *S. aureus*

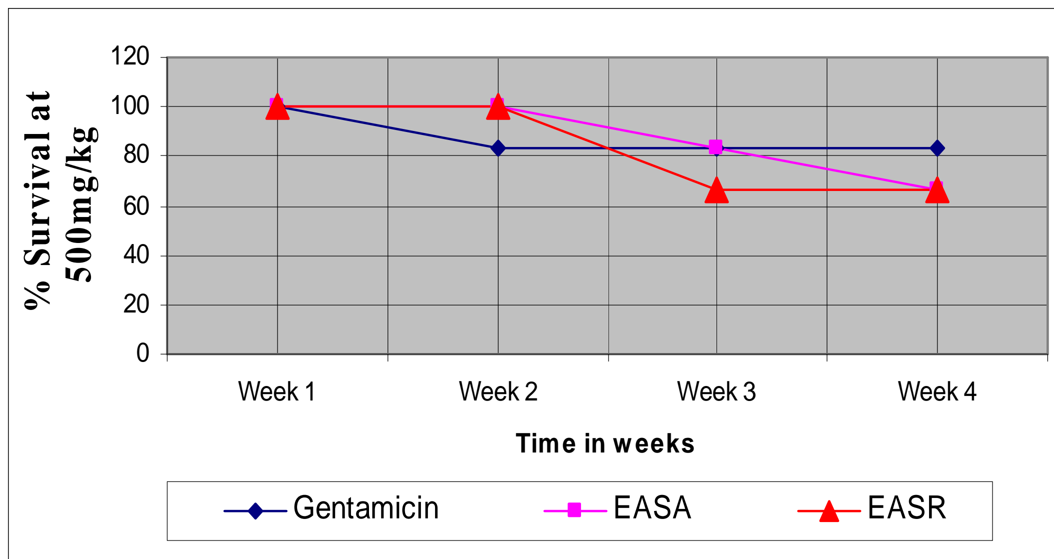

Figure 4. 5: Survival of rats treated with 500mg/kg ethanolic extracts of *A. setaceous* aerial part and root and exposed to *S. aureus*

The methanolic extracts of *C. volkensii* leaf, stem and root and methanolic extracts of *A. setaceous* aerial part and root at a concentration of 500mg/kg were also effective in prolonging the survival of rats exposed to *S. aureus*. The animals treated with the extracts survived for up to 4 weeks with percent survival ranging from 100% by the end of week 1, 80% to 100% by end of week 2, 50% to 66% at the end of week 3 and 50% to 66% at the end of week 4. Positive control (gentamicin treated group) exhibited percent survival of over 80% up to the end of week 4 (Fig 4.6 and Fig 4.7).

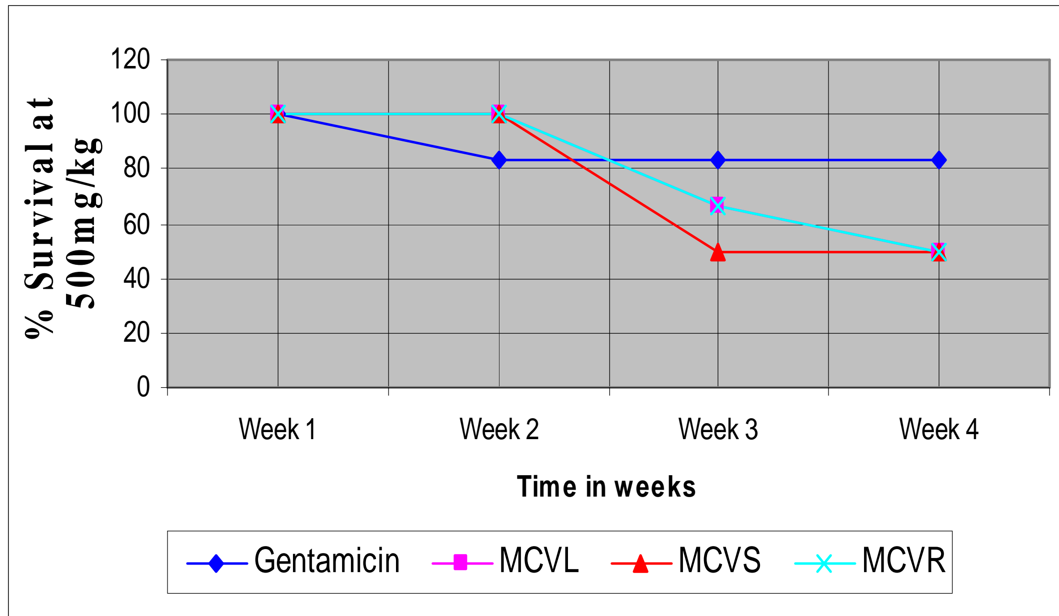

Figure 4. 6: Survival of rats treated with 500mg/kg methanolic extracts of *C. volkensii* leaf, stem and root and exposed to S. aureus

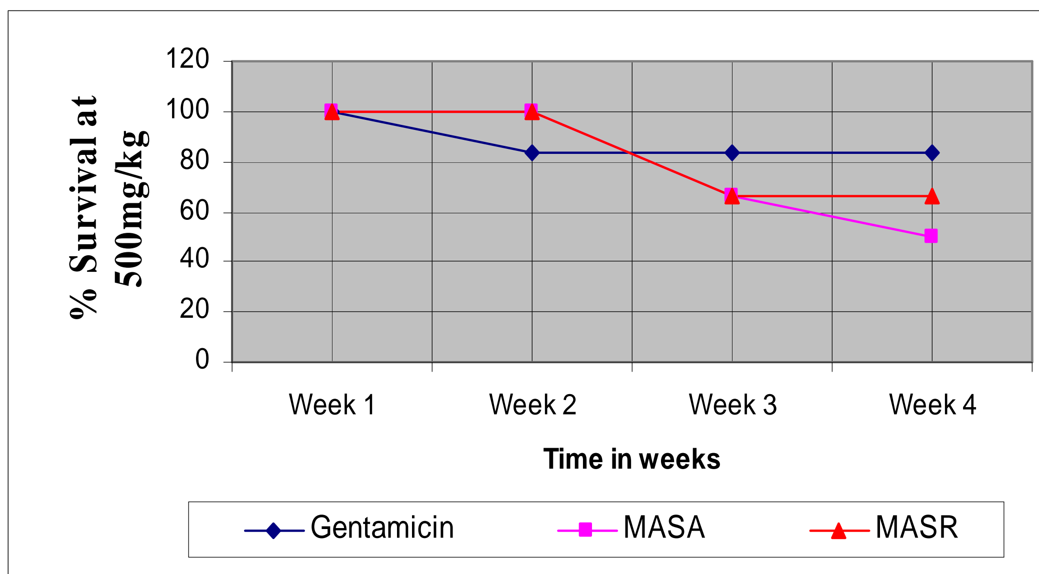

Figure 4.7: Survival of rats treated with 500mg/kg methanolic extracts of *A. setaceous* aerial part and root and exposed to S. aureus

The aqueous extracts of *C. volkensii* leaf, stem and root and aqueous extracts of *A. setaceous* aerial part and root at a concentration of 500mg/kg prolonged the survival of rats exposed to *S. aureus*. The animals treated with the extracts survived for up to 4 weeks with percent survival ranging from 100% by the end of week 1, 80% to 100% by end of week 2, 66% to 80% at the end of week 3 and 50% to 80% at the end of week 4. Positive control exhibited percent survival of over 80% up to the end of week 4 (Fig 4.8 and Fig 4.9).

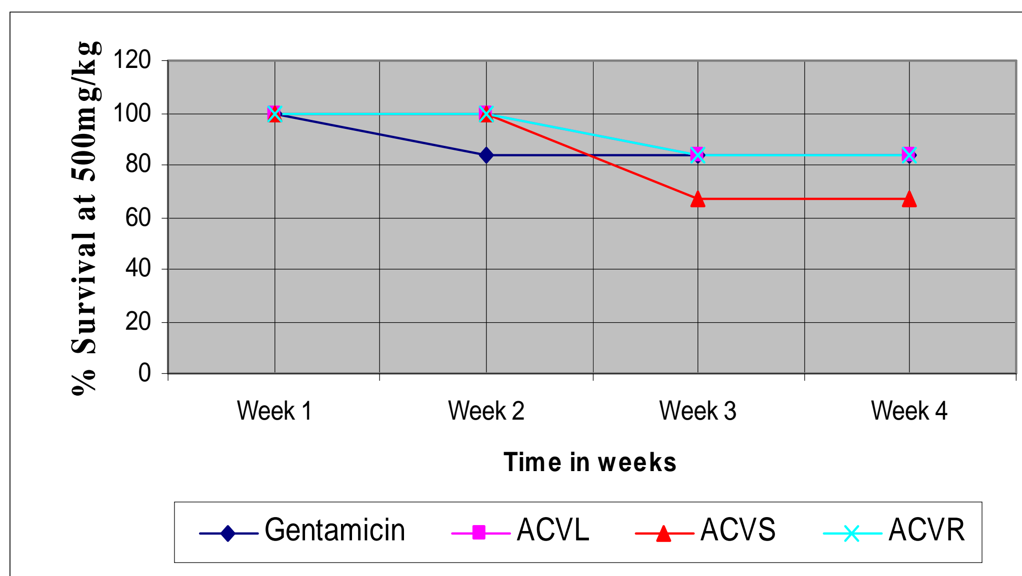

Figure 4. 8: Survival of rats treated with 500mg/kg aqueous extracts of *C. volkensii* leaf, stem and root and exposed to *S. aureus*

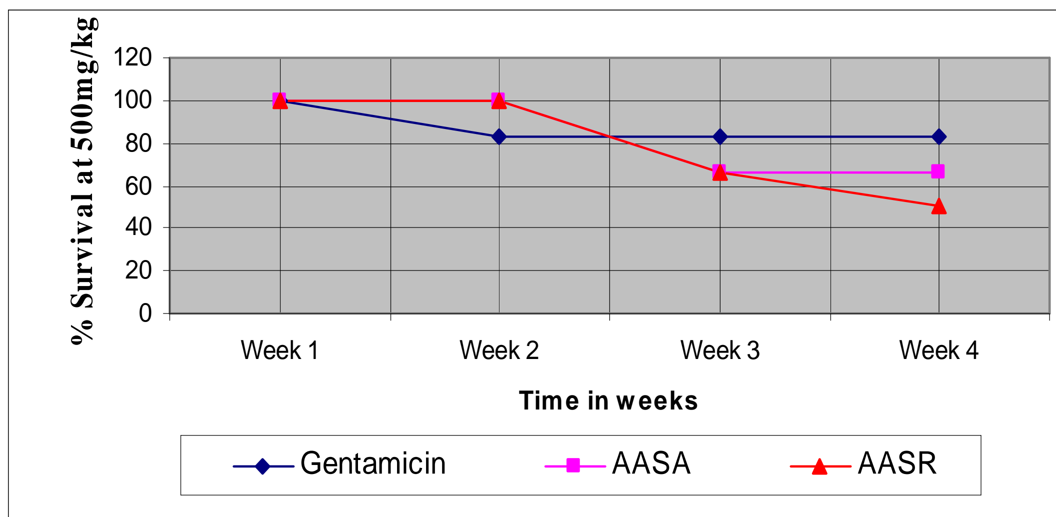

Figure 4. 9: Survival of rats treated with 500mg/kg aqueous extracts of *A. setaceous* aerial part and root and exposed to *S. aureus*

4.5. The effect of extracts of *C. volkensii* and *A. setaceous* on total and differential leukocyte counts

4.5.1. Effect of dichloromethane, methanol, ethanol, hexane and aqueuos extracts of *C. volkensii* and *A. seta*ceous on total leukocyte counts

Before treatment of groups of rats with various extracts of *C. volkensii* leaf, the mean WBC count was between 9100 and 10500 cells/mm^3. Nine days after treatment the mean WBC count increased to between 10400 and 14200 cells/mm^3 (Fig 4.10). ANOVA indicated that there was a significant difference between the mean leucocyte number of all groups (ANOVA, $F_{4,20} = 18.313$, $P = 0.000$). Dunn Bonferroni multiple comparison revealed that the most effective extracts in elevating WBC counts were dichloromethane, methanol, aqueous, and ethanol with $p<0.05$.

Before treatment of groups of rats with extracts of *C. volkensii* stem extracted with dichloromethane, methanol, ethanol, hexane and aqueous mean WBC count ranged

from 9500 to 11600 cells/ mm^3. Nine days after the treatment with the various extracts, it ranged between 11900 and 14400 cells /mm^3 (Fig 4.11). There was a significant difference between the means of all groups (ANOVA, $F_{4, 20} = 11.885$, P=0.000). Dunn Bonferroni multiple comparison revealed that the most effective extracts in increasing WBC counts were methanol, aqueous and ethanol with $p<0.05$**.**

Before treatment with dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* root, WBC count ranged from 9600 to 10900 cells/ mm^3 but nine days after the treatment with the various extracts, it ranged from 10450 to 13500 cells/mm^3 (Fig 4.12). There was a significant difference between the mean leucocyte number of all groups (ANOVA, $F_{4, 20} =3.442$, P=0.023). Dunn Bonferroni multiple comparison revealed that the most effective extracts in increasing WBC counts were methanol, aqueous and ethanol with $p<0.05$**.**

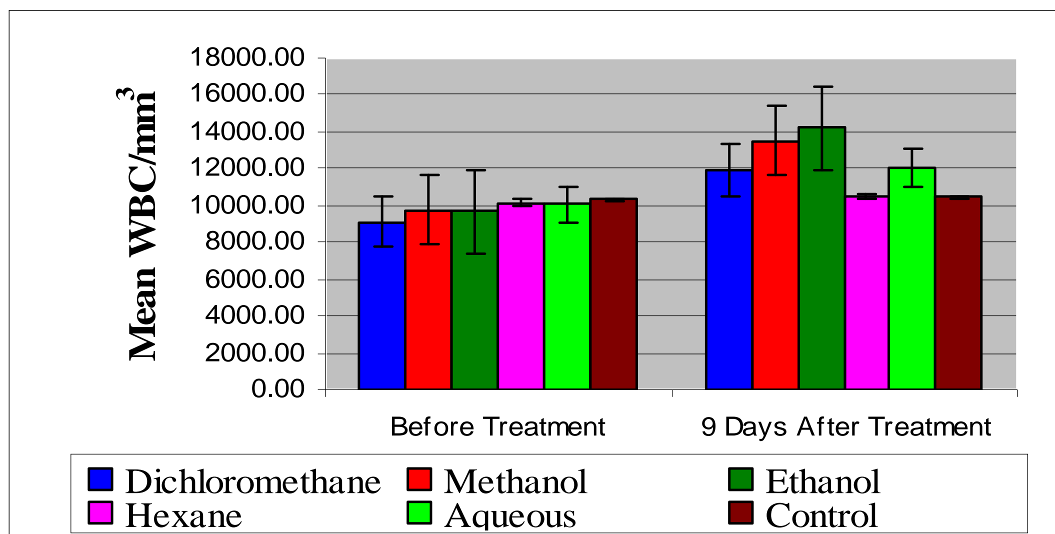

Figure 4. 10: Effects of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* leaf on WBCs counts in rats

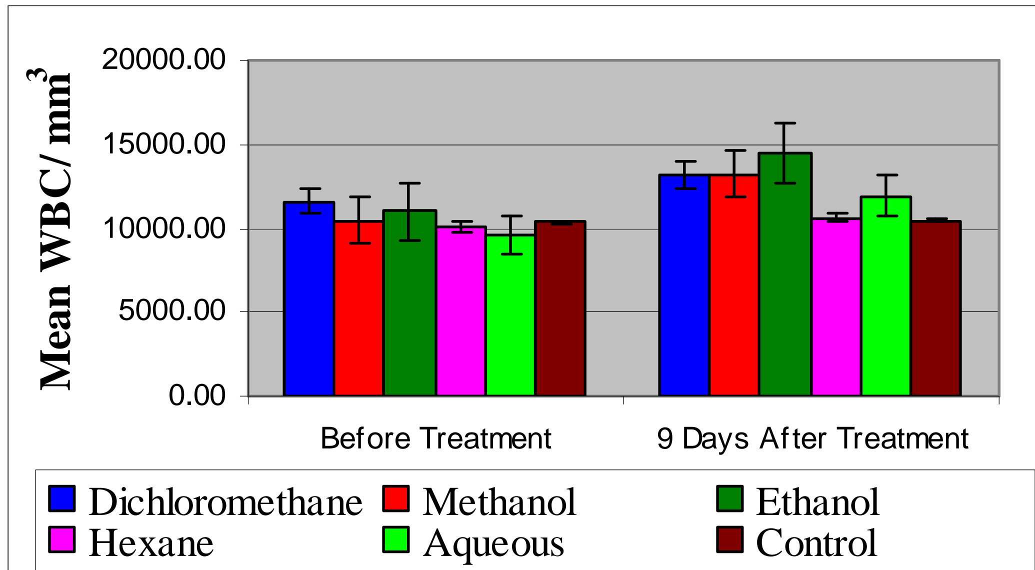

Figure 4. 11: Effects of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* stem on WBCs counts in rats

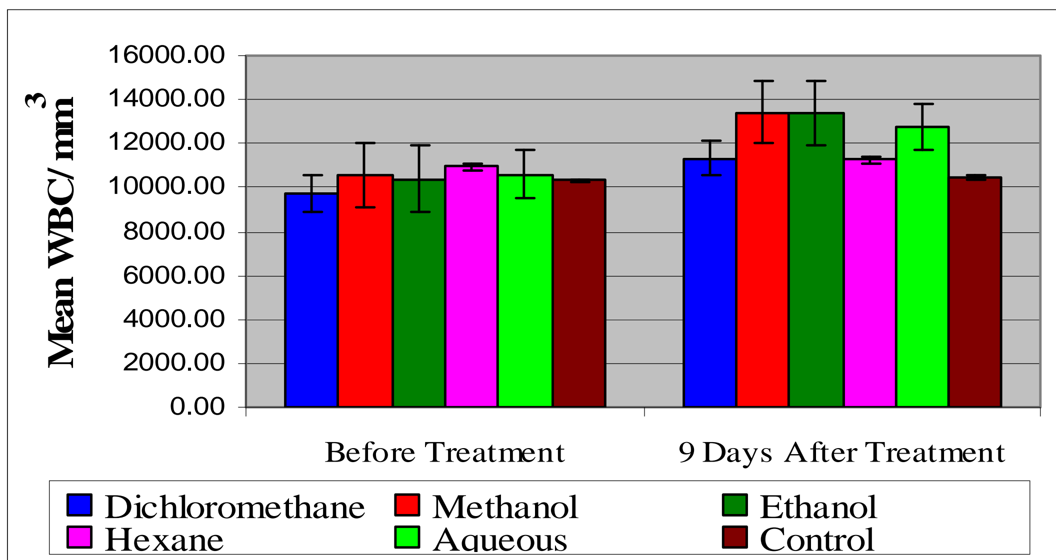

Figure 4. 12: Effects of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* root on WBCs counts in rats

For groups of rats treated with extracts of *A. setaceous* aerial part and root extracted with dichloromethane, methanol, ethanol, hexane and aqueous, nine days after the treatment with the various extracts, WBC counts ranged from 10100 to 14600 cells/mm3 up from 9100 to 10900 cells/mm^3 (Fig4.13). A significant differences between the mean WBC count of all groups (ANOVA, $F_{4, 20}$ =10.326, P=0.000) was observed. Dunn Bonferroni multiple comparison revealed that there was a significant difference between the ethanol and dichloromethane, methanol and ethanol, ethanol and hexane on WBC counts with $p<0.05$. With the extracts of *A. setaceous* root extracted with dichloromethane, methanol, ethanol, hexane and aqueous, WBC count was between 9000 and 10800 cells/mm^3. Nine days after the treatment with the various extracts, it ranged from 11600 to15500 cells/mm^3 (Fig 4.14). A significant differences between the means of all groups (ANOVA, $F_{4, 20}$ =10.161, P=0.000) was observed. Dunn Bonferroni multiple comparison revealed that there was a significant difference between the ethanol and dichloromethane, methanol and dichloromethane, ethanol and hexane, and methanol and hexane after extract treatment on WBC counts with $p<0.05$.

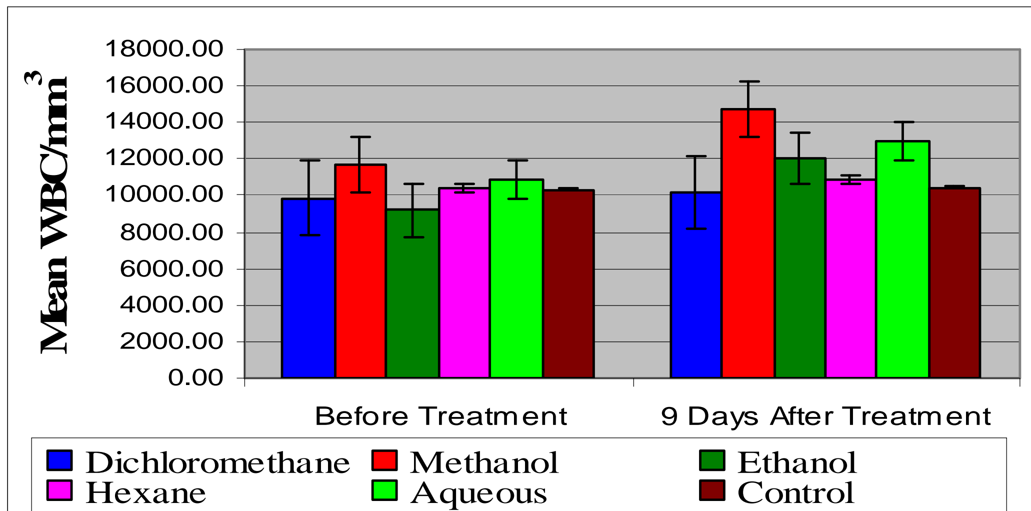

Figure 4. 13: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *A. setaceous* aerial part on WBCs counts in rats

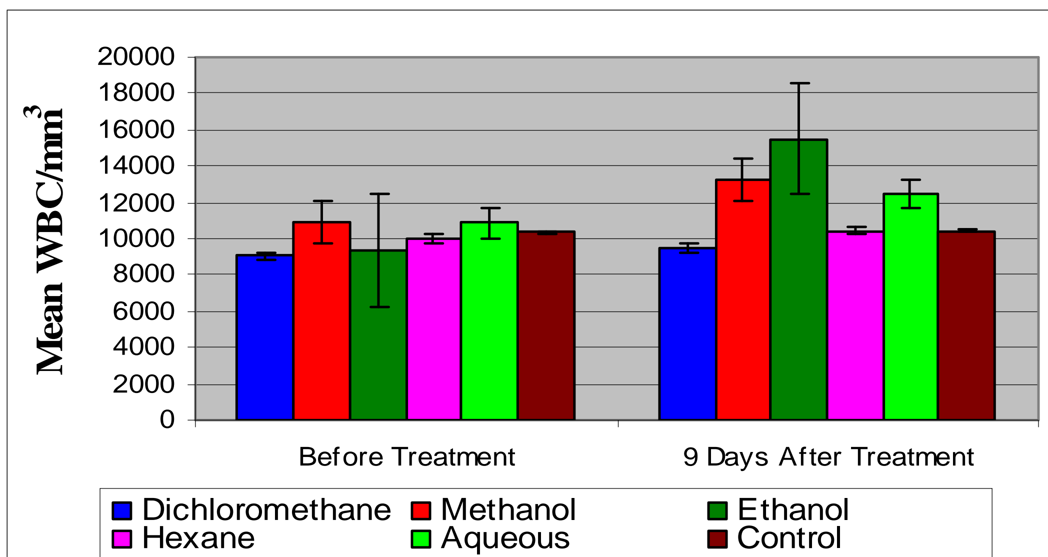

Figure 4. 14: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *A. setaceous* root on WBCs counts in rats

Mean WBC count ranged from 9000 to 11600 cells/mm^3, but nine days after treatment with the extracts of different plant parts of *C. volkensii* and *A. setaceous* with dichloromethane as the solvent of extraction, the mean WBC count increased to between 10450 and13100 cells/mm^3 (Fig 4.15). ANOVA indicated that there was a significant difference between the group means after the extracts treatment (ANOVA, $F_{5,\ 24}$ =11.728, P=0.000). Dunn-Bonferroni revealed that there was a significant difference between the leaves extract of *C. volkensii* and other plant parts from the two plants in increasing WBC counts (p<0.05).

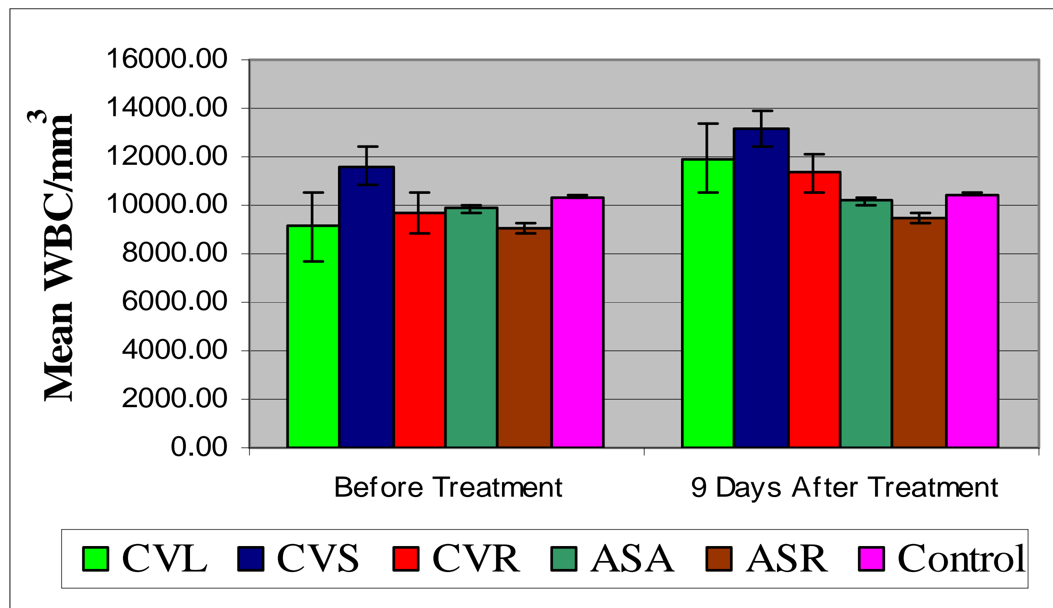

Figure 4. 15: Effects of dichloromethane extracts of leaf, stem, roots of *C. volkensii* and aerial part and root of *A. setaceous* on WBCs counts in rats

***CVL = *C. volkensii* leaf, CVS = *C. volkensii* stem, CVR = *C. volkensii* root, ASA = *A. setaceous* aerial part and ASR = *A. setaceous* root**
***Control group was administered with dimethylsulfoxide and WBC count done after 9 days**

Mean WBC count ranged from 9700 to 11700 cells/mm^3 before treatment but 9 days after treatment with the different plant parts extracted with methanol, it had increased to between 10450 and 14700 cells/mm^3 (Fig 4.16). ANOVA revealed that there was a significant difference between the group means after the treatment (ANOVA, $F_{5, 24}$ = 15.374, P= 0.000). Dunn-Bonferroni revealed that there was a significant difference between the leaves extract of *C. volkensii* and aerial parts, roots of *A. setaceous* as well as with the control. There was also a significant difference between CVS and ASA, ASR and control and between CVR and ASR, in increasing WBC counts among the groups of rats (p<0.05).

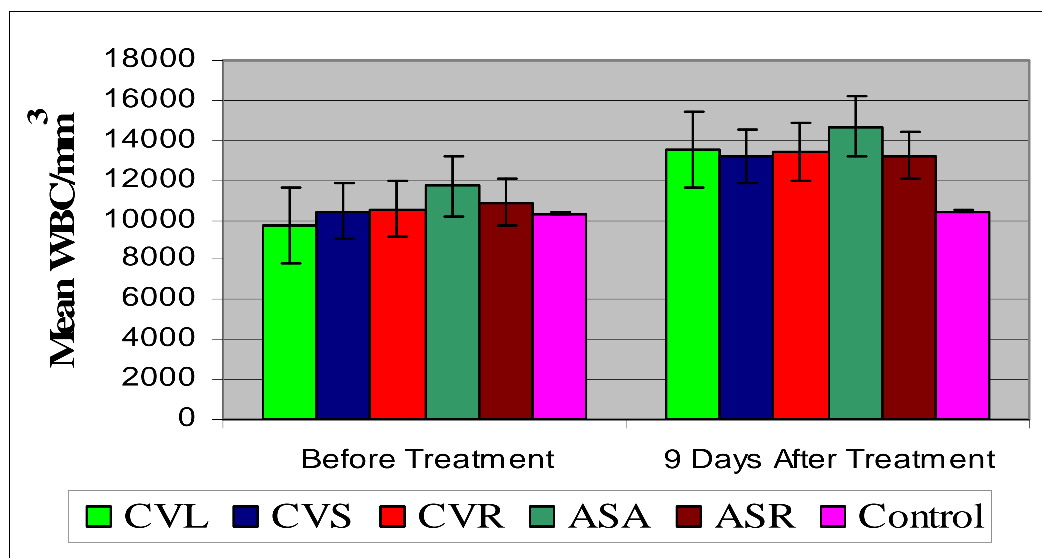

Figure 4. 16: Effects of methanol extracts of leaf, stem, roots of *C. volkensii* and aerial part and root of *A. setaceous* on WBCs counts in rats

CVL = *C. volkensii* leaves, CVS = *C. volkensii* stem, CVR = *C. volkensii* roots, ASA = *A. setaceous* aerial parts and ASR = *A. setaceous* roots

***Control group was administered with dimethylsulfoxide and WBC count done after 9 days**

Mean WBC count ranged from 9300 to 11000 cells/ mm^3 before treatment with ethanol extracts of the different parts of the two plants, but 9 days after treatment, WBC count increased to between 10450 and 15500 cells/mm^3 (Fig 4.17). ANOVA indicates that there was a significant difference between the group means after the treatment (ANOVA, $F_{5, 24} = 6.054$, P= 0.001). Dunn-Bonferroni revealed that there was a significant difference between the leaves extract of *C. volkensii* and control as well as aerial parts of *A. setaceous* with the control raising WBC counts ($p<0.05$).

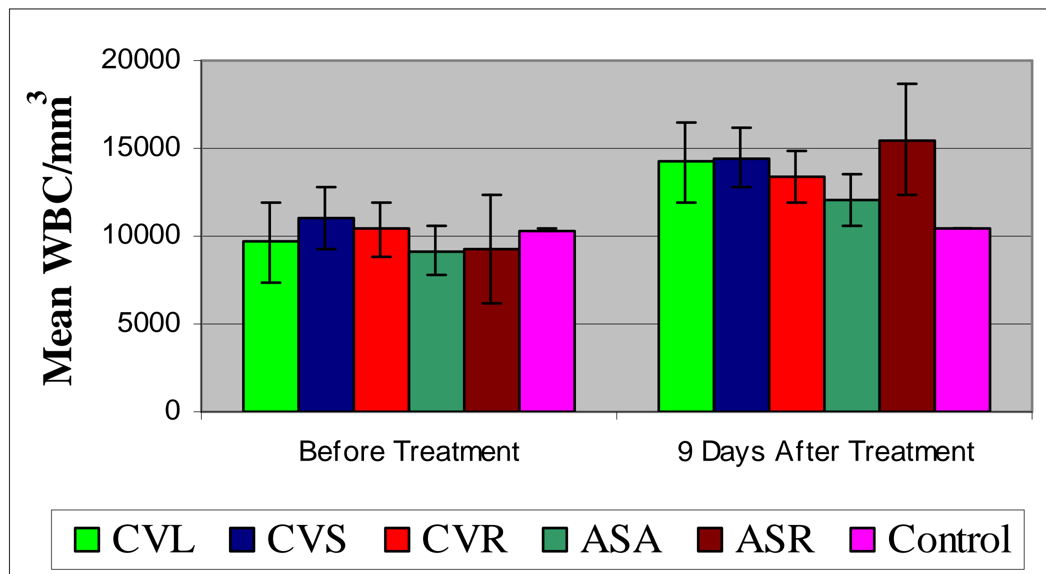

Figure 4. 17: Effects of ethanol extracts of leaf, stem, roots of *C. volkensii* and aerial part and root of *A. setaceous* on WBCs counts in rats

CVL = *C. volkensii* leaf, CVS = *C. volkensii* stem, CVR = *C. volkensii* root, ASA = *A. setaceous* aerial part and ASR = *A. setaceous* root

***Control group was administered with dimethylsulfoxide and WBC count done after 9 days**

Mean WBC count ranged from 9900 to 10900 cells/mm^3 before treatment but 9 days after treatment with hexane extracts of leaf, stem, roots of *C. volkensii* and aerial part and root of *A. setaceous*, it ranged between 10400 to 11200 cells/mm^3 (Fig 4.18). ANOVA indicates that there was no significant difference between the group means after treatment (ANOVA, $F_{5, 24}$ =4.317, P= 0.05). Dunn-Bonferroni revealed that there was a significant difference between the stem extract of *C. volkensii* and aerial part of *A. setaceous* as well as *C. volkensii* root and aerial part of *A. setaceous* in raising WBC counts (p<0.05).

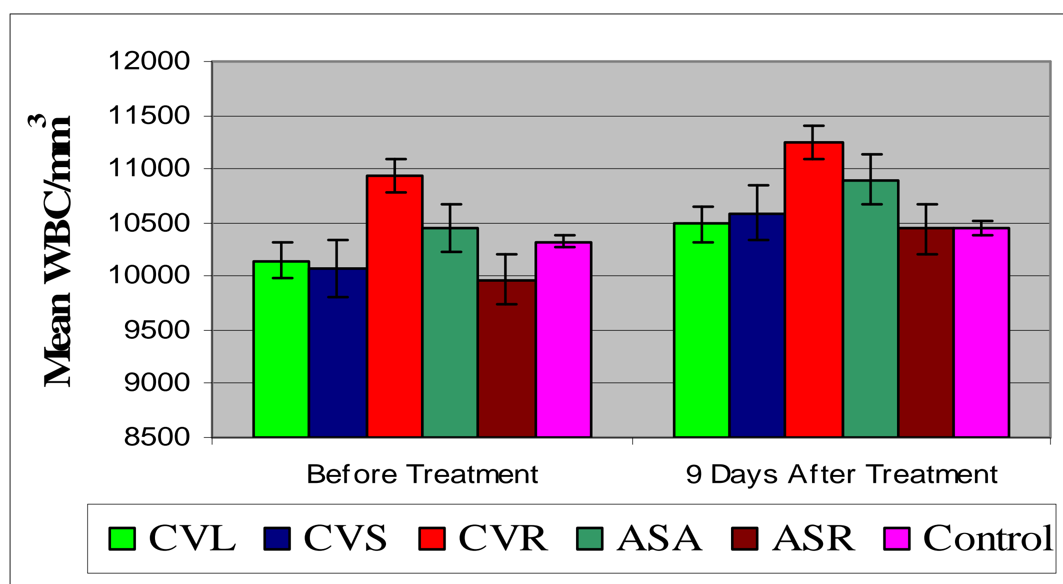

Figure 4. 18: Effects of hexane extracts of leaf, stem, roots of *C. volkensii* and aerial part and root of *A. setaceous* on WBCs counts in rats

CVL = *C. volkensii* leaves, CVS = *C. volkensii* stem, CVR = *C. volkensii* root, ASA = *A. setaceous* aerial part and ASR = *A. setaceous* root

***Control group was administered with dimethylsulfoxide and WBC count done after 9 days**

4.5.2. Effect of extracts of *C. volkensii* and *A. setaceous* on total and differential leukocyte counts

Table 4.9 shows the number of different WBC population before extract treatments and the number of the different WBC population after extract treatment. In DCVL treated rats, mean neutrophil number increased from 4120 to 4965 after extract treatment. This was found to be statistically significant ($t_{4(1)}$ = -20.0832, P = 0.00003). The drop in mean lymphocyte number from 1026 to 918 after extract treatment was statistically significant ($t_{4(1)}$ =4.4312, P = 0.01) as was the drop in eosinophils number ($t_{4(1)}$ = 8.55236, P = 0.001). Changes in basophils was statistically insignificant ($t_{4(1)}$ = 0.8846, P = 0.426).

In DCVS treated rats, the mean neutrophil number rose from 3990 to 4990 after treatment with extract and this was found to be statistically significant ($t_{4(1)}$ = -9.6896, P = 0.0006) while drop in mean lymphocyte numbers from 1110 to 786 was also significant ($t_{4(1)}$ = 2.0181, P = 0.1137). Changes in monocytes, eosinophil and basophils were insignificant after treatment with p values greater than the set significance level ($p>0.05$).

In rats treated with MCVL, increase in mean neutrophil number from 4020 to 4995 after extract treatment was found to be significant ($t_{4(1)}$ = -18.3848, P = 0.00005). The reduction in mean lymphocyte number from 1098 to 852 was also significant ($t_{4(1)}$ =2.6666, P = 0.0034) as was with mean eosinophil number from 17 to 5 which

was also significant ($t_{4(1)}$ = 5.7154, P = 0.0046). The extract had no effect on monocytes and basophils (p>0.05).

In rats treated with MCVS, increase in mean neutrophil number from 4020 to 4995 was found to be significant ($t_{4(1)}$ = -7.91155, P = 0.0001). There was a significant reduction in mean lymphocytes from 1134 to 894 ($t_{4(1)}$ = 5.6568, P = 0.004) as was with mean monocyte numbers which decreased from 46 to 26 after extract treatment which was significant ($t_{4(1)}$ = 9.7979, P = 0.0006). Extract had no effect on basophils (p=0.208).

In MCVR treated rats, mean neutrophil number increased from 3855 to 5015 and this increase was found to be significant ($t_{4(1)}$ = -38.5, P = 0.00027). The reduction in mean lymphocyte number from 1122 to 750 was significant ($t_{4(1)}$ = 7.90595, P = 0.00138). Changes in monocytes, eosinophils and basophils were statistically insignificant with their p values greater than 0.05.

There was a significant increase in mean neutrophil number from 3885 to 4260 in rats following treatment with MASA ($t_{4(1)}$ = -3.371, P = 0.028). The reduction in number lymphocyte from 1158 to 972 was also significant ($t_{4(1)}$ = 3.392489, P = 0.027467). Changes in monocytes, eosinophil and basophils were statistically insignificant with their p values greater than 0.05.

Following treatment of rats with MASR, an increase in mean neutrophil number from 3990 to 4560 was observed and this was found to be significant ($t_{4(1)}$ = -3.08729, P = 0.00366). The reduction in mean lymphocyte number from 1104 to 882 was significant ($t_{4(1)}$ = 3.0833, P = 0.036812). Changes in monocytes, eosinophils and basophils were statistically insignificant with their p values greater than 0.05.

ECVL treated group of rats showed an increase in mean neutrophil number which rose to 5160 from 3975 nine days following treatment. This was found to be significant ($t_{4(1)}$ = -18.3671, P = 0.0000517). The reduction in mean lymphocyte number from 1074 to 732 was significant ($t_{4(1)}$ = 14.03243, P = 0.00015). Changes in monocytes, eosinophils and basophils were statistically insignificant with their p values greater than 0.05.

In ECVS treated rats there was an increase in mean neutrophil number from 3298 to 5052 that was observed to be significant ($t_{4(1)}$ = -16.5072, P = 0.000000789). The reduction in mean lymphocyte from 1128 to 858 was also significant ($t_{4(1)}$ = 6.8333, P = 0.0023) as was with monocytes (t, 4(1) = 4.82418, P = 0.0084) and eosinophils ($t_{4(1)}$ = 2.95419, P = 0.0417). Extract had no effect on basophils (p=0.3045).

In rats treated with EASA, increase in the mean number of neutrophils from 3915 to 5205 was statistically significant ($t_{4(1)}$ = -12.0424, P = 0.0002). Drop in mean lymphocytes level from 1110 to 786 was also significant ($t_{4(1)}$ = 4.8107, P = 0.0085).

A reduction in mean monocytes from 60 to 30 and eosinophils from 16 to 4 was also found to be significant ($t_{4(1)} = 3.5856$, $P = 0.02305$ and $t_{4(1)} = 5.09908$, $P = 0.006987$). Change in basophil was statistically insignificant ($p>0.05$).

Following treatment of rats with EASR, an increase in the mean number of neutrophil from 4080 to 5130 was observed and this was statistically significant ($t_{4(1)} = -16.7332$, $P = 0.00812$). Drop in lymphocytes level from 1050 to 762 was also significant ($t_{4(1)} = 4.88627$, $P =0.0081$). Changes in monocytes and basophils were statistically insignificant with p values of 0.2113 and 0.7489 respectively. Decrease in eosinophil was considered to be significant ($t_{4(1)} = 3.3541$, $P = 0.028$).

In group of rats used as dry controls, changes in the mean number of neutrophils, lymphocytes, monocytes, eosinophils and basophils were statistically insignificant after the treatment with their p values greater than 0.05. This also applied to animals in treated control groups where changes in all the parameters were considered to be statistically insignificant with p values greater than 0.05.

In group of rats treated with ACVL, increase in mean neutrophil number from 53 to 67 was significant ($t_{4(1)} = -17.9065$, $P = 0.0000572$) while the decrease in mean lymphocytes from 35 to 26 was statistically insignificant ($t_{4(1)} = 3.477003$, $P = 0.2543$). The extract had no effect on the other population of WBC with their p values equal to or greater than 0.05.

In rats treated with ACVS, mean neutrophil number increased from 3960 to 4950 and this was considered statistically significant ($t_{4(1)}$ = -11.8539, P = 0.00029). Drop in mean lymphocytes from 1140 to 750 was also significant ($t_{4(1)}$ = 11.8673, P = 0.000289). Changes in the other parameters were insignificant with P =0.3739, 0.6701 and 0.62308 for monocytes, eosinophils and basophils respectively.

Following treatment of rats with ACVR, significant increase in mean neutrophil number from 3885 to 5040 was observed and this was significant ($t_{4(1)}$ = -62.8702, P = 0.000000383). Drop in mean lymphocytes from 1122 to 678 was also observed to be significant ($t_{4(1)}$ = 11.21985, P = 0.000359). Changes in the other parameters were insignificant with P =0.54147, 0.7488 and 0.3739 for monocytes, eosinophils and basophils respectively.

In rats treated with AASA, an increase in mean neutrophil number from 3825 to 4965 after was observed and this was significant ($t_{4(1)}$ = -7.29623, P = 0.001876. Drop in mean lymphocytes from 1188 to 738 was also found to be significant ($t_{4(1)}$ = 5.144958, P = 0.006768). Changes in the other parameters were insignificant with p values greater than the chosen level of significance ($p>0.05$).

In group of rats treated with AASR, increase in mean neutrophil number from 3855 to 5010 was seen after treatment and this was significant ($t_{4(1)}$ = -17.9284, P = 0.0000569). Drop in lymphocytes from 1152 to 708 after treatment was also

significant ($t_{4(1)} = 25.38179$, $P = 0.0000143$). No significant changes were seen in the numbers of monocytes, eosinophils and basophils with their p values equal to or greater than the chosen level of significance ($p>0.05$).

Table 4. 9: The effects of plant extracts on differential WBCs count on rats before and after treatment
N= Neutrophil, L= Lymphocytes, M= monocytes, E= eosinophils, B= basophil

	Before Treatment					After Treatment				
Groups	**N**	**L**	**M**	**E**	**B**	**N**	**L**	**M**	**E**	**B**
DCVL	4120±59.90	1026±33.41	50±4.47	19.4±1.78	1.2±0.49	4965±80.08	918±22.45	14±5.10	5.0±1.00	0.6±0.24
MCVL	4020±102.29	1098±43.06	50±5.48	17.63±1.60	1.2±0.37	4995±102.29	852±20.35	34±5.10	5.20±1.71	0.2±0.2
MCVS	4020±102.29	1134±39.57	46±2.45	13.2±2.37	1.2±0.37	4995±69.55	894±25.81	22±3.74	3.4±1.66	0.6±0.24
MCVR	3855±107.65	1122±55.80	54±5.10	21±2.49	1.2±0.37	5015±92.40	750±49.30	44±5.10	13.2±3.71	0.8±0.37
MASA	3885±212.86	1158±40.91	46±4.00	19.4±2.18	1±0.32	4260±96.05	972±55.80	64±5.10	64±5.10	0.8±0.2
MASR	3990±117.12	1104±41.79	50±4.47	16.8±1.71	1.2±0.37	4560±236.46	882±78.57	56±8.12	13.2±2.37	0.6±0.24
ECVL	3975±97.79	1074±41.79	54±4.00	19.2±2.63	1.2±0.37	5160±93.07	732±30.89	42±8.60	8.8±2.37	0.6±0.24
ECVS	3298±753.69	1128±15.30	54±5.10	17.6±3.04	1±0.45	5025±85.51	858±33.67	22±8.60	7.2±1.80	0.4±0.24
ECVR	3945±122.32	1092±43.06	52±3.74	20.4±1.60	1.2±0.37	4425±171.03	942±77.42	62±5.83	10.6±0.98	1±0.32
EASA	3915±64.52	1110±47.43	60±3.16	15.8±1.83	1.2±0.37	5205±56.12	786±29.09	30±7.07	4.2±1.43	0.4±0.24
EASR	4080±93.67	1050±51.96	56±4.00	19.4±2.18	0.6±0.4	5130±60.93	762±58.17	40±7.07	6±2.95	0.8±0.2
Dry Control	3945±80.78	1086±34.73	54±5.10	18.60±1.66	1.6±0.51	4020±77.22	1056±25.81	70±7.07	12.40±1.66	1.2±0.37
Treated Control	4005±38.24	1098±27.82	44±6.78	19.4±1.78	1.4±0.24	3990±76.49	1116±37.23	70±4.47	8.8±1.43	1±0.32
ACVL	3990±55.11	1062±54.17	50±4.47	19.4±1.78	0.8±0.37	4995±77.22	792±43.05	42±3.74	19.40±2.89	0.4±0.24
ACVS	3960±80.08	1140±28.46	50±4.47	15±2.26	0.8±0.2	4950±82.16	750±41.35	46±4.00	16.80±2.56	0.6±0.24
ACVR	3885±64.52	1122±30.89	54±5.10	20.2±2.20	0.8±0.37	5040±49.75	678±33.67	50±7.07	21.2±1.50	0.4±0.24
AASA	3825±106.07	1188±54.17	44±6.00	15.8±1.83	0.6±0.24	4965±80.08	738±54.17	46±5.10	18.6±1.66	0.6±0.24
AASR	3885±80.08	1152±40.91	54±6.00	15.8±1.83	0.8±0.37	5010±49.75	708±27.82	54±.5.10	16±2.26	0.6±0.24

4.6. Screening the extracts for enhancement of neutrophil activity

The results on the screening of the extracts on their ability to affect neutrophil adhesion, phagocytic index and nitroblue tetrazolium reduction are presented in the table 4.10 below. The extracts had different effect on the various neutrophil functions. There was a statistical difference between the means of % neutrophil adhesion of all the 27 groups of experimental animals after the extract treatment which was significant (ANOVA, $F_{25,\ 104}$ = 72.90623, P = 0.00000). The means of the extracts treated groups were then compared to the mean of the control group using the t- test for two samples assuming unequal variances. The mean percent neutrophil adhesion for the DCVL treated group with a mean of 30±1.6 compared to the mean of the control 13.4 ±2.5 was enhanced and this was significant ($t_{8(2)}$ = 12.3087, P = 0.000005). The mean percent neutrophil adhesion in DCVS treated group was 17.4±2.05 which when compared to that of the control group, was observed to be significant ($t_{8(2)}$ = 2.8612, P = 0.021). The mean percent neutrophil adhesion was observed to be significantly depressed in DCVR group ($t_{8(2)}$ = -2.12675, P = 0.071), DASA group ($t_{8(2)}$ = -0.3599, P = 0.072) and DASR group ($t_{8(2)}$ = 0.37853, P = 0.7180).

The mean percent neutrophil adhesion in MCVL with a mean of 38.5± 3.8, MCVS with mean of 34.5±3.2, MCVR with mean of 31.9 ±4.5, MASA with mean of 21.5±4.4, MASR with mean of 24.1±3.34 was greatly enhanced when compared to the mean of the control. This enhancement was observed to be

statistically significant in MCVL treated group ($t_{8(2)}$ = 12.3068, P = 0.00000), MCVS treated group ($t_{8(2)}$ = 11.6183, P = 0.00000), MCVR treated group ($t_{8(2)}$ = 7.9375, P = 0.00021), MASA group ($t_{8(2)}$ = 3.6064, P = 0.011) and in MASR group ($t_{8(2)}$ = 5.760521, P = 0.0006).

The mean percent neutrophil adhesion was found to be 36.2±3.4 for ECVL, 37.7±1.55 for ECVS, 22.5± 6.1 for ECVR, 37.9± 4.3 for EASA and 35.1± 4.2 for EASR. Compared to the mean of the control group this enhancement was found to be significant in ECVL treated group ($t_{8(2)}$ = 12.0250, P = 0.0000627), ECVS group ($t_{8(2)}$ = 18.5045, P = 0.00000), ECVR group ($t_{8(2)}$ = 3.08678, P = 0.0272), EASA group ($t_{8(2)}$ = 10.9672, P = 0.000034) and in EASR treated group ($t_{8(2)}$ = 9.78815, P = 0.00006).

Percent neutrophil adhesion was significantly depressed in HCVL treated group ($t_{8(2)}$ = -5.307215, P = 0.00072), HCVS group ($t_{8(2)}$ = -2.76079, P = 0.0246), HCVR group ($t_{8(2)}$ = -3.10300, P = 0.014), HASA treated group ($t_{8(2)}$ = -1.66185, P = 0.0145) and in HASR treated group ($t_{8(2)}$ = -2.73513, P = 0.02564). Neutrophil adhesion was significantly enhanced in ACVL treated group ($t_{8(2)}$ = 18.50702, P = 0.00000), ACVS group ($t_{8(2)}$ = 12.9957, P = 0.00000), ACVR group (t, 8(2) = 13.7924, P = 0.00000), AASA group ($t_{8(2)}$ = 14.2474, P = 0.00000) as well as in AASR treated group ($t_{8(2)}$ = 19.0588, P = 0.000000).

The result on phagocytic index (PI) is also given in table 4.10. ANOVA

revealed that there was a difference between mean % phagocytic index of all the groups after the extracts treatment (ANOVA, $F_{26,\ 107}$ = 31.723, P = 0.0000). The means of the groups were then compared to the mean of the control group using t- test for two samples assuming unequal variances. The mean phagocytic index for DCVL group at 85.8±1.9 when compared to the control mean at 74.4±3.6 was statistically significant ($t_{8(2)}$ = 6.1825, P = 0.0008). PI was insignificant for DCVS group ($t_{8(2)}$ = 1.35027, P= 0.2138), DCVR group ($t_{8(2)}$ = -0.9594, P = 0.3744), DASA group ($t_{8(2)}$ = -1.17456, P = 0.13697) and DASR group ($t_{8(2)}$ = -0.70352, P = 0.50445).

The mean PI was 87±2.4 for MCVL, 88.2±1.6 for MCVS, 80.4±1.6 for MCVR, 80.8± 1.4 for MASA and 80.8±2.6 for MASR. Compared to the mean of the control, these means were observed to be significantly elevated in MCVL group ($t_{8(2)}$ = 6.48152, P = 0.0003), MCVS treated group ($t_{8(2)}$ = 7.71443, P = 0.00024), MCVR group ($t_{8(2)}$ = 3.3436, P = 0.0155), MASA group ($t_{8(2)}$ = 3.6349, P = 0.01498), and MASR group ($t_{8(2)}$ = 3.16073, P = 0.0159)

The mean PI was 87±4.6 for ECVL, 89±2.5 for ECVS, 85±0.8 for ECVR, 89.2±1.9 for MASA and 84.8±2.0 for MASR. Compared to the mean of the control, these means were observed to be significantly raised in ECVL treated group ($t_{8(2)}$ = 5.01788, P = 0.00102), ECVS group ($t_{8(2)}$ =7.4711, P = 0.000149), ECVR group ($t_{8(2)}$ = 6.30476, P = 0.00032), EASA group ($t_{8(2)}$ = 8.02642, P = 0.00019) and EASR treated group ($t_{8(2)}$ = 5.4366, P = 0.00167).

The PI were not significantly different from those of control values in HCVL group ($t_{8(2)}$ = 1.05847, P = 0.32074), HCVS group ($t_{8(2)}$ = 1.06600, P = 0.3274), HCVR group ($t_{8(2)}$ = 1.84390, P = 0.1147) as well as in HASA group ($t_{8(2)}$ = 0.90007, P = 0.40933) and HASR treated group ($t_{8(2)}$ = 0.103695, P = 0.92031).

The mean PI was seen to be 89.8±3.1 for ACVL, 75.4±3.5 for ACVS, 89.8±1.9 for ACVR, 88±2 for AASA and 89±2.5 for AASR. Compared to the mean of the control, these means were observed to be significantly raised in ACVL group ($t_{8(2)}$ = 7.18028, P = 0.00000), ACVR group ($t_{8(2)}$ = 8.3518, P = 0.000160), AASA group ($t_{8(2)}$ = 7.311405, P = 0.00033) and AASR group ($t_{8(2)}$ = 7.4, P = 0.00014) but not in ACVS group ($t_{8(2)}$ = 0.44194, P = 0.6702).

The extracts were also evaluated on their ability to affect nitroblue tetrazolium reduction in neutrophils and result presented in table 4.10. The mean NBT values of the experimental groups were found to be statistically different from one another (ANOVA, $F_{26,\ 108}$ = 35.91119, P = 0.0000) following extracts treatment. The differences between the means were also compared to the mean of the control group using the t- test for two samples assuming unequal variances. The mean NBT was 36.5±2.3 for DCVL, 25.6±3.2 for DCVS, 25.6±2.8 for DCVR, 26.8±1.9 for EASA and 26.4±2.4 for DASR. Compared to the mean of the control which was 26.6 4.0, these means were observed to be significantly raised only in DCVL group ($t_{8(2)}$ = 4.33012, P = 0.0049) but not in DCVS group ($t_{8(2)}$ = -0.43355, P = 0.6760),

DCVR group ($t_{8(2)}$ = - 0.45083, P = 0.6657), DASA group ($t_{8(2)}$ = 0.1, P = 0.9236) and DASR group ($t_{8(2)}$ = - 0.0951, P = 0.92360).

The mean % NBT was 37.8±2.7 for MCVL, 39.0±1.5 for MCVS, 38±1.5 for MCVR, 30±1.9 for EASA and 31±1.5 for MASR. Compared to the mean of the control which was 26.6±4.0, these means were observed to be raised in some of the groups after extract treatment. NBT reduction was significantly enhanced in MCVL group ($t_{8(2)}$ = 5.46504, P = 0.00156), MCVS group ($t_{8(2)}$ = 6.3948, P = 0.00138). It was not enhanced in MCVR group ($t_{8(2)}$ = 2.26912, P = 0.0725), MASA group ($t_{8(2)}$ = 2.1, P = 0.08) and MASR group ($t_{8(2)}$ = 2.2691, P = 0.072).

The mean % NBT was 39.5±2.5 for ECVL, 39.4±2 for ECVS, 33.2±1.6 for ECVR, 40.2±2.3 for EASA and 34.2±1.6 for DASR. Compared to the mean of the control which was 26.6± 4.0, these means were observed to be elevated in ECVL group ($t_{8(2)}$ = 5.87478, P = 0.00061), ECVS group ($t_{8(2)}$ = 6.306107, P = 0.0007), ECVR group ($t_{8(2)}$ =3.38572, P = 0.0195), EASA group ($t_{8(2)}$ = 6.48354, P = 0.00063) and in EASR group ($t_{8(2)}$ = 3.8987, P = 0.01142).

The mean % NBT was 40.2±2.3 for ACVL, 35.0±2.2 for ACVS, 40±1.5 for ACVR, 36±2 for AASA and 41±1.5 for AASR. Compared to the mean of the control which was 26.6±4.0, these means were observed to be raised in all the groups after extract treatment. NBT reduction was significantly enhanced in ACVL group (t

$_{8(2)}$ = 6.48354, P = 0.00063), ACVS group (t $_{8(2)}$ = 4.0698, P = 0.0065), ACVR group (t $_{8(2)}$ = 6.91052, P = 0.0009), AASA group (t $_{8(2)}$ = 4.6651, P = 0.0034) and in AASR group (t $_{8(2)}$ = 7.42623, P = 0.00069.

Table 4.10: The effects of the various extracts of *C. volkensii* and *A. setaceous* on percent neutrophil adhesion, phagocytic index and NBT reduction. The values represent the mean ±SEM

	% Neutrophil Adhesion	**% Phagocytic index**	**% NBT Reduction**
DCVL	30±1.69	85.8±1.92	36.5±2.30
DCVS	17.5±2.05	77.2±2.86	25.6±3.21
DCVR	10.4±1.82	72.6±2.07	25.6±2.88
DASA	12.7±2.93	71.8±3.35	26.8±1.92
DASR	14.3±4.96	73±2.55	26.4±2.41
MCVL	38.5±3.83	87±2.48	37.8±2.71
MCVS	34.5±3.21	88.2±1.64	39.0±1.58
MCVR	31.9±4.58	80.4±1.67	38.0±1.58
MASA	21.5±4.44	80.8±1.48	30.0±1.92
MASR	24.1±3.34	80.8±2.68	31.0±1.58
ECVL	36.2±3.43	87.0±4.62	39.2±2.59
ECVS	37.7±1.55	89.0±2.59	39.4±2.07
ECVR	22.5±6.13	85.0±0.82	33.2±1.64
EASA	37.9±4.35	89.2±1.92	40.2±2.39
EASR	35.1±4.28	84.6±2.07	34.2±1.64
ACVL	40.5±2.11	89.8±3.11	40.2±2.39
ACVS	34.1±2.54	75.4±3.50	35.0±2.24
ACVR	39.7±3.47	89.8±1.92	40.0±1.58
AASA	32.2±1.58	88.0±2.00	36.0±2.00
AASR	41.9±2.24	89.0±2.59	41.0±1.58
DRY CONTROL	13.4±2.5	74.4±3.65	26.6±4.04
TREATED CONTOL	13.6±2.7	74.4±2.41	25.0±2.24

***Treated control group was administered with dimethylsulfoxide while the dry control was not manipulated**

4.7 Effects of the extracts of *C. volkensii* and *A. setaceous* on reversing WBC reduction in cyclophosphamide treated rats

Four days after CP treatment, there was a significant drop in the mean WBC count to about 6000cells/mm3 in all the groups of rats ($t_{4(1)}$ =15.332, P = 0.000). A significant increase in mean WBC counts was observed 9 days later after the administration of dichloromethane extract of the *C. volkensii* leaves ($t_{4(1)}$ = -7.683, P = 0.002). A significant reduction in WBC counts was seen 4 days after CP treatment ($t_{4(1)}$ = 57.601, P = 0.000: $t_{4(1)}$ =16.437, P = 0.000) but following treatment of rats with methanol and ethanol extracts of *C. volkensii* leaves, WBC counts was observed to increase significantly 9 days later following administration ($t_{4(1)}$ = -63.513, P = 0.000 and $t_{4(1)}$ = -13.501, P = 0.000). With hexane extract, a significant drop was seen after CP injection ($t_{4(1)}$ = 21.414, P = 0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP injection ($t_{4(1)}$ = -0.861, P = 0.438) (Fig 4.19)

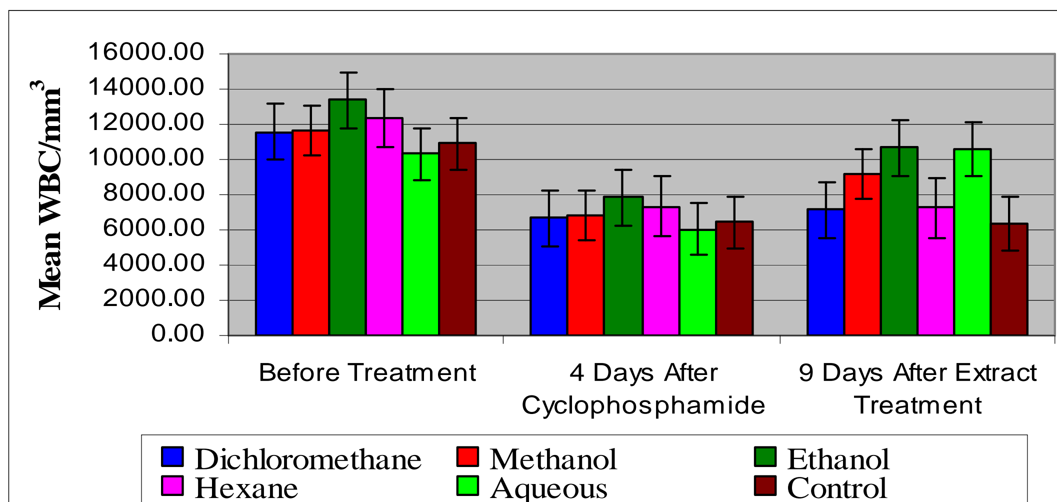

Figure 4. 19: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* leaves on WBCs counts in rats 4 days after cyclophosphamide treatment and 9 days later after extracts treatment

Four days following CP treatment, there was a significant drop in the WBC count ($t_{4(1)}$ =13.350, P = 0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the *C. volkensii* stem ($t_{4(1)}$ = 0.000, P =1.000). With the methanol and ethanol extracts, significant reduction in WBC counts were observed 4 days after CP treatment ($t_{4(1)}$ =10.021, P = 0.001: $t_{4(1)}$ = 11.925, P = 0.000). Methanol extracts of *C. volkensii* stem caused the WBC counts to significantly rise after 9 days following their administering while ethanol did not have any effect ($t_{4(1)}$ = - 10.772, P = 0.000 and $t_{4(1)}$ = -1.823, P = 0.142). With hexane extract, a significant drop was seen after CP injection ($t_{4(1)}$ = 14.403, P = 0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP treatment ($t_{4(1)}$ = - 0.154, P = 0.885) (Fig 4.20).

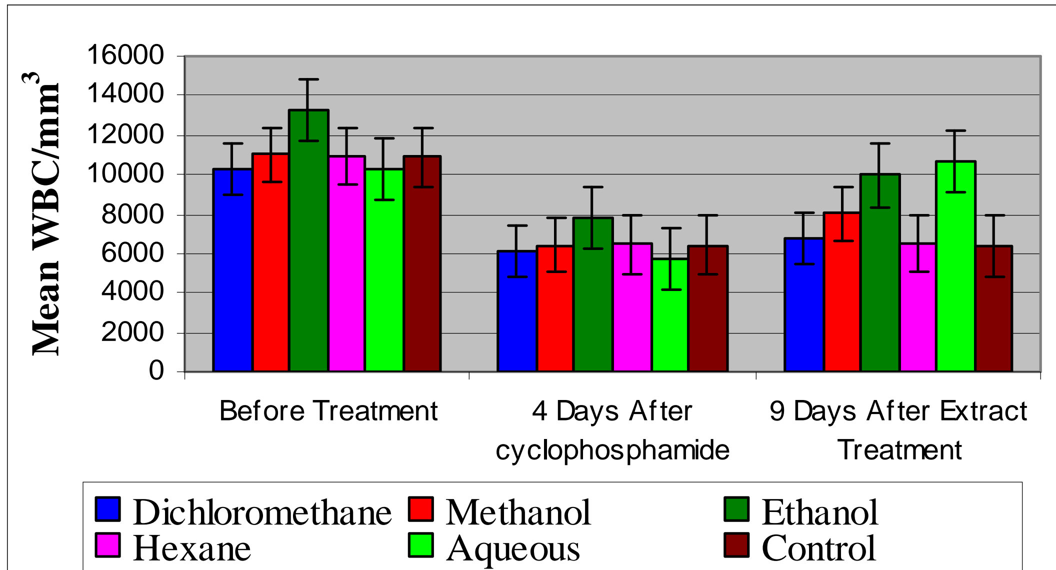

Figure 4. 20: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* stem on WBCs counts in rats treated with cyclophosphamide

WBC count decreased significantly 4 days after CP treatment ($t_{4(1)}$ = 16.584, P = 0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the *C. volkensii* roots ($t_{4(1)}$ = -0.211, P = 0.843). With the methanol and ethanol extracts, significant reduction in WBC counts were seen 4 days after CP treatment ($t_{4(1)}$ =22.145, P = 0.000: $t_{4(1)}$ =12.590, P = 0.000). The two extracts caused the WBC counts to significantly rise after 9 days following administering ($t_{4(1)}$ = - 8.929, P = 0.001 and $t_{4(1)}$ = -10.177, P = 0.001). With hexane extract, a significant drop was seen after CP injection ($t_{4(1)}$ = 21.457, P =0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following CP injection ($t_{4(1)}$ = - 1.264, P = 0.275) (Fig 4.21).

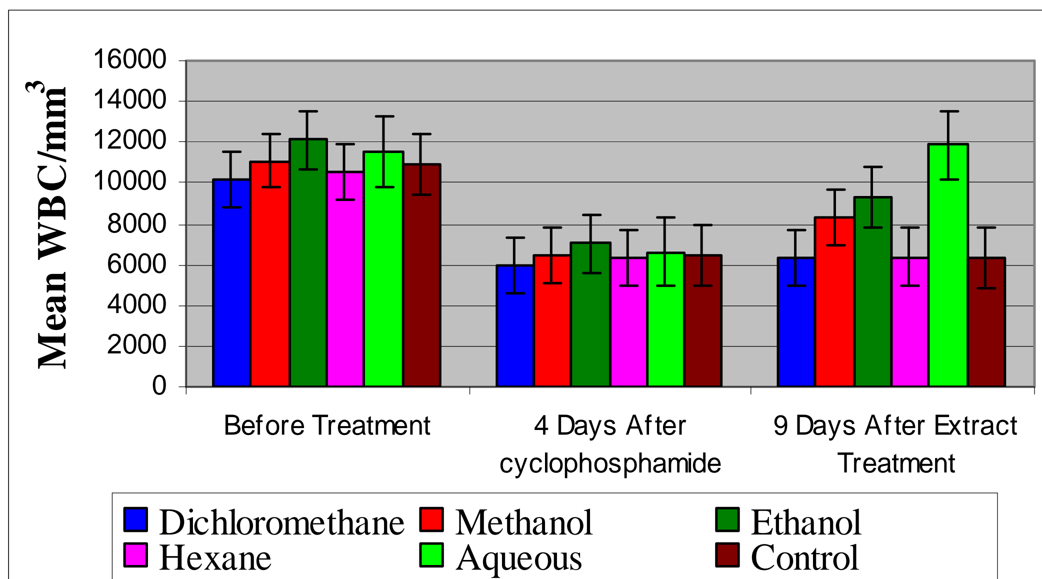

Figure 4. 21: Effect of dichloromethane methanol, ethanol and hexane extracts of *C. volkensii* root on WBCs counts in rats treated with cyclophosphamide

***Control group was administered with dimethylsulfoxide**

Four days following CP treatment, there was a significant drop in the WBC count ($t_{4(1)} = 18.40$, P = 0.000). There was no alteration in WBC counts 9 days later after the administration of dichloromethane extract of the aerial parts of *A. setaceous* ($t_{4(1)} = 1.847$, P = 0.138). With the methanol and ethanol extracts, significant reduction in WBC counts were seen 4 days after CP treatment ($t_{4(1)} = 15.821$, P = 0.000: $t_{4(1)} = 14.348$, P = 0.000). The two extracts caused the WBC counts to significantly rise after 9 days following their administering ($t_{4(1)} = -13.534$, P = 0.000 and $t_{4(1)} = -16.571$, P = 0.000). With hexane extract, a significant drop was seen after CP injection ($t_{4(1)} = 24.348$, P = 0.000) whereas the extract was ineffective in

reversing the decrease in WBC counts following CP injection ($t_{4(1)} = -0.512$, P = 0.636) (Fig 4.22).

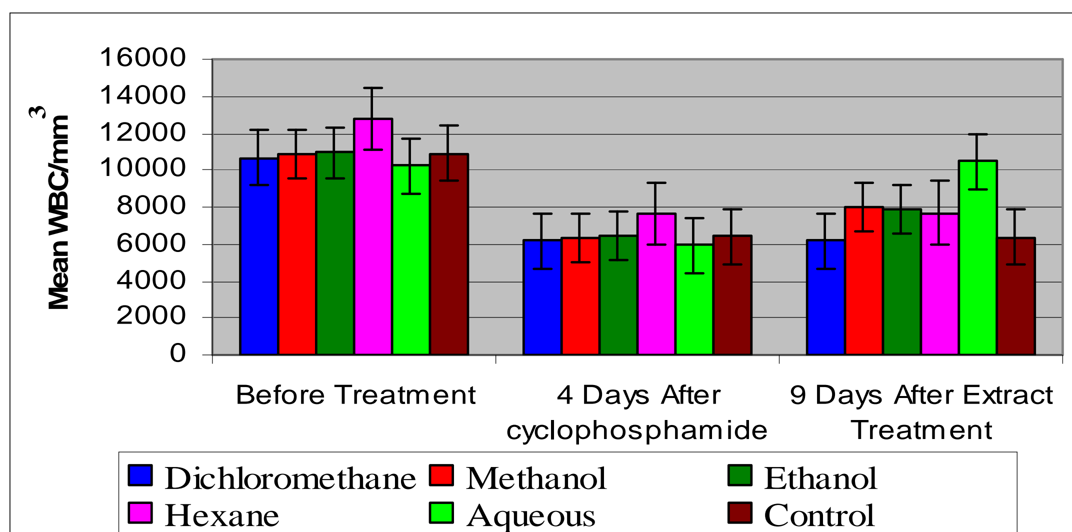

Figure 4. 22: Effect of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *A. setaceous* aerial part on WBCs counts in rats treated with cyclophosphamide

***Control group was administered with dimethylsulfoxide**

After 4 days following CP treatment there was a significant drop in the WBC count ($t_{4(1)} = 11.885$, P = 0.000). There was no change in WBC counts 9 days later after the administration of dichloromethane extract of the roots of *A. setaceous* ($t_{4(1)} = -0.945$, P = 0.398). With the methanol and ethanol extracts, significant reduction in WBC counts were observed 4 days after CP treatment ($t_{4(1)} = 11.852$, P = 0.000: $t_{4(1)} = 21.261$, P = 0.000). The two extracts caused the WBC counts to significantly rise after 9 days ($t_{4(1)} = -12.357$, P = 0.000 and $t_{4(1)} = -20.750$, P = 0.000). With hexane

extract, a significant drop was seen after CP injection ($t_{4(1)}$ = 11.657, P = 0.000) whereas the extract was ineffective in reversing the decrease in WBC counts following its injection ($t_{4(1)}$ = - 2.465, P = 0.069) (Fig 4.23).

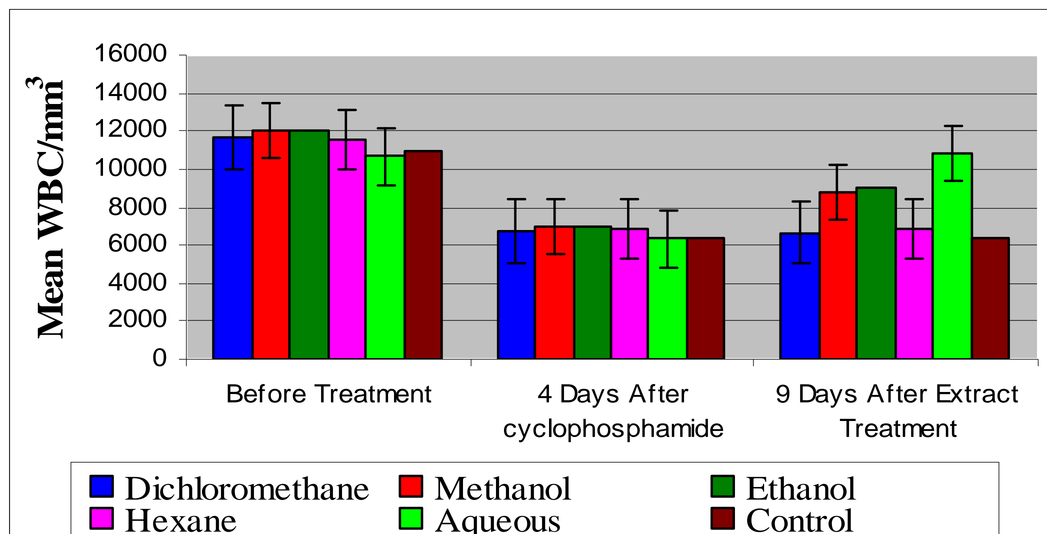

Figure 4. 23: Effect of dichloromethane methanol, ethanol hexane and aqueous extracts of *A. setaceous* roots on WBCs counts in rats treated with cyclophosphamide

***Control group was administered with dimethylsulfoxide**

CP treatment was observed to cause a significant drop in the WBC count 4 days after administering ($t_{5(1)}$ = 15.332, P = 0.000). There was a rise in WBC counts 9 days later after administration of dichloromethane extract of the leaves which was highly significant ($t_{5(1)}$ = -7.683, P = 0.002). With the dichloromethane extracts of *C. volkensii* stem and root, significant reduction in WBC counts were seen 4 days after CP treatment ($t_{5(1)}$ =13.3350, P = 0.000: $t_{5(1)}$ =16.584, P = 0.000). The two extracts

did not have any effect on the WBC counts 9 days after administration ($t_{5(1)}$ = 0.000, P =1.000 and $t_{5(1)}$ = -0.211, P = 0.843). With the dichloromethane extract of *A. setaceous* aerial parts, a significant drop was seen after CP injection ($t_{5(1)}$ = 18.401, P = 0.000) whereas the extract was ineffective in reversing the decrease in WBC counts ($t_{5(1)}$ = 1.847, P = 0.138). The dichloromethane extract of *A. setaceous* root was also ineffective in reversing the decrease in WBC count ($t_{5(1)}$ = - 0.945, P = 0.398) after a significant drop ($t_{5(1)}$ = 11.885, P = 0.000) following CP treatment (Fig 4.24)

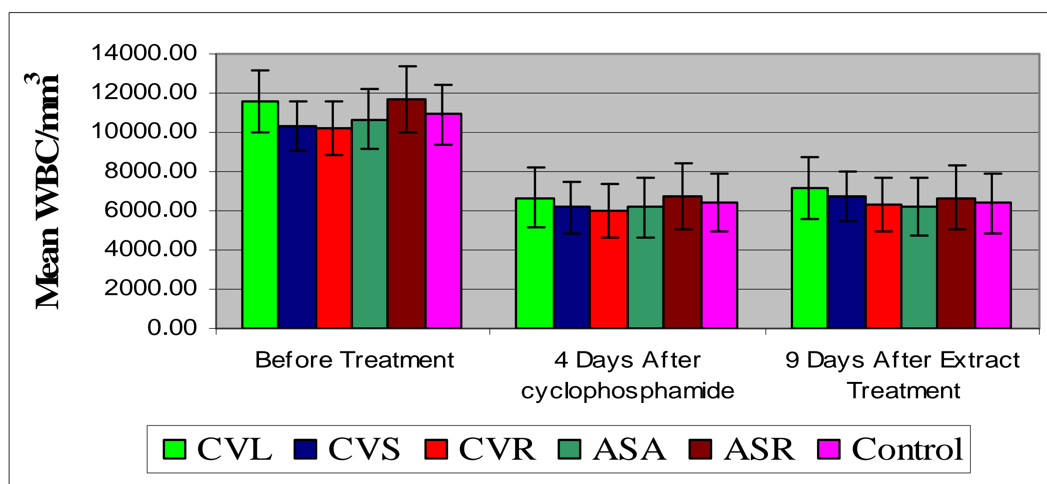

Figure 4. 24: Effects of dichloromethane extracts of *C. volkensii* and *A. setaceous* on WBC counts in rats treated with cyclophosphamide

***Control group was administered with dimethylsulfoxide**

A significant drop in the WBC count was observed 4 days after CP treatment, ($t_{5(1)}$ =57.601, P = 0.000). There was a rise in WBC counts 9 days later after the administration of methanol extracts of the *C. volkensii* leaf which was highly significant ($t_{5(1)}$ = -63.515, P = 0.000). With the methanol extracts of *C. volkensii*

stem and root, significant reductions in WBC counts were seen 4 days after CP treatment ($t_{5(1)}$ =10.021, P = 0.000: $t_{5(1)}$ = 22.145, P = 0.000). The two extracts caused an increase in WBC counts 9 days after administration ($t_{5(1)}$ = -10.772, P = 0.000 and $t_{5(1)}$ = -8.929, P = 0.001). With the methanol extract of *A. setaceous* aerial part, a significant drop was seen after CP injection ($t_{5(1)}$ = 15.821, P = 0.000) whereas the extract was very effective in reversing the decrease in WBC counts following CP injection ($t_{5(1)}$ = 13.534, P = 0.000). The methanol extract of A. setaceous root was also effective in reversing the decrease in WBC count ($t_{5(1)}$ = - 12.357, P = 0.000) after a significant drop ($t_{5(1)}$ = 11.852, P = 0.000) following CP treatment (Fig 4.25).

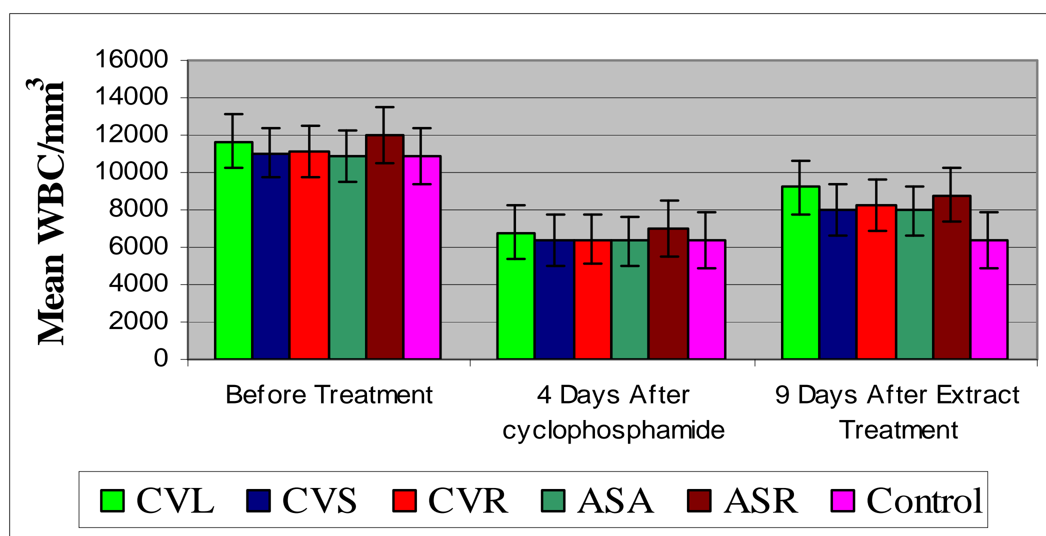

Figure 4. 25: Effects of methanol extracts of *C. volkensii* and *A. setaceous* on WBCs counts in rats treated with cyclophosphamide

Four days after CP treatment, there was a significant drop in the WBC count ($t_{5(1)}$ =16.437, P = 0.000). There was a rise in WBC counts 9 days later after the administration of ethanol extracts of the *C. volkensii* leaf which was highly significant ($t_{5(1)}$ = -13.501, P = 0.000). With the ethanol extracts of *C. volkensii* stem and

root, significant reduction in WBC counts were seen 4 days after CP treatment ($t_{5(1)}$ =11.925, P = 0.001: $t_{5(1)}$ =12.590, P = 0.000). The root extracts caused the WBC counts to rise significantly 9 days after their administration ($t_{5(1)}$ = -10.177, P = 0.001) while the stem extract did not have an effect ($t_{5(1)}$ = -1.823, P = 0.142). With the ethanol extract of *A. setaceous* aerial part, a significant drop was seen after CP injection ($t_{5(1)}$ = 14.348, P = 0.000) whereas the extract was very effective in reversing the decrease in WBC counts following CP injection ($t_{5(1)}$ = -16.571, P = 0.000). The ethanol extract of *A. setaceous* root was also effective in reversing the decrease in WBC count ($t_{5(1)}$ = - 20.750, P = 0.000) after a significant drop ($t_{5(1)}$ = 21.261, P = 0.000) following CP treatment (Fig 4.26).

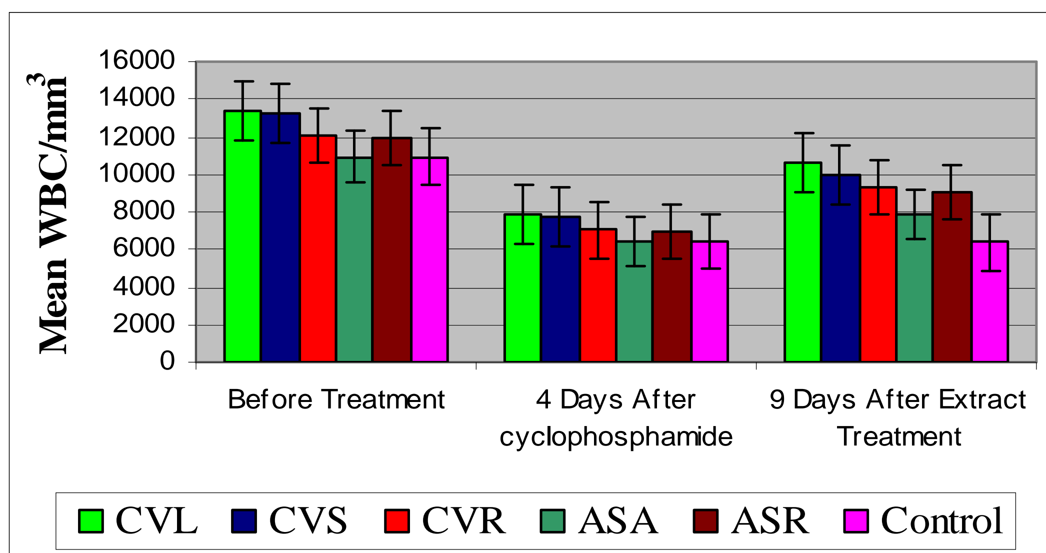

Figure 4. 26: Effects of ethanol extracts of *C. volkensii* and *A. setaceous* on WBCs counts in rats treated with cyclophosphamide

Four days following CP treatment, there was a significant drop in the WBC count

($t_{5(1)}$ =21.414, P = 0.000). There was no effect on WBC counts 9 days later after administration of hexane extracts of *C. volkensii* leaf ($t_{5(1)}$ = -0.861, P = 0.438). With the hexane extracts of *C. volkensii* stem and root, significant reduction in WBC counts were seen 4 days after CP treatment ($t_{5(1)}$ =14.403, P = 0.000: $t_{5(1)}$ = 21.457, P = 0.000). The two extracts did not cause any rise in the WBC counts 9 days after administration ($t_{5(1)}$ = -0.154, P = 0.885 and $t_{5(1)}$ = -1.264, P = 0.275). With the hexane extracts of *A. setaceous* aerial part, a significant drop was seen after CP injection ($t_{5(1)}$ = 24.348, P = 0.000) whereas the extract was very ineffective in reversing the decrease in WBC counts following CP injection ($t_{5(1)}$ = -0.512, P = 0.636). The hexane extract of *A. setaceous* root was also ineffective in reversing the decrease in WBC count ($t_{5(1)}$ = - 2.465, P = 0.069) after a significant drop ($t_{5(1)}$ = 11.657, P = 0.000) following CP treatment (Fig 4.27).

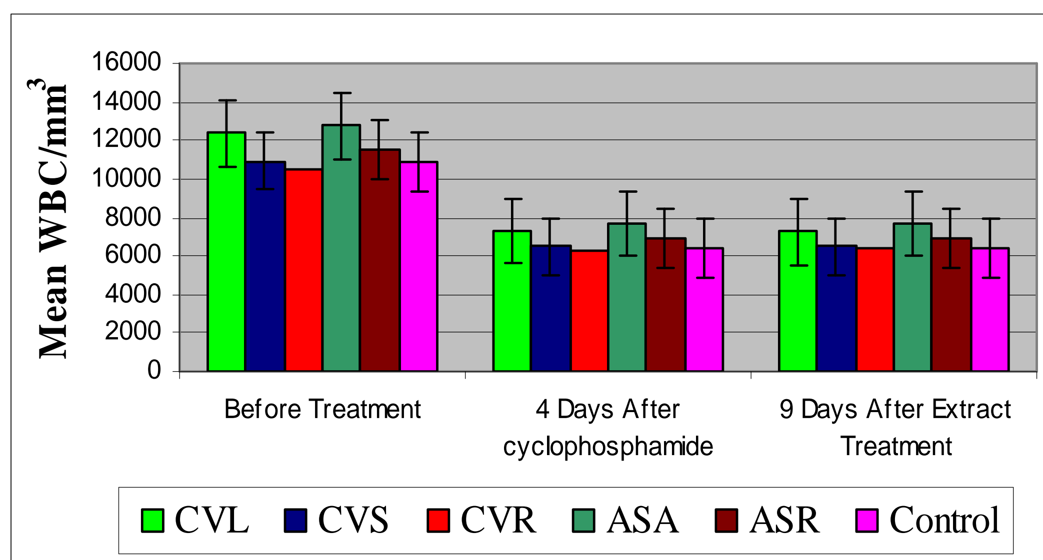

Figure 4. 27: Effects of hexane extracts of *C. volkensii* and *A. setaceous* on WBC counts in rats treated with cyclophosphamide

***Control group was administered with dimethylsulfoxide**

4.8. Assessing the time taken by methanolic, ethanolic and aqueous extracts of *C. volkensii* and *A. setaceous* in reversing the effects of cyclophosphamide

WBC count was done before any treatment, then on the fourth day after CP treatment. WBC counts were again performed starting on the 3RD day, and then on the 6TH, 9TH, 12TH and 15th day after extracts treatment. One group was injected with lithium carbonate and acted as positive control. CP injection caused the WBC counts to drop to 6000cells/ mm^3 in all groups of experimental animals and this was found to be statistically significant (ANOVA, $F_{6, 42} = 6.4594$, P=0.0001). It was observed that the methanolic extracts of C. volkensii and A. setaceous were effective in reversing a decline in WBC counts induced by CP injection. Most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, $F_{6, 42} =5.7807$, P=0.0002). Lithium carbonate took a few days in reversing the suppressive effect of CP. The control group injected with CP only experienced decline in WBC counts with levels going below 2500 cells/mm^3 by day 12 (Fig 4.28).

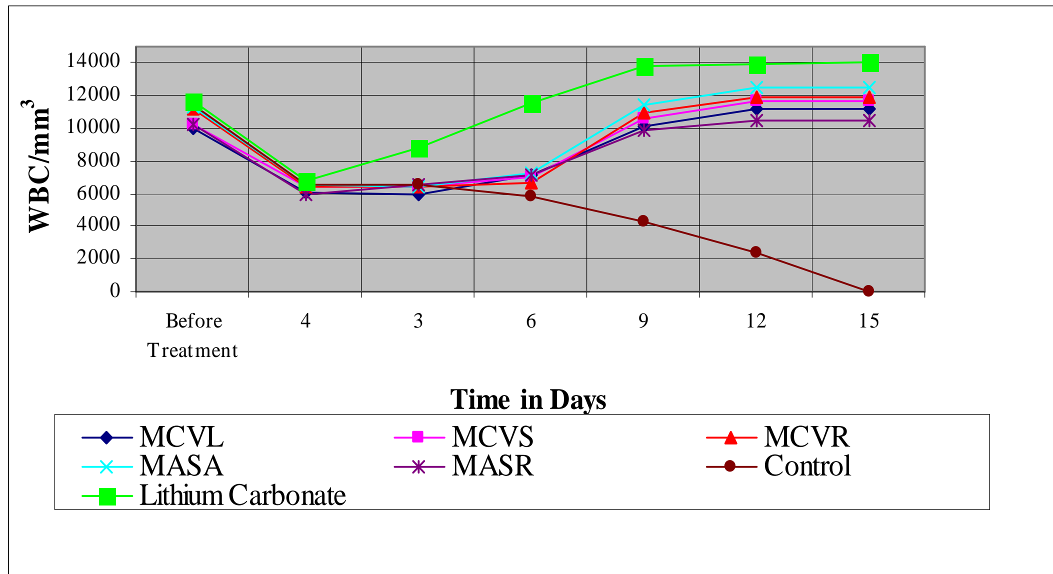

Figure 4. 28: Effects of methanol extracts of *A. setaceous* and *C. volkensii* on cyclophosphamide treated rats

***Control group was administered with dimethylsulfoxide while group administered lithium carbonate acted as positive control**

CP injection in groups of rats resulted in a significant drop in WBC counts to about 6000cells/mm^3 after 4 days and this reduction was observed to be statistically significant (ANOVA, $F_{6, 42}$ = 6.86105, P=0.0000). The ethanolic extracts of the two plants and lithium carbonate were very effective in reversing the decline in WBC counts following CP injection. Lithium carbonate took few days in reversing the suppressive effect of CP whereas most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, $F_{6, 42}$ = 6.40513, P=0.0001). There was a rapid decline in WBC counts of the negative control group of rats which never recovered from CP treatments with their WBC

counts going below 2500mm^3 by day 12 (Fig 4.29).

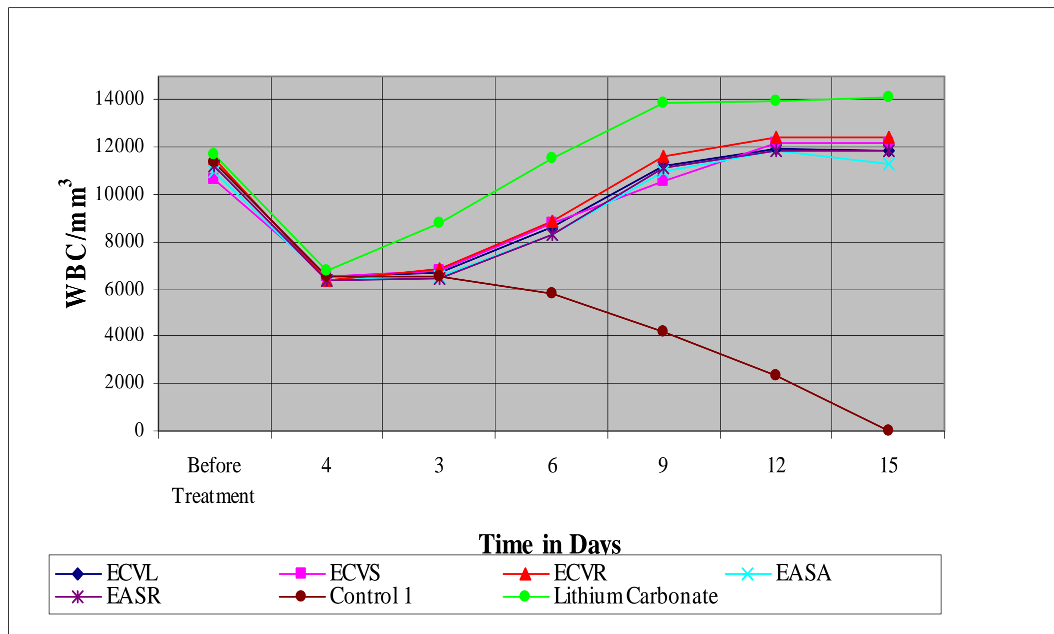

Figure 4.29: Effects of ethanol extracts of *A. setaceous* and *C. volkensii* in cyclophosphamide treated rats

***Control 1 group was administered with dimethylsulfoxide while group administered lithium carbonate acted as positive control**

A drop in WBC counts was seen following CP injection (ANOVA, F6, 42(1) =5.772427, P=0.0002). From figure 4.30, it can be seen that the aqueous extracts and lithium carbonate were very effective in reversing a decline in WBC counts and lithium carbonate took a few days in reversing the suppressive effect of CP whereas most of the extracts had reversed the negative effect of CP drugs by day 9 which was also found to be statistically significant (ANOVA, F6, 42(1) =7.080849, P=0.000049). There was a rapid decline in the negative control group with WBC

counts levels going below 2500 mm^3 by day 12.

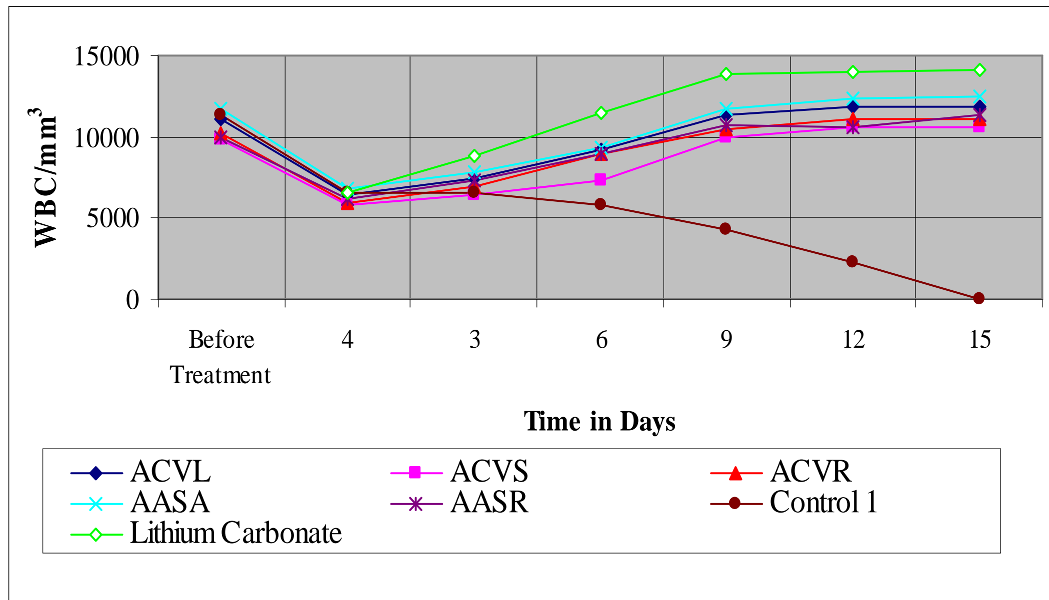

Figure 4. 30: Effects of aqueous extracts of *A. setaceous* and *C. volkensii* in cyclophosphamide treated rats

4.9. Evaluating the effect of extracts of *C. volkensii* and *A. setaceous* against the myelosuppressive action of cyclophosphamide in pretreated rats

The extracts of aqueous, ethanol and methanol of *C. volkensii* leaves, stem and roots and A. setaceous aerial parts and roots were also evaluated on their ability to protect pretreated rats against cyclophosphamide induced myelosuppression and to determine whether pretreatment is necessary. The methanolic extracts of *C. volkensii* and *A. setaceous* caused a rise in WBC counts by day 9 following their administration. Cyclophosphamide treatment caused a drop in the WBC counts three days later in all groups of experimental animals which then remained almost at the same level at day

6, 9 and 12. When compared to the negative control, the extracts performed well in protecting the rats from the effects of CP. Lithium carbonate proved to be superior to all the extracts (Fig 4.31).

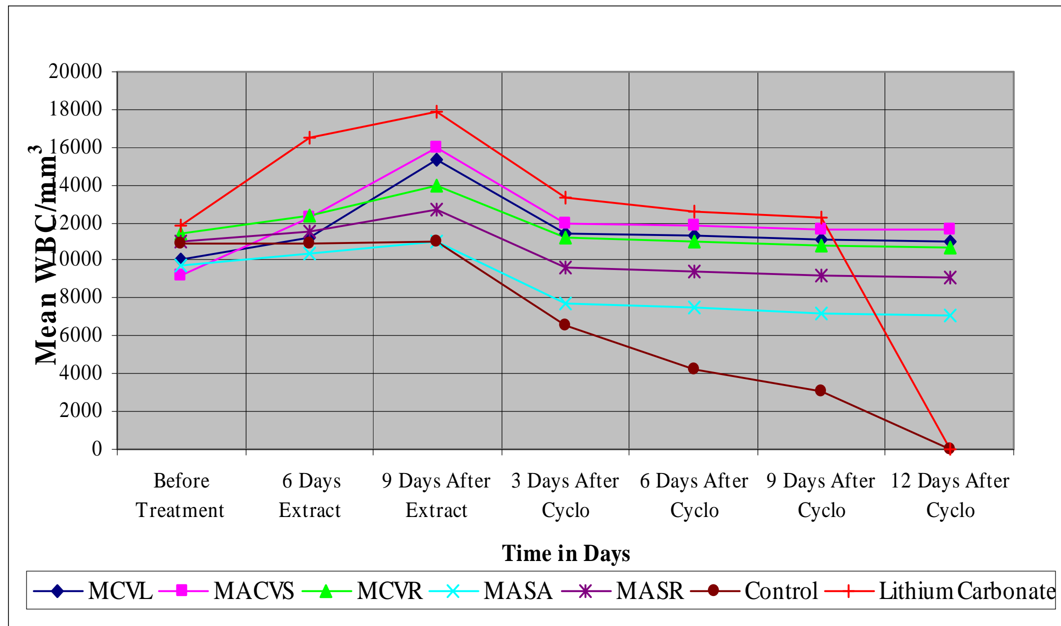

Figure 4. 31: The effect of methanolic extracts of *C. volkensii* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pretreated rats

***Control group was administered with dimethylsulfoxide while group administered lithium carbonate acted as positive control**

All the ethanolic extracts caused an increase in WBC counts by day 9 (Fig 4.32). Cyclophosphamide treatment caused a drop in the WBC counts 3 days after its administration. For most methanolic extracts WBC counts then stabilized by day 6 and remained at the same level at day 9 and 12. WBC counts decreased to very low levels in the negative control group with the count dropping to about 3000 cells/mm^3

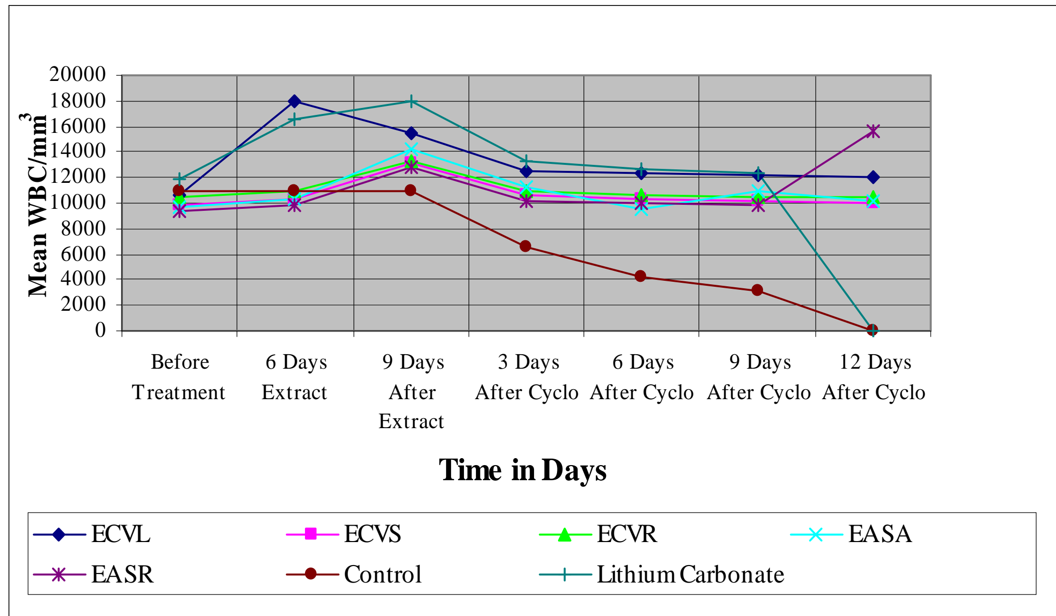

Figure 4. 32: The effect of ethanolic extracts of *C. volkensii* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pretreated rats

***Control group was administered with dimethylsulfoxide while group administered lithium carbonate acted as positive control**

All the aqueous extracts caused a rise in WBC counts by day 9. Cyclophosphamide treatment caused a drop in the WBC counts 3 days after its injection and this continued to drop slightly but had stabilizes by day 6 and then remained stable at day 9 and 12. WBC count at day 12 after cyclophosphamide injection was almost at the same level of WBC counts at the beginning of the experiments before the rats were treated with any extracts. This was an indication that the aqueous extracts could actively protect the rats from the suppressive effects of CP (Fig 4.33).

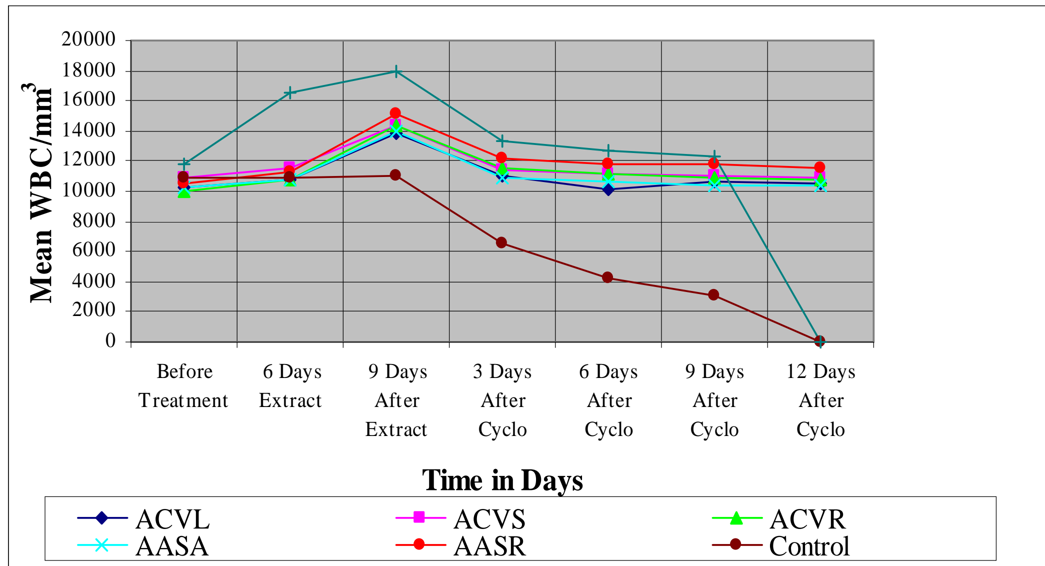

Figure 4. 33: The effect of aqueous extracts of *C. volkensii* and *A. setaceous* against myelosuppression induced by cyclophosphamide in pretreated rats

***Control group was administered with dimethylsulfoxide while group administered lithium carbonate acted as positive control**

4.10. The effect of varying the different concentrations of aqueous, ethanol and methanol extracts on percent neutrophil adhesion

In enhancement of percent neutrophil adhesion, the leaf aqueous extracts of *C. volkensii* was found to be very effective at all the concentrations followed by the root extracts which was active as from concentration of 250mg/kg. The stem extract was only active at higher doses of 500mg/kg and above (Fig 4.34). This dose- dependent enhancement was found to be significant for ACVL (ANOVA, $F_{4, 20}$= 54.2811, P = 0.0000), ACVS (ANOVA, $F_{4, 20}$ = 119.7087, P = 0.0000) and ACVR (ANOVA, $F_{4, 20}$ = 264.9691, P = 0.0000).

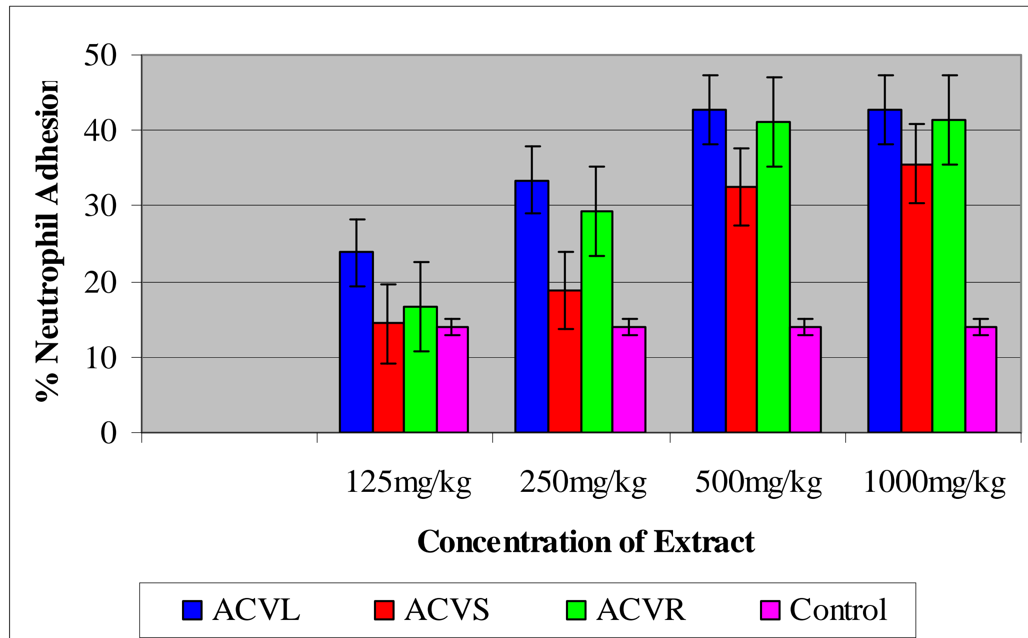

Figure 4.34: The effects of aqueous extracts of *C. volkensii* leaf, stem and root on neutrophil adhesion

***Control group was administered with dimethylsulfoxide**

Comparison analysis indicated that the aqueous extracts of aerial parts and roots of *A. setaceous* were active from concentrations of 250mg/kg and their activity increased with the increasing doses of 500mg/kg and 1000mg/kg though there was no difference between the two higher doses (Fig 4.35). The extracts were inactive at the lowest dose tested. Dose enhancement was found to be statistically significant for AASA (ANOVA, $F_{4,\ 20}$ = 175.682, P= 0.0000) and AASR (ANOVA, $F_{4,\ 20}$= 273.7494, P = 0.0000)

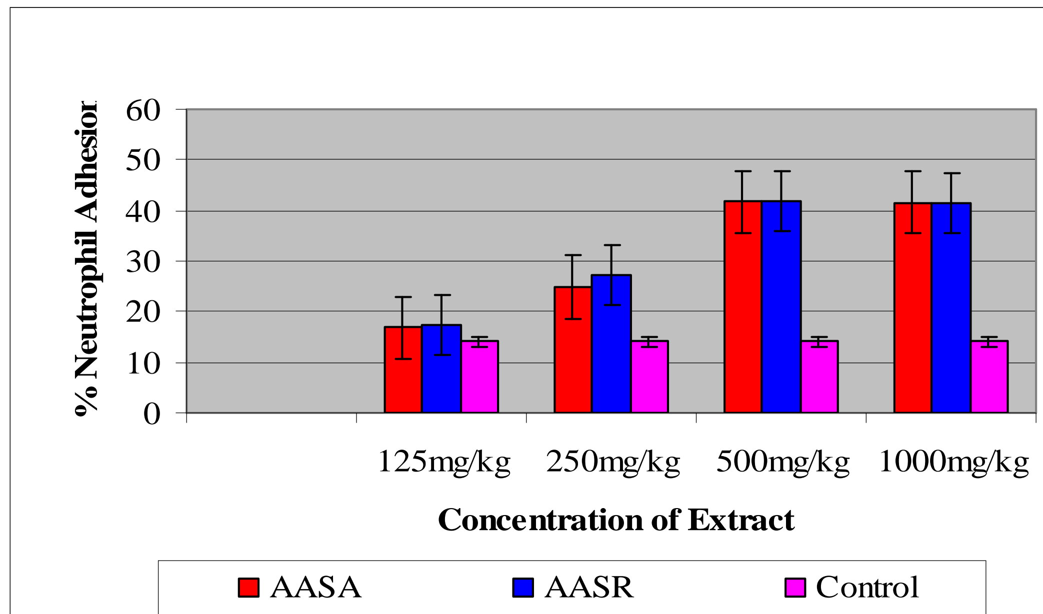

Figure 4. 35: The effects of aqueous extracts of *A. setaceous* aerial part and root on neutrophil adhesion

***Control group was administered with dimethylsulfoxide**

An increase in enhancement of neutrophil adhesion with increasing doses was observed with ethanolic extracts of *C. volkensii* and this was found to be significant for ECVL (ANOVA, $F_{4, 20}$ = 205.1109, P = 0.0000), ECVS (ANOVA, $F_{4, 20}$ = 288.4429, P = 0.0000) and ECVR (ANOVA, $F_{4, 20}$ = 167.6529, P = 0.0000). With the ethanol extracts of *C. volkensii* leaf, stem and root, the leaf and the root were observed to be more active and an increase in activity of the two extracts that was dose dependent was seen. The stem was only active at higher doses of 500 and 1000mg/kg (Fig 4.36).

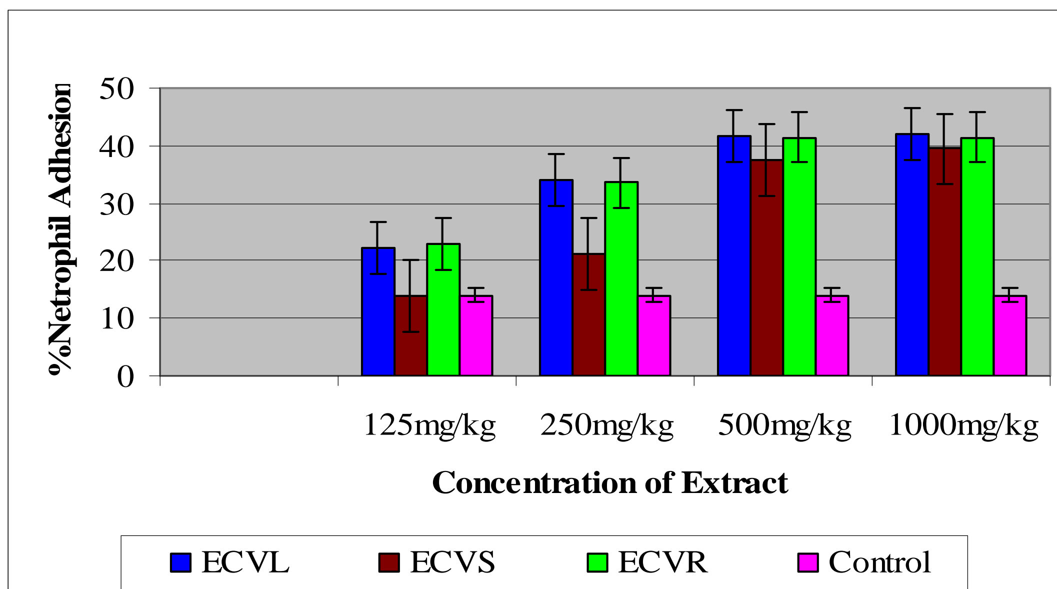

Figure 4.36: The effects of ethanolic extracts of *C. volkensii* leaf, stem and root on neutrophil adhesion

***Control group was administered with dimethylsulfoxide**

Dose enhancement was found to be statistically significant for EASA (ANOVA, $F_{4,\ 20}$ = 151.3929, P= 0.0000) and EASR (ANOVA, $F_{4,\ 20}$= 137.981, P = 0.0000). The ethanolic extracts of *A. setaceous* aerial part and root were effective from concentrations of 250mg/kg and the activity increased with increasing concentrations although the activity of the two higher doses was similar (Fig 4.37).

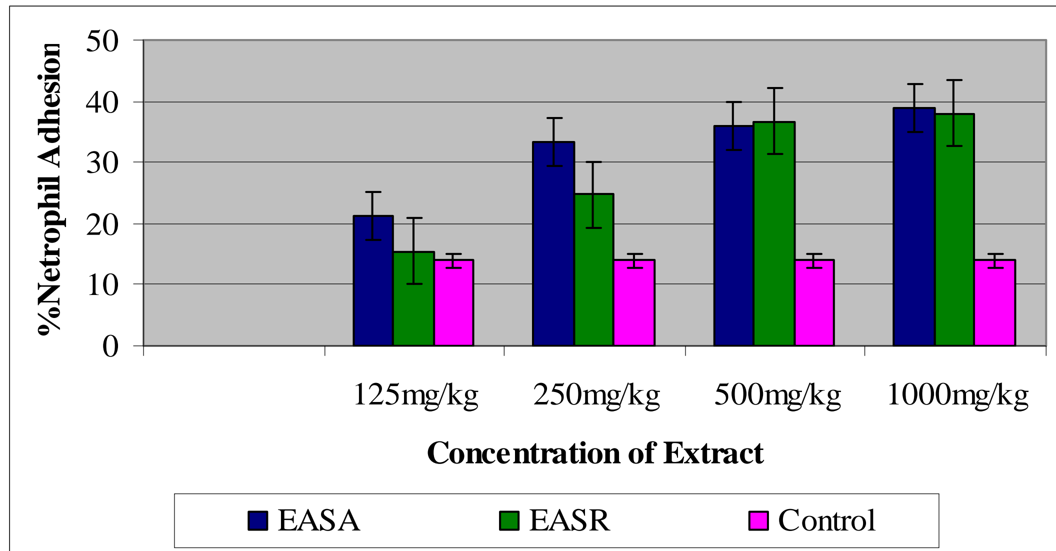

Figure 4. 37: The effects of ethanolic extracts of *A. setaceous* aerial part and root on neutrophil adhesion

A significant dose dependent increase in neutrophil adhesion was observed with MCVL (ANOVA, $F_{4, 20} = 203.9075$, P = 0.0000), MCVS (ANOVA, $F_{4, 20} = 205.8716$, P = 0.0000) and MCVR (ANOVA, $F_{4, 20} = 171.6529$, P = 0.0000). With the methanol extracts of *C. volkensii* leaves, stem and roots, the extracts were active starting from a concentration of 250mg/kg and increasing with increasing concentrations (Fig 4.38).

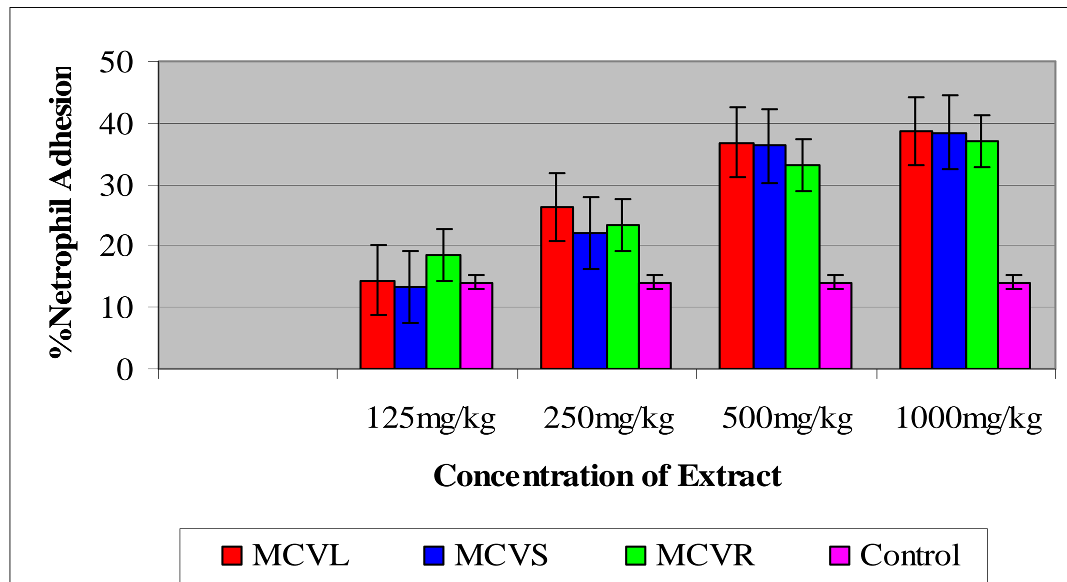

Figure 4.38: The effect of methanolic extracts of *C. volkensii* leaf, stem and root on neutrophil adhesion

***Control group was administered with dimethylsulfoxide**

A significant increase in enhancement of neutrophil adhesion with increasing doses was observed for MASA (ANOVA, $F_{4, 20}$ = 102.5006, P = 0.0000), and MASR (ANOVA, $F_{4, 20}$ = 153.3773, P = 0.0000). The methanolic extracts of *A. setaceous* aerial part and root were effective from a concentration of 250mg/kg and the activity increased with increasing concentration (Fig 4.39).

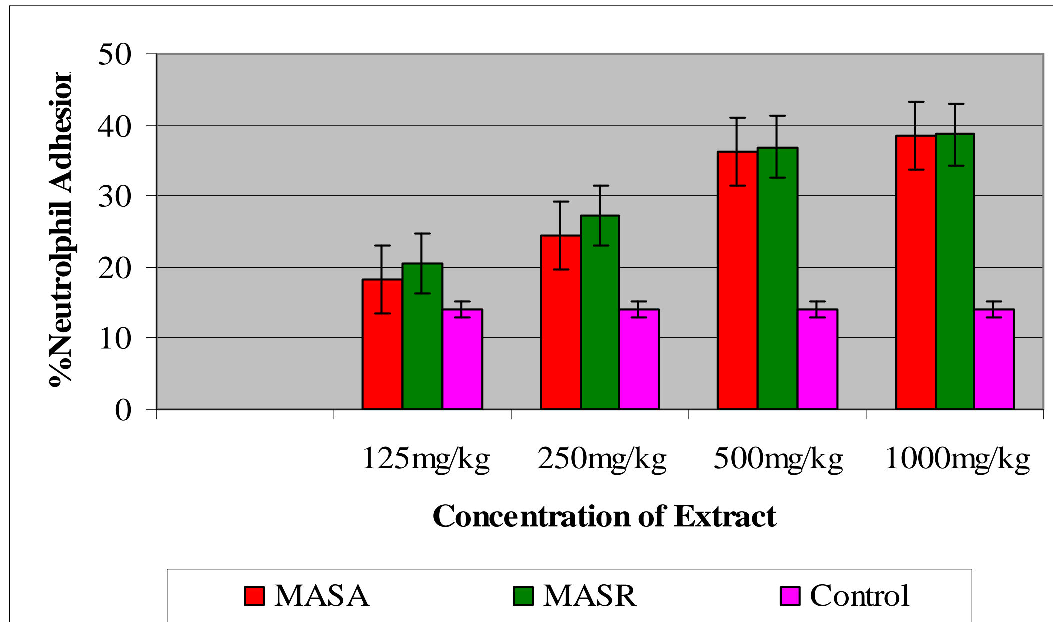

Figure 4.39: The effect of methanolic extracts of *A. setaceous* aerial part and root on neutrophil adhesion

***Control group was administered with dimethylsulfoxide**

4.10.1 The effect of different concentrations of aqueous, ethanol and methanol extracts of *A. setaceous* and *C. volkensii* on phagocytic index

Fig 4.40 shows that the aqueous extracts of *C. volkensii* leaf, stem and root were active at high concentrations of 500 mg/kg and 1000 mg/kg. An increase in enhancement of phagocytic index with increasing doses was observed and this was significant for ACVL (ANOVA, $F_{4, 20} = 130.950$, P = 0.0000), ACVS (ANOVA, $F_{4, 20} = 104.\ 4538$, P = 0.0000) and ACVR (ANOVA, $F_{4, 20} = 132.1633$, P = 0.0000).

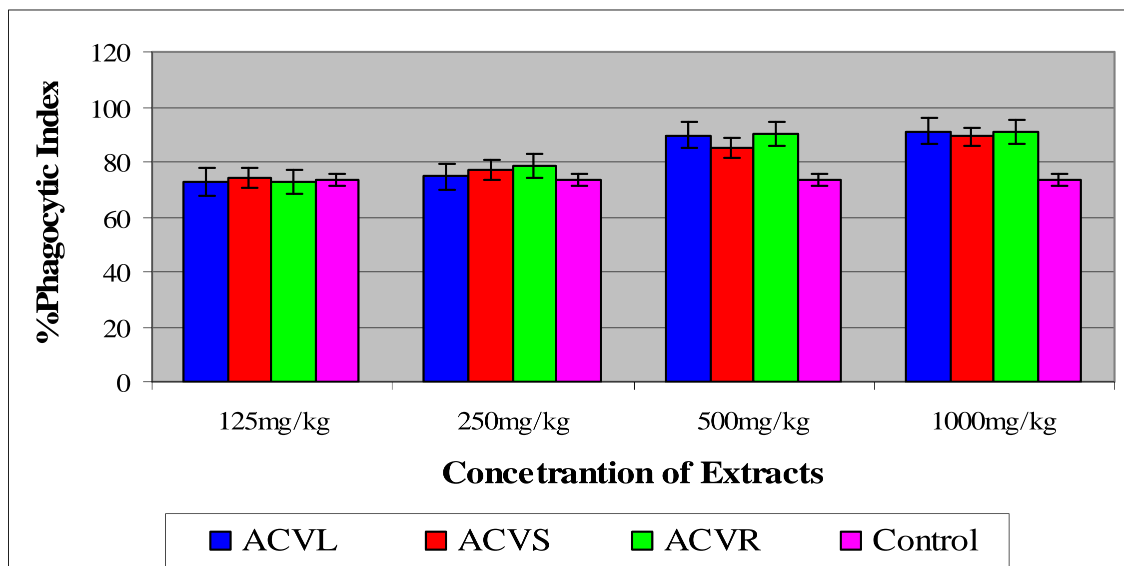

Figure 4.40: The effects of aqueous extracts of *C. volkensii* leaf, stem and root on phagocytic index

***Control group was administered with dimethylsulfoxide**

A significant enhancement of phagocytic index with increasing doses was observed for AASA (ANOVA, $F_{4, 20} = 61.5683$, P = 0.0000), and AASR (ANOVA, $F_{4, 20} = 109.8541$, P = 0.0000). The aqueous extracts of *A. setaceous* aerial part and root were active at higher concentrations of 500 mg/kg and 1000 mg/kg (Fig 4.41).

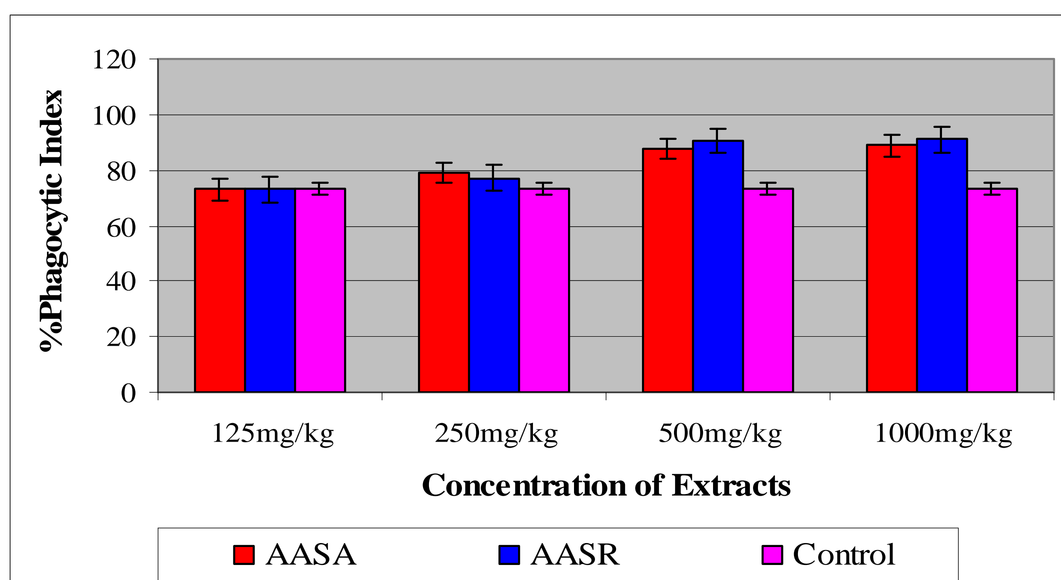

Figure 4. 41: The effects of aqueous extracts of *A. setaceous* aerial part and root on phagocytic index

***Control group was administered with dimethylsulfoxide**

An enhancement of phagocytic index with increasing doses was observed and this was significant for ECVL (ANOVA, $F_{4, 20} = 99.1264$, P = 0.0000), ECVS (ANOVA, $F_{4, 20} = 120.7313$, P = 0.0000) and ECVR (ANOVA, $F_{4, 20} = 107.2378$, P = 0.0000). Fig 4.42 shows that the ethanolic extracts of *C. volkensii* leaf, stem and root were

active at higher concentrations of 500 mg/kg and 1000 mg/kg.

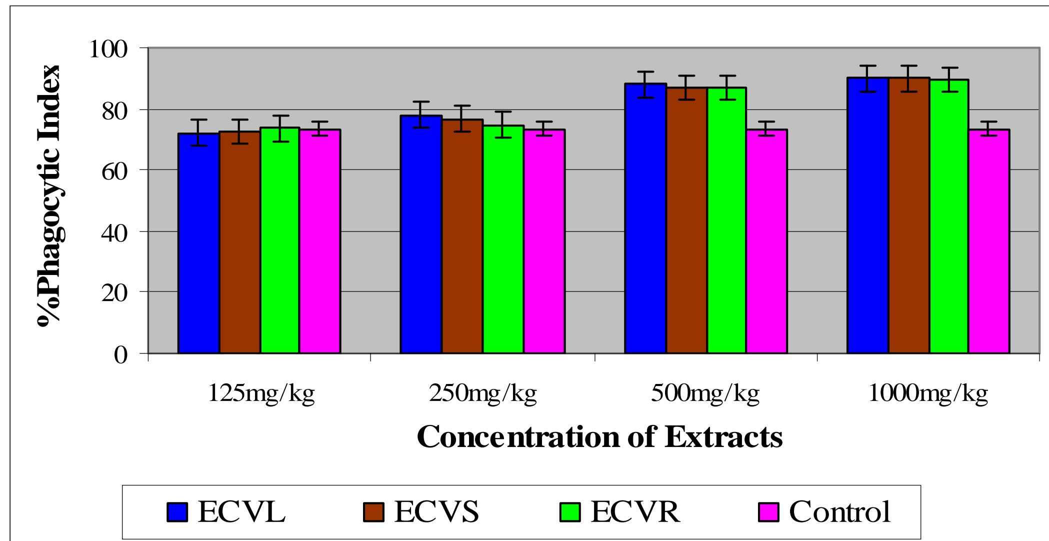

Figure 4. 42: The effect of ethanolic extracts of *C. volkensii* leaf, stem and root on phagocytic index

***Control group was administered with dimethylsulfoxide**

From figure 4.43, an enhancement in phagocytic index with ethanolic extracts of *A. setaceous* aerial parts with increasing doses was observed and this was significant for EASA (ANOVA, $F_{4,\ 20}$ = 127.6892, P = 0.0000) and EASR (ANOVA, $F_{4,\ 20}$ = 72.5988, P = 0.0000).

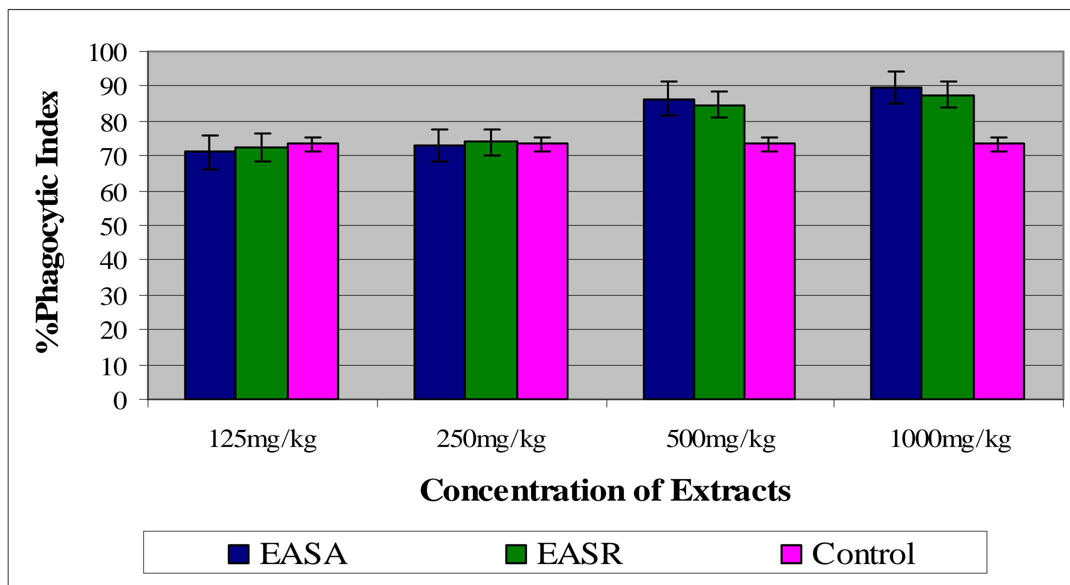

Figure 4. 43: The effects of ethanolic extracts of *A. setaceous* aerial part and root on phagocytic index

A significant enhancement in phagocytic index with increasing doses was observed for MCVL (ANOVA, $F_{4, 20} = 61.3115$, P = 0.0000), MCVS (ANOVA, $F_{4, 20} = 144.8$, P = 0.0000) and MCVR (ANOVA, $F_{4, 20} = 49.2582$, P = 0.0000). The methanolic extracts of *C. volkensii* leaf, stem and root were found to be active at higher concentrations of 500mg/kg and 1000mg/kg (fig 4.44).

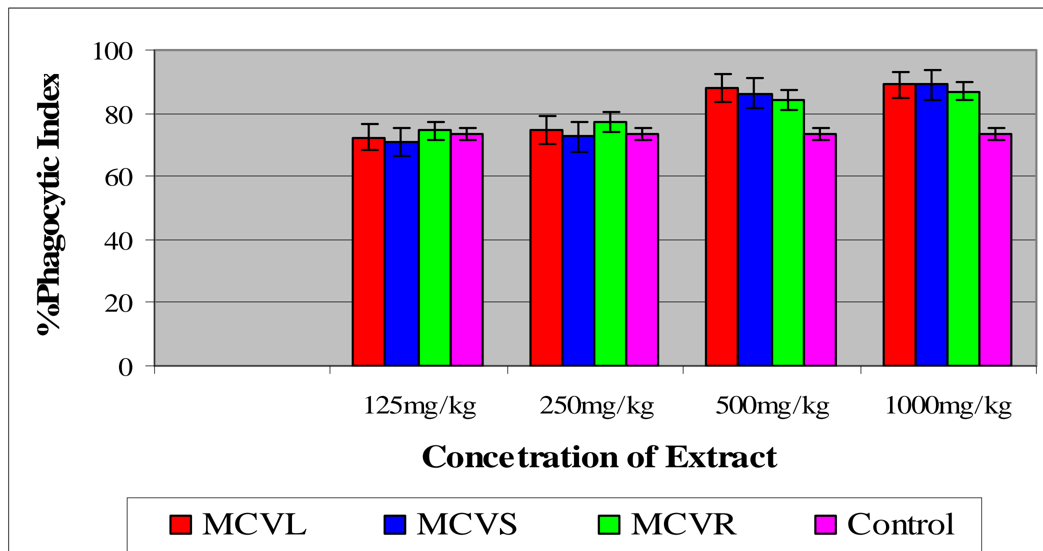

Figure 4. 44: The effects of methanolic extracts of *C. volkensii* leaf, stem and root on phagocytic index

An enhancement in phagocytic index with increasing doses was observed and this was significant for MASA (ANOVA, $F_{4,\ 20}$ = 45. 2043, P = 0.0000) and MASR (ANOVA, $F_{4,\ 20}$ = 107.4149, P = 0.0000). The methanolic extracts of *A. setaceous* aerial part and root were active at higher concentrations of 500 mg/kg and 1000 mg/kg but lacked activity at lower concentrations of 125 and 250 mg/kg (Fig 4.45).

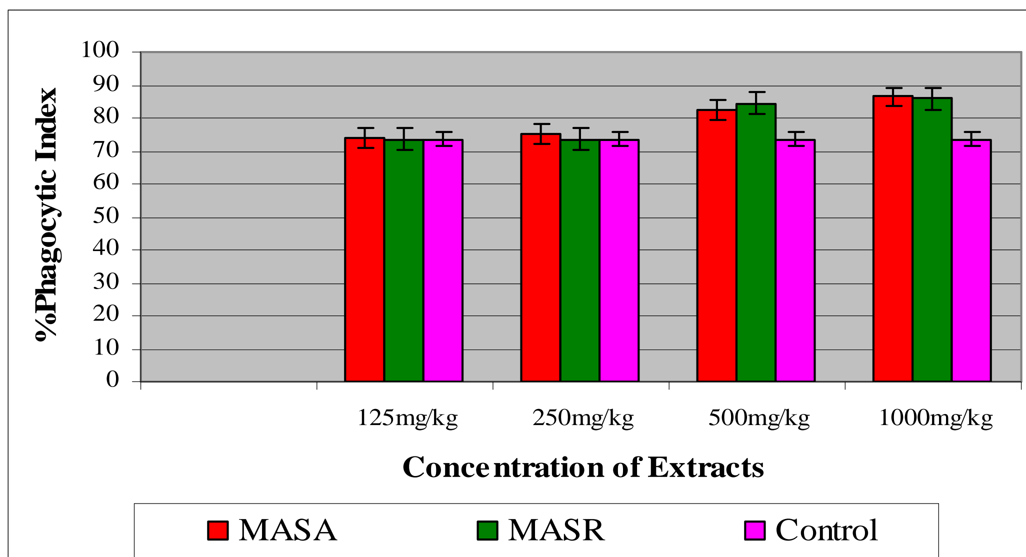

Figure 4.45: The effects of methanolic extracts of *A. setaceous* aerial part and root on phagocytic index

***Control group was administered with dimethylsulfoxide**

4.10.2 The effects of different concentrations of aqueous, ethanol and methanol extracts of *A. setaceous* and *C. volkensii* on NBT reduction by neutrophils

An increase in NBT reduction in a dose dependent manner was observed in rats treated with aqueous extracts of *C. volkensii* leaf, stem and root. This increment was significant for ACVL (ANOVA, $F_{4, 20} = 80.3395$, P = 0.0000), ACVS (ANOVA, $F_{4, 20} = 28.2272$, P = 0.0000) and ACVR (ANOVA, $F_{4, 20} = 101.0944$, P = 0.0000). Although the lower doses of aqueous extracts of *C. volkensii* leaf, stem and root had negligible activity at a concentration of 250 mg/kg, they were active at the higher concentrations used as can be observed in fig 4.46.

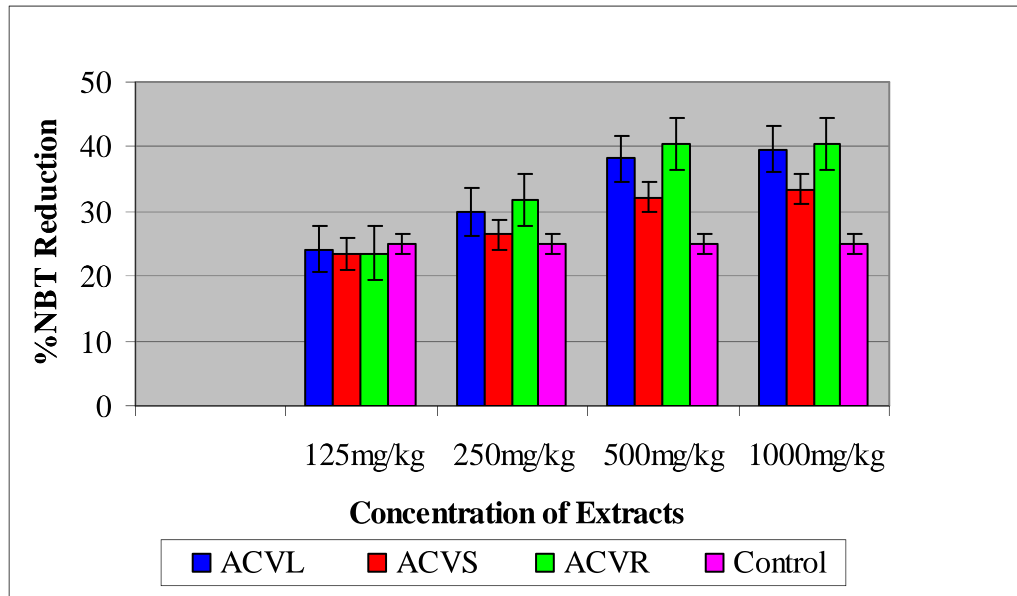

Figure 4. 46: The effects of aqueous extracts of *C. volkensii* leaf, stem and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

A significant increase in NBT reduction in a dose dependent manner was observed in animals orally dosed with aqueous extract of *A. setaceous* aerial part and root for AASA (ANOVA, $F_{4, 20}$ = 110.3097, P = 0.0000) and AASR (ANOVA, $F_{4, 20}$ = 117.7682, P = 0.0000). The aqueous extracts of *A. setaceous* aerial part and root were active at high concentrations of 500 mg/kg and 1000 mg/kg (Fig 4.47).

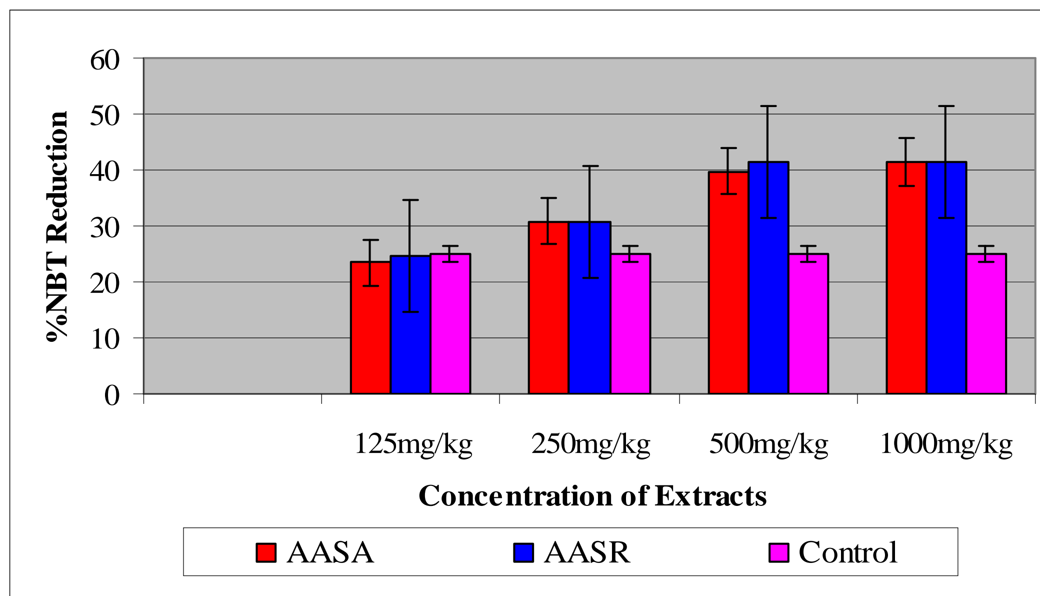

Figure 4.47: The effects of aqueous extracts of *A. setaceous* aerial part and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

An increase in NBT reduction in a dose dependent manner was observed and this was found to be significant for ECVL (ANOVA, $F_{4, 20}$ = 104.4406, P = 0.0000), ECVS (ANOVA, $F_{4, 20}$ = 104.4846, P = 0.0000) and ECVR (ANOVA, $F_{4, 20}$ = 148.422, P = 0.0000). The ethanolic extracts of *C. volkensii* leaf, stem and root were active at higher concentrations of 500 mg/kg and 1000 mg/kg with minimal activity observed at a concentration of 250 mg/kg while the lowest dose of 125 mg/kg used proved to be very ineffective (Fig 4.48).

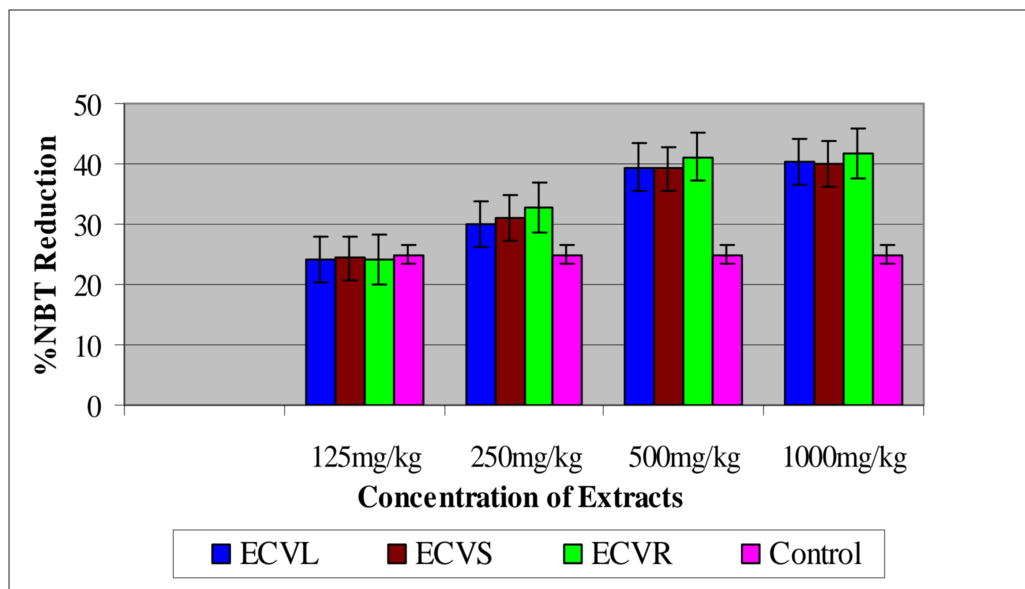

Figure 4. 48: The effects of ethanolic extracts of *C. volkensii* leaf, stem and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

In rats treated with ethanolic extracts of *A. setaceous* aerial part and root, an increase in NBT reduction in a dose dependent manner was observed and this was found to be significant for EASA (ANOVA, $F_{4, 20} = 102.0724$, P = 0.0000), EASR (ANOVA, $F_{4, 20} = 101.5089$, P = 0.0000). The ethanolic extracts of *A. setaceous* aerial part and root were observed to be active at higher concentrations of 500 mg/kg and 1000 mg/kg with minimal activity also observed with the dose of 250 mg/kg but not with the 125 mg/kg dose (Fig 4.49).

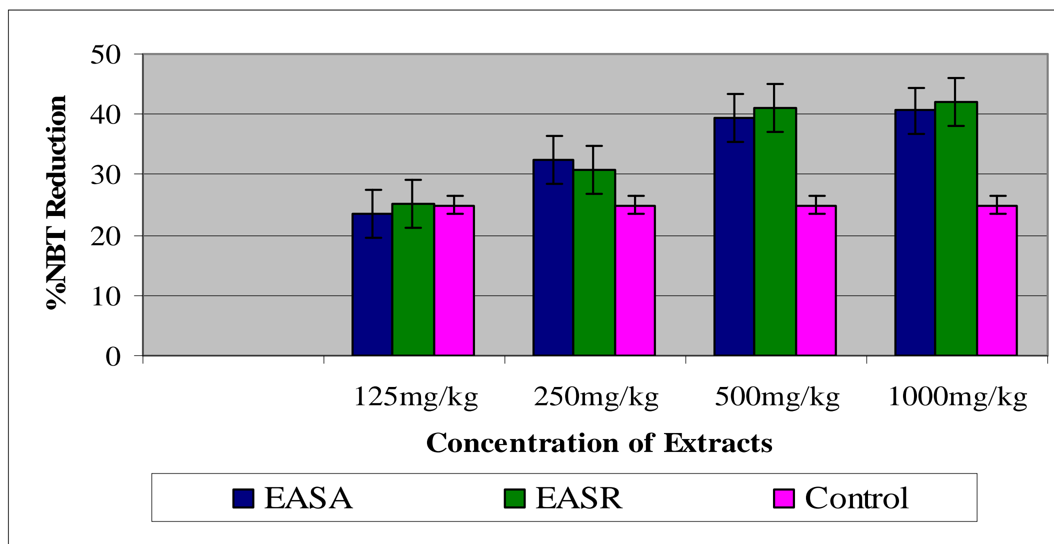

Figure 4. 49: The effects of ethanolic extracts of *A. setaceous* aerial part and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

An increase in NBT reduction in a dose dependent manner was observed in group of rats treated with methanolic extracts of *C. volkensii* leaf, stem and root and this was found to be significant for MCVL (ANOVA, $F_{4, 20}$ = 122.7248, P = 0.0000), MCVS (ANOVA, $F_{4, 20}$ = 119.2754, P = 0.0000) and MCVR (ANOVA, $F_{4, 20}$ = 45.1532, P = 0.0000). The methanolic extracts of *C. volkensii* leaf, stem and root were active at higher concentrations of 500 mg/kg and 1000 mg/kg but also showed activity at a dose of 250 mg/kg but were ineffective at the lowest dose of 125 mg/kg. The leaf extract was the most active, followed by the stem and finally the root extracts (Fig 4.50).

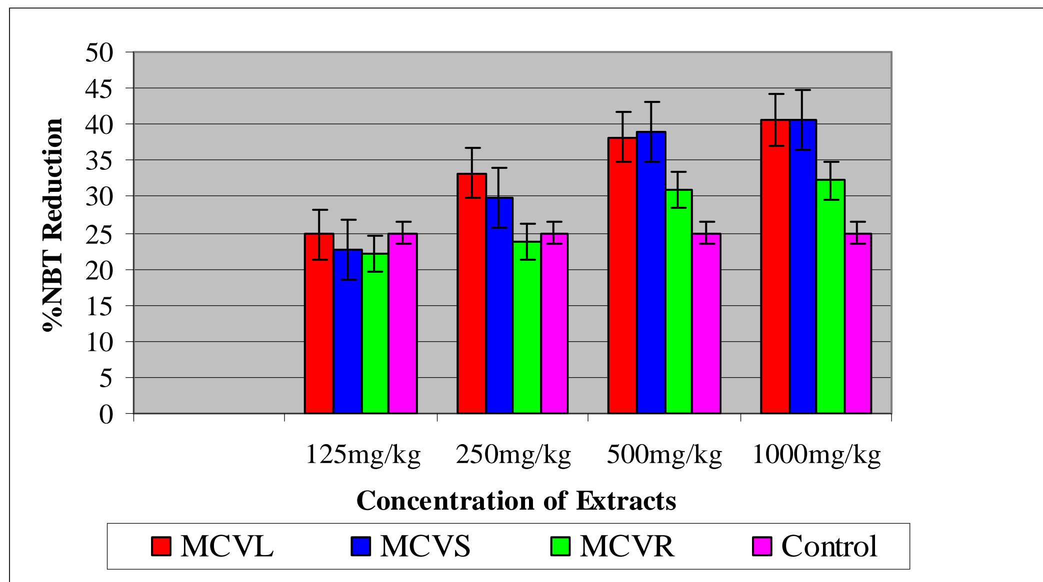

Figure 4. 50: The effects of methanolic extracts of *C. volkensii* leaf, stem and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

In rats treated with methanolic extracts of *A. setaceous* aerial part and root, a significant increase in NBT reduction that was dose dependent was also observed, MASA (ANOVA, $F_{4, 20}$ = 63.0937, P = 0.0000) and MASR (ANOVA, $F_{4, 20}$ = 66.9780, P = 0.0000). The methanolic extracts of *A. setaceous* aerial parts and roots were active at higher concentrations of 500 mg/kg and 1000 mg/kg with minimal activity at lower concentrations (Fig 4.51).

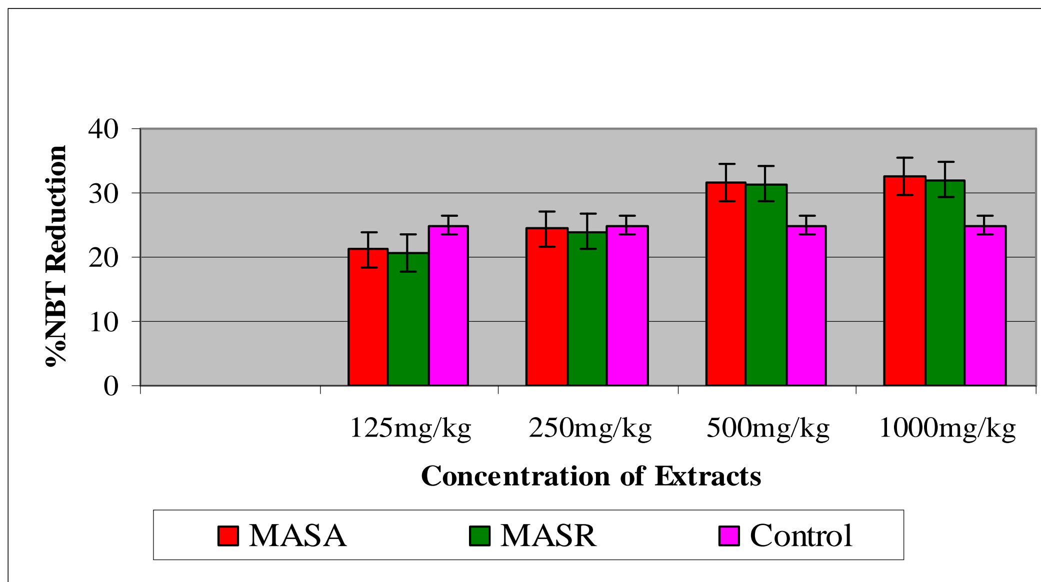

Figure 4. 51: The effects of methanolic extracts of *A. setaceous* aerial part and root on NBT reduction

***Control group was administered with dimethylsulfoxide**

4.11. The effect of aqueous, ethanol and methanol extracts of *C. volkensii* and *A. setaceous* on macrophage phagocytic activity

An increase in macrophage phagocytosis that was dose dependent was observed and this was found to be significant for ACVL (ANOVA, $F_{4,\ 20} = 529.2593$, P = 0.0000), ACVS (ANOVA, $F_{4,\ 20} = 130.3248$, P = 0.0000) and ACVR (ANOVA, $F_{4,\ 20} = 238.4524$, P = 0.0000). Aqueous extracts of C. volkensii leaf, stem and root were very effective at higher doses of 500 mg/kg and above but not at 250 mg/kg and below (Fig 4.52).

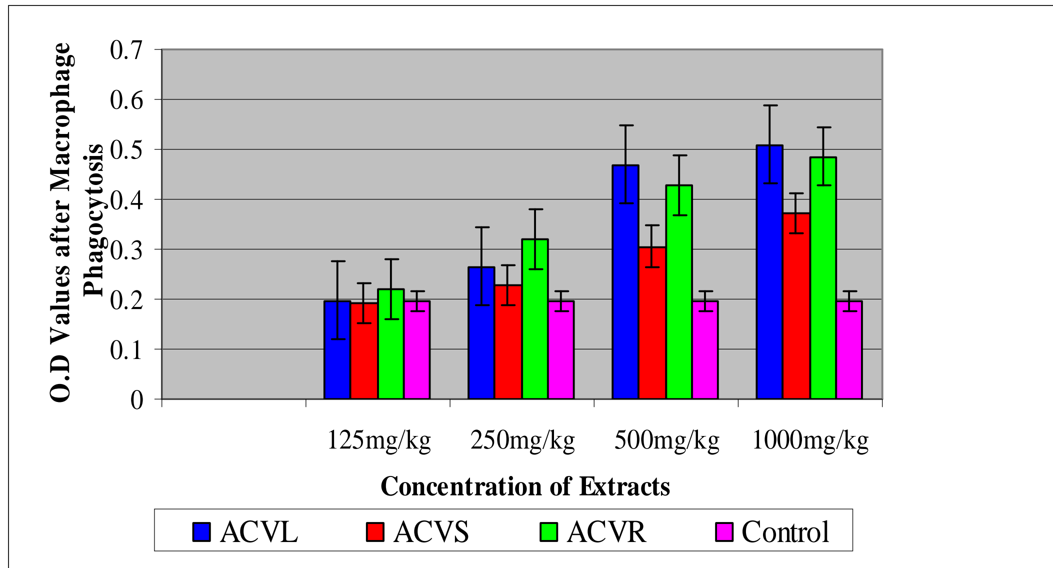

Figure 4. 52: The effects of aqueous extracts of *C. volkensii* leaf, stem and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

There was a significant increase in macrophage phagocytosis that was dose dependent in rats treated with *A. setaceous* aerial part and root, AASA (ANOVA, $F_{4, 20}$ = 268.5613, P = 0.0000) and AASR (ANOVA, $F_{4, 20}$ = 384.5, P = 0.0000). Aqueous extracts of A. setaceous aerial part and root were also active at higher doses (Fig 4.53).

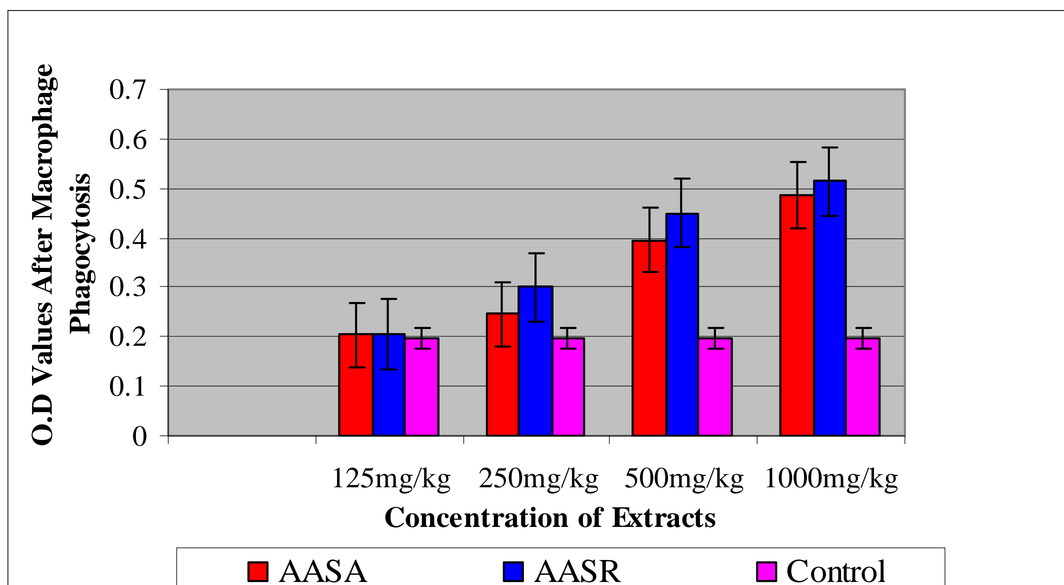

Figure 4. 53: The effects of aqueous extracts of *A. setaceous* aerial part and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

For rats orally dosed with ethanolic extracts of *C. volkensii* leaf, stem and root, an increase in macrophage phagocytosis that was dose dependent was observed and this was found to be significant for ECVL (ANOVA, $F_{4, 20}$ = 313.8789, P = 0.0000), ECVS (ANOVA, $F_{4, 20}$ = 155.2823, P = 0.0000) and ECVR (ANOVA, $F_{4, 20}$ = 322.6607, P = 0.0000). Ethanolic extracts of *C. volkensii* leaf, stem and root were also only active at higher concentrations with the root and leaves being the most active (Fig 4.54).

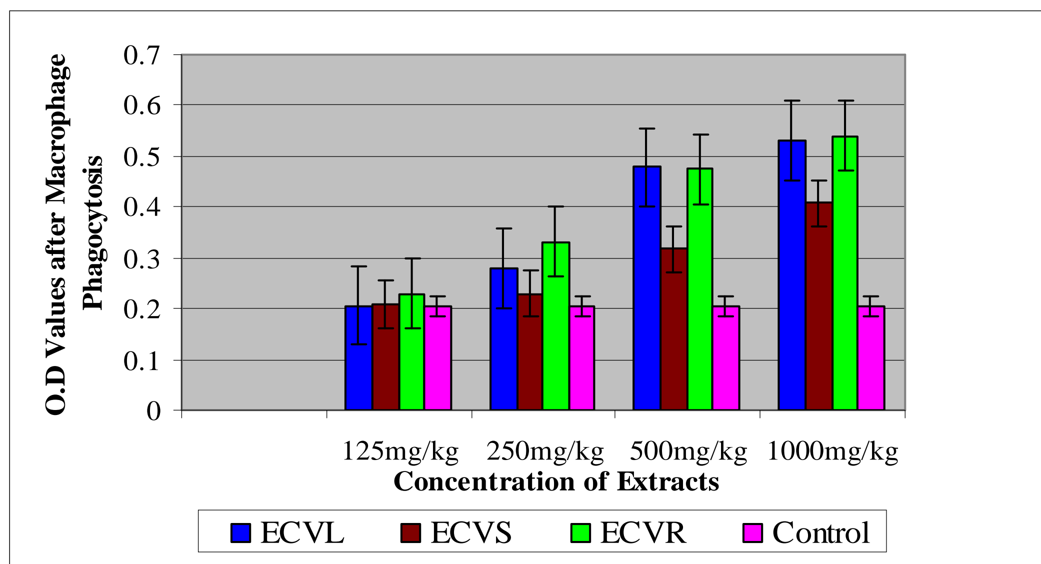

Figure 4. 54: The effects of ethanol extracts of *C. volkensii* leaf, stem and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

An increase in macrophage phagocytosis in rats treated with ethanolic extracts of A. *setaceous* aerial part and root that was dose dependent was observed and this was found to be significant for EASA (ANOVA, $F_{4, 20} = 341.2467$, P = 0.0000) and EASR (ANOVA, $F_{4, 20} = 265.7654$, P = 0.0000). Ethanol extracts of *A. setaceous* aerial part and root enhanced macrophage phagocytosis at higher concentrations but also showed enhancement at a dose of 250 mg/kg (Fig 4.55).

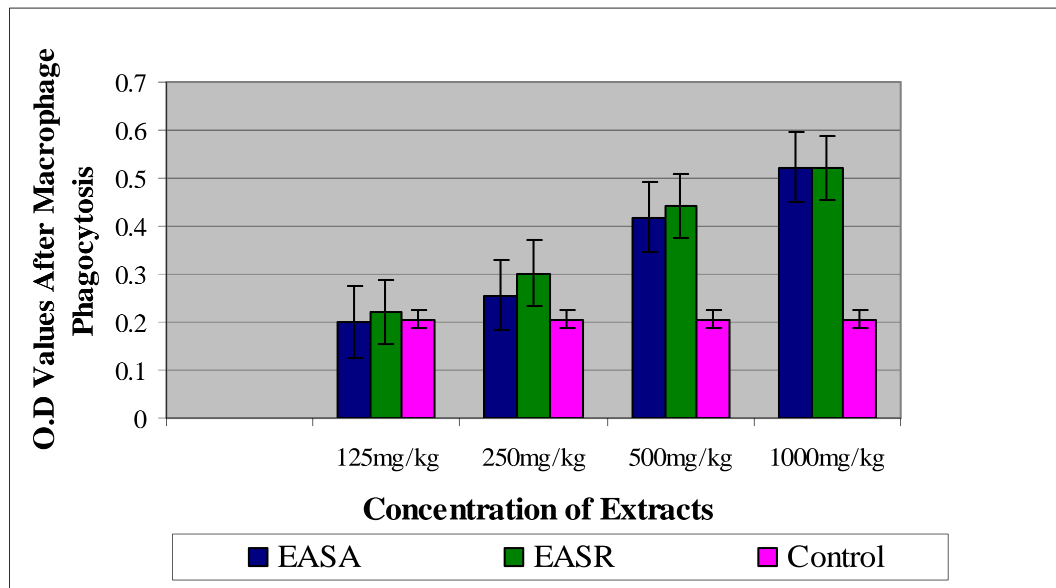

Figure 4. 55: The effects of ethanol extracts of *A. setaceous* aerial part and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

A significant increase in macrophage phagocytosis that was dose dependent was observed in rats treated with methanolic extracts *C. volkensii* leaf, stem and root. This for MCVL (ANOVA, $F_{4, 20}$ = 74.0680, P = 0.0000), MCVS (ANOVA, $F_{4, 20}$ = 74.0680, P = 0.0000) and MCVR (ANOVA, $F_{4, 20}$ = 310.3072, P = 0.0000). Methanol extracts of *C. volkensii* leaf, stem and root showed higher activity at higher concentrations of 500 and 1000 mg/kg (fig 4.56).

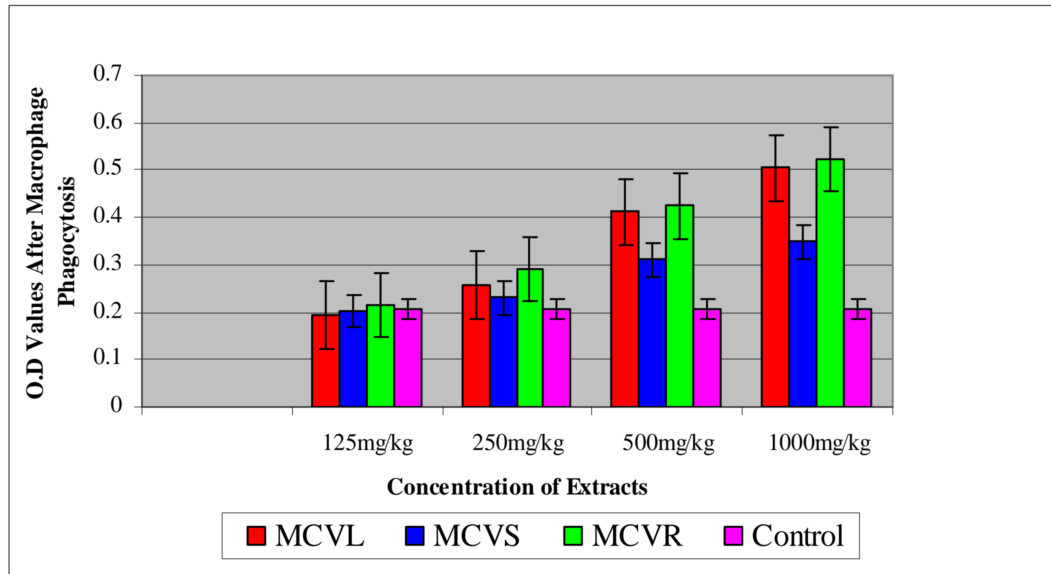

Figure 4. 56: The effects of methanol extracts of *C. volkensii* leaf, stem and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

A significant increase in macrophage phagocytosis that was dose dependent was also observed with rats treated with methanol extracts of *A. setaceous* aerial part and root, MASA (ANOVA, $F_{4, 20} = 292.4379$, $P = 0.0000$) and MASR (ANOVA, $F_{4, 20} = 274.6879$, $P = 0.0000$). Methanol extracts of *A. setaceous* aerial part and root enhanced macrophage phagocytosis at higher concentrations of 500 and 1000 mg/kg (Fig 4.57).

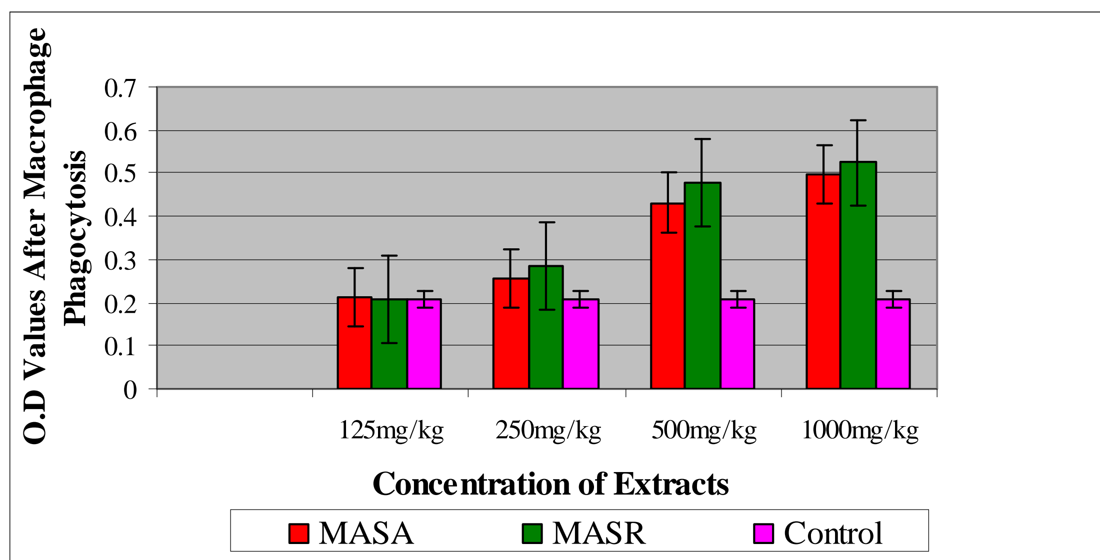

Figure 4. 57: The effects of varying concentrations of methanol extracts of *A. setaceous* aerial part and root on macrophage phagocytosis

***Control group was administered with dimethylsulfoxide**

4.12. The effects of aqueous, ethanol and methanol extracts of *C. volkensii* and *A. setaceous* on delayed type hypersensitivity (DTH) response in treated rats

As depicted in fig 4.58, ethanolic extracts of *C. volkensii* leaf and root were very effective in enhancing the delayed type hypersensitivity especially at higher concentration while the stem caused a depression in delayed type hypersensitivity response and this was more depressed at the highest concentration used which was statistically significant (ANOVA, $F_{4, 20} = 9.6194$, P = 0.0001). Enhancement in DTH by extracts of *C. volkensii* leaf and root observed was dose dependent and this was found to be significant for ECVL (ANOVA, $F_{4, 20} = 137.1905$, P = 0.0000), and

ECVR (ANOVA, $F_{4, 20}$ = 281.611, P = 0.0000).

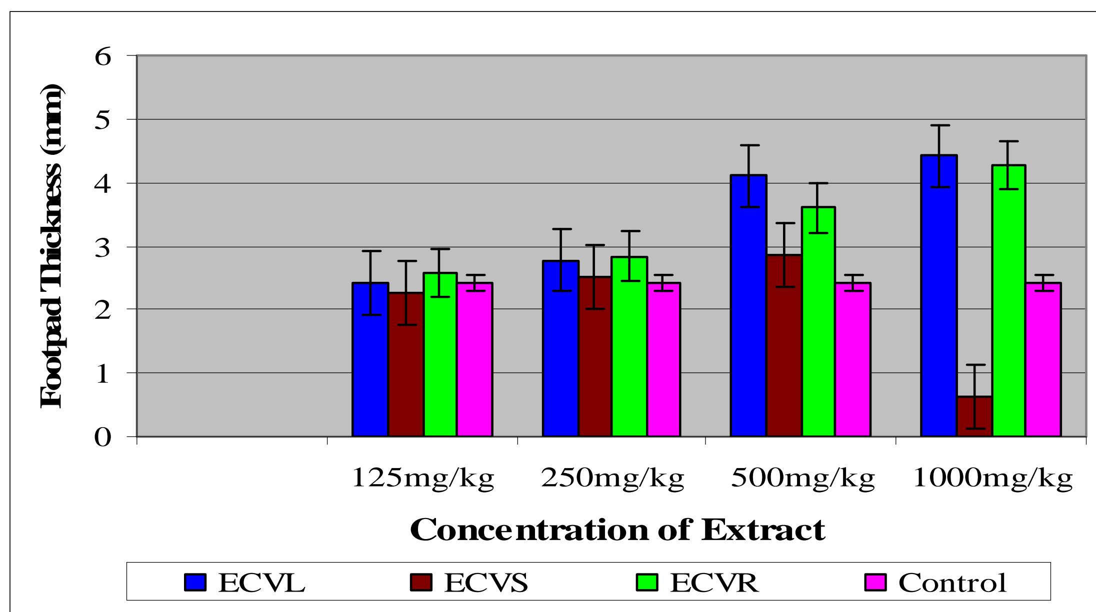

Figure 4. 58: The effects of varying concentrations of ethanolic extracts of *C. volkensii* leaf, stem and root on delayed type hypersensitivity

***Control group was administered with dimethylsulfoxide**

A significant enhancement of DTH in rats treated with ethanolic extracts of *A. setaceous* aerial part and root that was dose dependent was observed, EASA (ANOVA, $F_{4, 20}$ = 199.8652, P = 0.0000) and EASR (ANOVA, $F_{4, 20}$ = 214.1807, P = 0.0000). Lower doses were ineffective in enhancing DTH response (Fig 4.59).

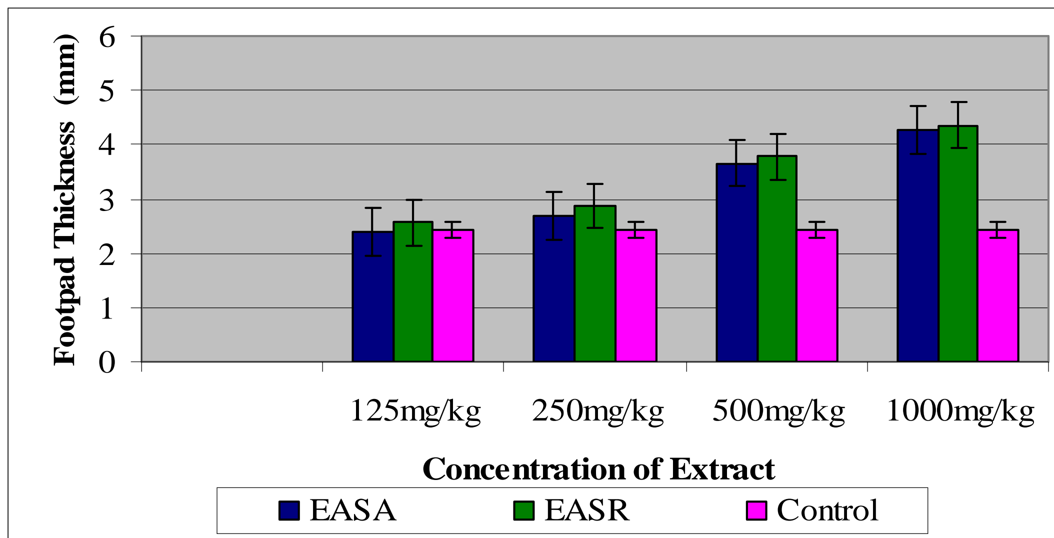

Figure 4. 59: The effects of ethanol extracts of *A. setaceous* aerial part and root on delayed type hypersensitivity

***Control group was administered with dimethylsulfoxide**

Methanolic extracts of *C. volkensii* leaf and root were significantly effective in enhancing DTH at higher concentrations tested while the stem extract did not have any effect (Fig 4.60), MCVL (ANOVA, $F_{4, 20} = 214.1807$, $P = 0.0000$), MCVS (ANOVA, $F_{4, 20} = 32.25$, $P = 0.0001$) and MCVR (ANOVA, $F_{4, 20} = 278.8616$, $P = 0.0000$).

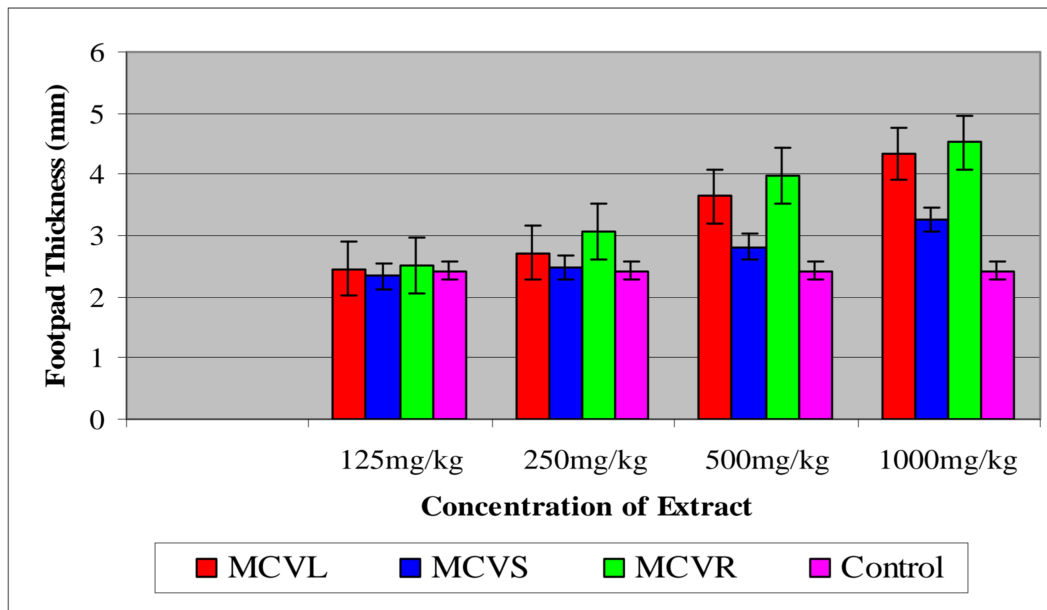

Figure 4. 60: The effects of methanol extracts of *C. volkensii* leaves, stem and roots on delayed type hypersensitivity

A significant enhancement in DTH in treated rats that was dose dependent was observed for MASA (ANOVA, $F_{4, 20} = 110.2701$, $P = 0.0000$) and MASR (ANOVA, $F_{4, 20} = 139.7676$, $P = 0.0000$). Methanolic extracts of *A. setaceous* aerial part and root enhanced DTH response at higher doses. Lower doses were ineffective in enhancing DTH response (Fig 4.61)

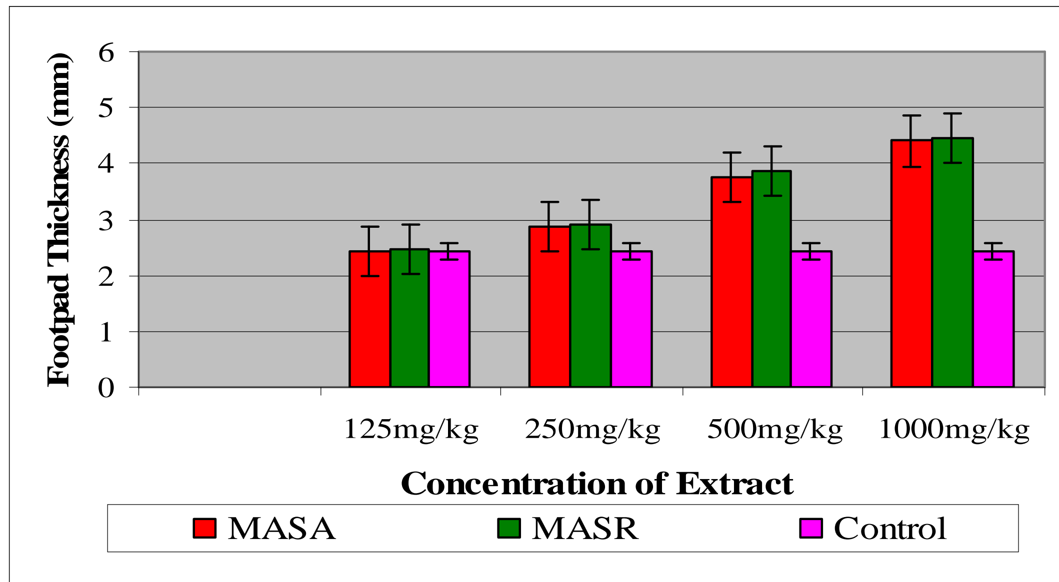

Figure 4. 61: The effects of methanol extracts of *A. setaceous* aerial part and root on delayed type hypersensitivity

A significant enhancement in DTH that was dose dependent was observed for ACVL (ANOVA, $F_{4, 20}$ = 199.9271, P = 0.0000), ACVS (ANOVA, $F_{4, 20}$ = 22.0064, P = 0.0001) and ACVR (ANOVA, $F_{4, 20}$ = 116.2687, P = 0.0000). Aqueous extracts of *C. volkensii* leaf and root enhanced DTH response in rats at higher doses. Lower doses were ineffective in enhancing DTH response. The aqueous stem extract also was ineffective at all the tested concentrations (Fig 4.62).

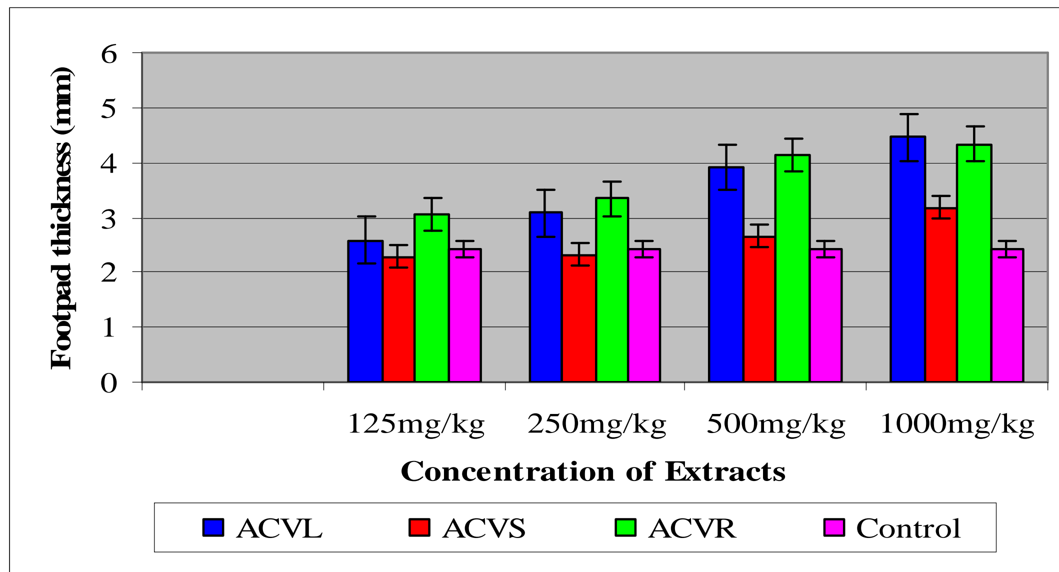

Figure 4. 62: The effects of aqueous extracts of *C. volkensii* leaf, stem and root on delayed type hypersensitivity

A significant enhancement in DTH response that was dose dependent was observed for AASA (ANOVA, $F_{4, 20}$ = 98.0329, P = 0.0000) and AASR (ANOVA, $F_{4, 20}$ = 186.037, P = 0.0000). Ethanolic extracts of *A. setaceous* aerial part and root enhanced DTH response in rats at higher doses. Lower doses were ineffective in enhancing DTH response (Fig 4.63).

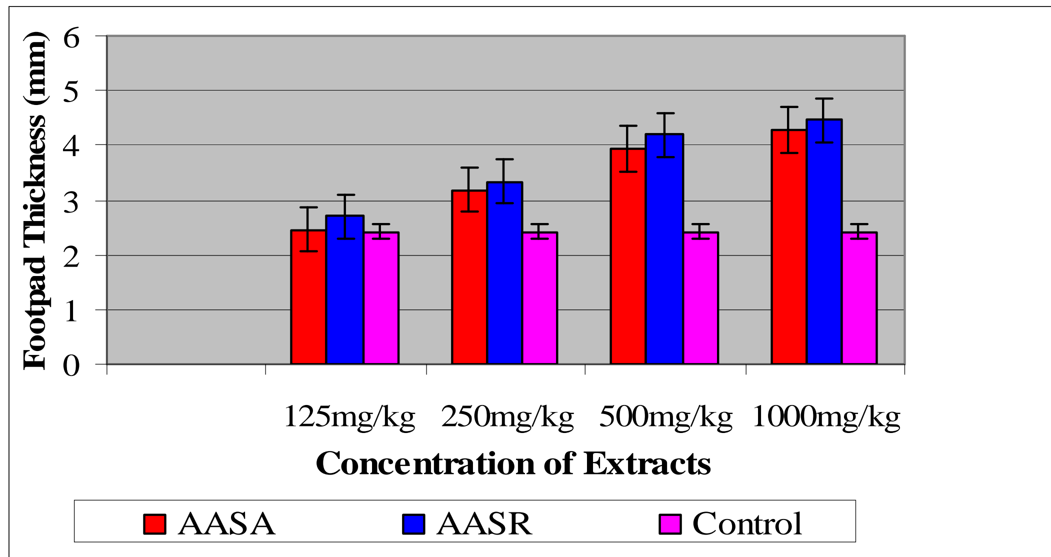

Figure 4. 63: The effects of aqueous extracts of *A. setaceous* aerial part and root on delayed type hypersensitivity

4.13 Phytochemicals screening of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* and *A. setaceous*

The results on phytochemicals screening of dichloromethane, methanol, ethanol, hexane and aqueous extracts of *C. volkensii* and *A. setaceous* are presented in table 4.11.

Alkaloids were present in the dichloromethane leaf, stem, and root extracts of *C. volkensii* but not in the aerial part and root of *A. setaceous*. Flavonoids were only present in both the dichloromethane root extracts of the two plants. Sterols, steroids and saponins were present in the dichloromethane extracts of *C. volkensii* stem and root and only in the dichloromethane root extracts of *A. setaceous*. Tannins were present in all the dichloromethane extracts of the two plants with the exception of the aerial part of *A. setaceous* (Table 4.11)

When methanolic extracts were screened for phytochemical compounds, it was found out that both alkaloids and flavonoids were present in all the methanolic extracts of *C. volkensii* leaf, stem and root and also in the aerial part and root of *A. setaceous*. Sterols and saponins were present in all the methanolic extracts except in that of *C. volkensii* leaf whereas steroids were lacking in all the methanolic extracts except in that of the root of *A. setaceous*. Tannins were only lacking in the extracts of *C. volkensii* root but present in the rest of the methanolic extracts (Table 4.11).

Among the ethanolic extracts, alkaloids were found to be present in the stem and root

of *C. volkensii* but not in the leaf while flavonoids were found only in the root of *C. volkensii* and *A. setaceous* ethanolic extracts. Sterols were present in all the ethanolic extracts with the exception of the ethanolic aerial part extract of *A. setaceous*. Steroids were absent in all the ethanolic extracts of the two plants. Saponins were only absent in the aerial part of *A. setaceous* while tannins were only absent in the ethanolic root extracts of *C. volkensii* (Table 4.11).

When the hexane extracts of *C. volkensii* leaf, stem and root and *A. setaceous* aerial part and root were subjected to phytochemical screening, presence of alkaloids were only detectable in stem extract of *C. volkensii* and aerial part of *A. setaceous*. Flavonoids could not be detected in any of the extracts whereas sterols and steroids were detected in all the parts of the plants screened. Saponins were only present in the leaf and stem extracts of *C. volkensii* while tannins were only present in the leaf and root of *C. volkensii* and aerial part of *A. setaceous* (Table 4.11).

With the aqueous extracts, alkaloids and steroids could not be detected in any of the extracts. Flavonoids were present in the stem and root extracts of *C. volkensii* while sterol was found in the stem and root of *C. volkensii* and also in the root of *A. setaceous*. Saponins were present in all the aqueous extracts of the leaves, stem and root of *C. volkensii* as well as in the aerial part and root of *A. setaceous*. Tannins were only absent in the aqueous root extract of *C. volkensii* (Table 4.11).

Table 4.11: Phytochemical components of leaf, stem, root of *C. volkensii* and aerial part and root of *A. setaceous* extracted with various solvent

Samples	Phytochemicals					
	Alkaloids	Flavonoids	Sterols	Steroids	Saponins	Tannins
DCVL	+	-	-	-	-	+
DCVS	+	-	+	+	+	+
DCVR	+	+	+	+	+	+
DASA	-	-	-	-	-	-
DASR	-	+	+	+	+	+
MCVL	+	+	-	-	-	+
MCVS	+	+	+	-	+	+
MCVR	+	+	+	-	+	-
MASA	+	+	+	-	+	+
MASR	+	+	+	+	+	+
ECVL	-	-	+	-	+	+
ECVS	+	-	+	-	+	+
ECVR	+	+	+	-	+	-
EASA	+	-	-	-	-	+
EASR	+	+	+	-	+	+
HCVL	-	-	+	+	+	+
HCVS	+	-	+	+	+	-
HCVR	-	-	+	+	-	+
HASA	+	-	+	+	-	+
HASR	-	-	+	+	-	-
ACVL	-	-	-	-	+	+
ACVS	-	+	+	-	+	+
ACVR	-	+	+	-	+	-
AASA	-	-	-	-	+	+
AASR	-	-	+	-	+	+

+ = Positive; - = Not detected

CHAPTER FIVE

5.0 DISCUSSION

In the present study, dichloromethane extracts showed antibacterial activity. The dichloromethane root extract of *C. volkensii* was the most active of all the dichloromethane extracts, showing a broad spectrum antibacterial activity against the tested microbes and even showing greater antifungal activity than the rest of dichloromethane extracts. Dichloromethane root extracts of *A. setaceous* exhibited greater antibacterial activity against *B. subtilis* and *S. faecalis* but had very weak antibacterial activity against *E. coli* and *P. aeruginosa* while it lacked activity against *S. aureus*. The most susceptible microorganism to dichloromethane extracts was *B. subtilis* whereas *S. aureus* and *P. aeruginosa* seems to be resistant to most of the extracts. It would appear that the dichloromethane root extracts of both *C. volkensii* and *A. setaceous* were the most active of the dichloromethane extracts with the *C. volkensii* root showing broad spectrum activity against both the Gram positive and negative bacteria. The antimicrobial activity observed could be due to varied phytochemicals present. These phytochemicals have been documented as major biological active components which also exhibit their effects on physiological activity. Many phytomedicines exert their effect through the additive or synergistic action of several compounds acting at a single or multiple target sites associated with physiological process (Junaid *et al*. 2008).

Phytochemical screening indicated that, alkaloids were present in the dichloromethane leaves, stem, and root extracts of *C. volkensii* but not in the aerial part and root of *A. setaceous*. Flavonoids were only present in both the dichloromethane root extracts of the two plants. Sterols, steroids and saponins were present in the dichloromethane extracts of *C. volkensii* stem and root and only in the dichloromethane root extracts of *A. setaceous*. Tannins were present in all the dichloromethane extracts of the two plants with the exception of the aerial part of *A. setaceous*. Alkaloids, flavonoids and tannins have been reported to possess antibacterial activity and it would therefore suffice to say that the antibacterial activity shown by some of these extracts could be attributed to these compounds. It could be possible that the antibacterial activity seen with the root extracts of *A. setaceous* and *C. volkensii* compared to the other dichloromethane extracts was due to the presence of both flavonoids and tannins acting synergistically. Although other extracts exhibited antibacterial activity, it wasn't as great as the root extracts. This could be explained by the fact that these extracts had only either one kind of phytochemical compound present in them and even so, the phytochemicals may have been present in minute amount to bring about the desired effect. It is important to note that dichloromethane extract of aerial part of *A. setaceous* when subjected to phytochemical screening, lacked all these phytochemicals. It is therefore suprising that it had some form of antibacterial activity against some of the pathogens. It is difficult to explain why some of the extracts were active against some strains of the Gram positive bacteria and not others as well as being active against some Gram

negative and not against all. The observed resistance of some of the Gram positive and Gram negative bacteria probably could be due to cell permeability or due to other genetic factors.

The methanolic extracts also exhibited antimicrobial activity against the tested microbes. The methanolic leaf extract of *C. volkensii* was active against *B. subtilis*, *P. aeruginosa*, *S. faecalis*, and *S. aureus* but completely lacked activity against *E. coli*. The methanolic extract of *C. volkensii* stem was more active against *B. subtilis* but had weaker activity against the rest of the microbes and this was also the case with the methanolic extract of *C. volkensii* root. The methanolic extract of *A. setaceous* aerial part had broad antibacterial activity against all the microbes tested. Methanolic extract of *A. setaceous* root was more active against *B. subtilis* and *S. faecalis* but lacked activity against *E. coli*. *B. subtilis* was the most susceptible microorganism while *E. coli* and *S. aureus* were the least susceptible. *C. volkensii* leaf seemed to be the most active plant part. When methanolic extracts of the two plants were screened for phytochemical compounds, it was found out that both alkaloids and flavonoids were present in all the methanolic extracts of *C. volkensii* leaf, stem and root and also in the aerial part and root of A. *setaceous*. Sterols and saponins were present in all the methanolic extracts except in that of *C. volkensii* leaf whereas steroids were lacking in all the methanolic extracts except in that of the root of *A. setaceous*. Tannins were only lacking in the extracts of *C. volkensii* root but present in the rest of the methanolic extracts. It is therefore possible to postulate that antibacterial activity seen

with the methanolic extracts could have been due to the presence of alkaloid, flavonoids and tannins.

These results seem to be in conformity with a number of other research work carried out by other researchers. Different concentrations (50, 100, 150 mcg/ml) of the methanolic extract of the root of A. racemosus showed considerable *In vitro* antibacterial activity against *E. coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Vibrio cholerae*, *S. typhi*, *Pseudomonas putida*, *S. aureus* and *B. subtilis* (Mandal *et al*., 2000). The root methanolic extracts of *A. africanus* and *A. racemosus* were demonstrated to be active against *Staphylococcus aureus* and *Neisseria gonorrhea* respectively but lacked activity against *Shigella boydii* and *P. aeruginosa*. The same study also showed that *Tamarindus indica* (Caesalpinaceae) leaf methanolic extracts were active against *Shigella boydii* and *P. aeruginosa* but not against *S. aureus* and *N. gonorrhea* (Chhabra and Uiso, 1995). The methanolic leaf extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cardifolia*, *Withania somnifera* and *Ziziphus mauritania* showed significant antibacterial activity against *B. subtilis*, *E. coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* (Mahesh and Satish, 2008). Sida cordifolia showed significant antibacterial activity against *B. subtilis* and *S. typhi* while *Mimosa pudica* and *Aegle marmelos* were found to be active against *B. subtilis*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *S. typhi* with maximum activity noted against *P. aeruginosa* and *S. typhi* (Balakrishnan *et al*., 2006). *T. pubescens* methanolic extract demonstrated antibacterial activity against Gram positive bacteria including *S. aureus*, MRSA, *Streptococcus pyogenes* and *E. faecalis* but not against Gram

negative *E. coli*, *P. aeruginosa* and *Salmonella* spp (Mehrgan *et al*., 2008).

The ethanolic extract of *C. volkensii* and *A. setaceous* exhibited various degrees of antibacterial activity. The ethanol extract of *C. volkensii* leaf showed weak activity against *E. coli*, *S. aureus*, *B. subtilis*, *S. faecalis* while *P. aeruginosa* was resistant to the ethanolic leaf extract of *C. volkensii*. The ethanolic stem extract of *C. volkensii* also showed weak activity against the tested microbes but was inactive against *P. aeruginosa*. The ethanolic root extracts of *C. volkensii* demonstrated weak activity against all the tested bacteria whereas the ethanolic extract of *A. setaceous* aerial part was particularly active against *E. coli* but showed weak activity against *S. aureus*, B. subtilis and *S. faecalis*. It was inactive against *P. aeruginosa*. The ethanolic root extract of *A. setaceous* was found to show some form of activity against *E. coli*, *S. aureus*, *B. subtilis*, and *S. faecalis*. It was inactive against *P. aeruginosa*. The least susceptible microorganism to ethanolic extracts of the two plants was the *P. aeruginosa* while the most susceptible was *B. subtilis*. Ethanolic root extract of *C. volkensii* exhibited broad spectrum activity showing some form of activity against all the tested microbes. These differences in antibacterial activity could be due to the nature and level of antimicrobial agents present in the plant parts and their mode of action. Among the ethanolic extracts of the various parts of the two plants screened for the presence of phytochemical compounds, alkaloids were found to be present in the stem and root of *C. volkensii* but not in the leaves while flavonoids were found only in the root of *C. volkensii* and *A. setaceous* ethanolic extracts. Sterols

were present in all the ethanolic extracts with the exception of the ethanolic aerial part extract of *A. setaceous*. Steroids were absent in all the ethanolic extracts of the two plants. Saponins were only absent in the aerial part of *A. setaceous* while tannins were only absent in the ethanolic root extracts of *C. volkensii*.

Gliricidia sepium leaf ethanol extract was shown to be particularly active against *E. coli* but not against other test microorganisms and the reason for the difference in sensitivity of the microorganisms was attributed to morphological differences between them (Nazli et al., 2008).

The hexane extracts of *C. volkensii* and *A. setaceous* exhibited antibacterial activity against all the tested microbes. Leaf, stem and root of *C. volkensii* showed activity against the microbes with the highest activity being demonstrated by the root extracts of *C. volkensii* against *B. subtilis* and *S. faecalis*. The hexane extracts of aerial part and root of *A. setaceous* were generally active against all the tested microbes with the highest activity being shown by the root extract of *A. setaceous* against *B. subtilis* and *S. faecalis*. The most susceptible microorganism to hexane extracts were *B. subtilis* and *S. faecalis* whereas the most resistant microbe to the hexane extracts was *E. coli*. The hexane leaf extract of *C. volkensii* seemed to be the most active plant part showing activity against most of the bacteria. When the hexane extracts of *C. volkensii* leaf, stem and root and *A. setaceous* aerial part and root were subjected to phytochemical screening, presence of alkaloids were only detectable in stem extract of *C. volkensii* and aerial part of *A. setaceous*. Flavonoids could not be

detected in any of the extracts whereas sterols and steroids were detected in all the parts of the plants screened. Saponins were only present in the leaves and stem extracts of *C. volkensii* while tannins were only present in the leaves and root of *C. volkensii* and aerial part of *A. setaceous*.

The aqueous extracts were generally found to be inactive against most of the microbes they were tested against. All the aqueous extracts lacked antibacterial activity against S. *faecalis*, *P. aeruginosa* and *E. coli*. The aqueous leaf extract of *C. volkensii* was only slightly active against *S. aureus* while aqueous stem extract showed slight activity against *S. aureus* and *B. subtilis* but were largely inactive against the rest. The aqueous root extracts of *C. volkensii* was slightly active against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa*. Aerial part of *A. setaceous* extracts showed minimal activity against *S. aureus*, *B. subtilis* and *P. aeruginosa* while root of *A. setaceous* aqueous extract also showed slight activity against *S. aureus* and *B. subtilis* only. *S. aureus* was the most susceptible microorganism to aqueous extracts followed by *B. subtilis*. The most resistant microbes to aqueous extracts were *S. faecalis*, *P. aeruginosa* and *E. coli*. With the aqueous extracts, alkaloids and steroids could not be detected in any of the extracts. This is not surprising as alkaloids readily decompose with time. Flavonoids were present in the stem and root extracts of *C. volkensii* while sterol was found in the stem and root of *C. volkensii* and also in the root of *A. setaceous*. Saponins were present in all the aqueous extracts of the leaf, stem and root of *C. volkensii* as well as in the aerial part and root of *A.*

setaceous. Tannins was only absent in the aqueous root of *C. volkensii*.

The result of the antibacterial activity of aqueous extracts obtained in this study would seem to be in agreement with other studies done before. Decotion of *Schizandre fructus* were reported to have antimicrobial activities on *Bacillus* spp and *S. aureus* (Lee *et al*., 2006). Water extracts of *Erythrina vogelii*, *Jatropha curcas*, *Garcinia kola* among others were found to be active against *S. aureus* but not against *E. coli*, *P. aeruginosa* and the Gram positive *Enterococcus faecalis* (Kamanzi *et al*., 2003). Gamboe and Chavez (2008) demonstrated that aqueous extracts of *Gobernadora* and *Mirto* produced zones of inhibition to *B. subtilis* and *S. aureus* while *Pimienta dulce* and *Palo* produced zones of inhibition against *P. aeruginosa* but all of these plants lacked activity against *E. coli* and *Micrococcus luteus*. Akinyemi *et al*. (2005) demonstrated that water extract of *T. avicennioides*, *P. discoideus* and *O. gratissimum* were active against *S. aureus* and MRSA.

Compared with standard drugs, the extracts had weak activity. The results of the standard antibiotic activity against the tested microbes indicated that all the standard antibiotics were highly active against the Gram negative *E. coli* with ampicilin showing the least activity. Nitrofurantoin and cotrimoxale were highly active against the *E. coli* while *S. aureus* proved to be more refractory to ampicilin, sulphamethoxale and cotrimoxale which showed no activity against it. Nitrofurantoin and tetracycline demonstrated the highest activity against *S. aureus*. It has

now been documented that *S. aureus* is increasingly becoming refractory to most antibiotics.

B. subtilis showed resistance against sulphamethoxale and cotrimoxale while ampicilin showed slight activity against it. Tetracycline exhibited the highest activity against *B. subtilis*. The rest of the remaining antibiotics also had high activity against *B. subtilis*. P. aeruginosa was resistant to ampicilin, sulphamethoxale and cotrimoxale. Nitrofurantoin showed the highest activity against *P. aeruginosa* followed by gentamicin while the rest of the other antibiotics were also highly active against *P. aeruginosa*.

S. faecalis was susceptible to all the antibiotics used in the study with the highest activity against it being demonstrated by gentamicin and tetracycline while sulphamethoxale showed the least activity against *S. faecalis*.

Tetracycline, streptomycin and gentamicin had broad spectrum activity acting against both the Gram positive and negative bacteria in addition to possessing antifungal activity against the fungus *C. albicans*. Nitrofurantoin and nalidixic acid also possessed broad spectrum activity against both the Gram positive and Gram negative bacteria but lacked antifungal activity against the fungus *C. albicans*. Sulphamethoxale and cotrimoxale were inactive against most microbes being only active against *E. coli* and *S. faecalis*.

In the current study Gram positive bacteria especially *B. subtilis*, were found to be more susceptible than Gram negative bacteria. Gram negative organisms tend to have a higher intrinsic resistance to most antimicrobial agents (Ndukwe *et al*., 2005). This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lacks the natural sieve effect against large molecules due to the small pores in their cell envelope whereas Gram negative bacterial cell wall is a multi layered structure bounded by an outer cell membrane (Astal *et al*., 2005; Ergene *et al*., 2006). The most sensitive bacterium was *B. subtilis* which was inhibited by all the extracts. It is also not surprising that there are differences in the antibacterial effects of plant extracts, which could have been due to the phytochemical differences between the plant extracts.

The organic extracts of these plants exerted antibacterial activity than corresponding water extracts at the same concentration. These observations may be attributed to two reasons; firstly the nature of biologically active components (Alkaloids, anthraquinone, saponins, tannins) which could be enhanced in presence of ethanol and other organic solvents. Secondly, the stronger extraction capacity of organic solvents such as ethanol and methanol could have produced greater active constituents responsible for antimicrobial activity (Hassan *et al*., 2009).

The aqueous extract appears to have less antibacterial activity than the other polar and non polar extracts. This was interesting given that the traditional method of

treating bacterial infection was by administering a decotion of the plant or part there of by boiling it in water, whereas according to the result of this study, organic solvents are better and hence these may be beneficial. Although these aqueous plant extracts may not be able to exert a direct physiological antimicrobial effect, it may be that their clinical efficacy lies in their associated activity in the stimulation of other systems, such as macrophages or nitric oxide.

This work showed that some of the extracts can be used as broad spectrum antibiotics since they are both active against both Gram positive and negative bacteria. Antibacterial effects of these plants on *S. aureus*, *E. coli* and *P. aeruginosa* showed that the plant extracts can be used in the treatment of gastrointestinal infection, urinary tract infection, skin diseases and diarrhea in man. They could also be used in treatment of boils, sores and wounds associated with *S. aureus* and *P. aeruginosa* that have been implicated as causative agents of these diseases (Aboaba *et al*., 2006; Liasu and Ayandele, 2008).

It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils. It is necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases (Banso and Adeyemo, 2006). The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. For example,

saponins are a special class of glycosides which have soapy characteristics and have been reported to be active antifungal agents. Antimicrobial properties of a number of tannins, flavonoids, alkaloids have been reported. Not only the antimicrobial properties have been ascribed to these plant phytochemicals, but other biological activities including modulation of the immune system have been assigned to these compounds in plants. Phytochemical screening of the extracts of *A. setaceous* and *C. volkensii* revealed the presence or absence of different phytochemical compounds in different plant parts. Indeed phytochemical investigations of the plant *Asparagus racemosus* have resulted in characterization of steroidal glycosides, pyrrolizidine alkaloid and dihydrophenanthrene derivatives (Ravikumar *et al*., 1987). Indole quinoline alkaloids, glycoalkaloids, berberine type alkaloids, indole-quinolizidine alkaloids have been reported to be active against a range of Gram positive and gram negative bacteria (Iwu *et al*., 1999). Chloroform extract of the leaves of *Caesalpinia pyramidalis* has yielded new biflavanoid named caesaflavone, as well as podocarpusflavone A, agathisflavone, apigenin and kaempferol whereas the chloroform extract of the wood trunk gave 4, 4'- dihydroxy-2- methoxychalcone, (-)-syringaresinol and methyl gallate (Bahia *et al*., 2005).

The secondary metabolites identified in the plant materials used in this study could be responsible for antimicrobial activity exhibited by some of the extracts of these two plants. Their antibacterial activity may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotics. Several metabolites from

herb-species, such as alkaloids, tannins, saponins and sterols have been previously associated with antimicrobial activity (Leven *et al*., 1979). Many studies indicate that in some plants, there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, phenols and water, ethanol, chloroform and butanol soluble compounds. Water extracts of miswak (*Salvadora persica*) roots and stems have been reported to contain potential antimicrobial anionic components such as chloride, sulfate, thiocyanate and nitrate (Astal *et al*., 2005). Many plants contain non toxic glycosides which can get hydrolysed to release phenolics which are toxic to microbial pathogens (Aboaba *et al*., 2006). It therefore suggests that constituents of the plant extracts could serve as source of drugs useful in the chemotherapy of some microbial infections.

Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. *T. pubescens* has been reported to contain various substances including terpenoids, tannins and polyphenolic compounds as well as flavonoids. Flavonoids' activity is probably due to their ability to form complex with extra cellular and soluble proteins, as well as bacterial cell wall. Lipophilic flavonoids may also disrupt bacterial membranes.

Numerous studies have shown an association between increased prevalence of HIV infection and the occurrence of opportunistic fungal infections. Among

the different HIV associated fungal infections, oral mucosal lesions caused by *Candida* species are by far the most frequent manifestations. Up to 90% of HIV-infected individuals suffer at least one episode during the course of their disease, and the incidence and severity of the episodes increase with decreasing immunity. *Candida albicans* is the causative agent accounting for more than 90% of cases. However, other *Candida* species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* may also cause symptomatic oral candidiasis in HIV-positive individuals. Other fungal infections seen in HIV- infected individuals include cryptococcosis due to *Cryptococcus neoformans* and Aspergillosis due to *Aspergillus flavus*, *A. fumigatus* and *A. niger* (Runyoro *et al*., 2006). In sub Saharan Africa, where 70% of the world cases of HIV/AIDS are found, oral candidiasis including oropharyngeal and oesophageal candidiasis, are very common, causing significant morbidity among patients.

Oral candidiasis is usually treated by topical antifungal agents that include nystatin, miconazole, fluconazole, itraconazole and amphoterin B. However, the management of *Candida* infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungals, relapse of *Candida* infections, and the high cost of antifungal agents (Runyoro *et al*., 2006). When relapses occur, the infections tend to be refractory to treatment. The difficulties associated with the management of *Candida* infections necessitate the discovery of new antifungal agents in

order to widen the spectrum of activity against *Candida* and combat strains expressing resistance to the available antifungal agents. In this case also, plant derived natural products may offer potential lead to new compounds, which act on these fungi.

All the dichloromethane extracts exhibited some form of antifungal activity against *C. albicans* with the dichloromethane root extract of *C. volkensii* showing the highest activity. All the methanolic extracts of the two plants had some form of activity against *C. albicans* albeit a weak one. The ethanolic root extracts of *C. volkensii* demonstrated weak activity against the fungus. The ethanolic root extract of *A. setaceous* was found to show some form of activity against the fungus *C. albicans*. Ethanolic root extract of *C. volkensii* exhibited activity against the tested fungus. The hexane extracts of all the plant parts showed activity against the fungus *C. albicans* with the *C. volkensii* leaf extract being the most active. All the aqueous extracts lacked antifungal activity against *C. albicans*. *C. albicans* was generally more resistant to most of the standard antibiotics. Could it be possible that the antifungal property of some of the extracts could be attributed to saponins, a special class of glycosides which have soapy characteristics and reported to be active antifungal agent (Iwu, 2000). Indeed, from the root of *Asparagus filicinus*, a folk medicine used in treatment of bronchitis, pneumonia and coughs, three saponins have been isolated and demonstrated to possess antiprotozoal and antifungal activity (Iwu , 2000). The interesting aspect is that all the aqueous extracts had saponins yet they failed to

exhibit any antifungal activity. This may be an indicator that apart from the saponins, there may be other phytochemicals responsible for the observed antifungal activity in the non aqueous extracts. Extraction of aqueous extracts by boiling could also have altered the structure and hence the activity of the resulting saponins. Runyoro *et al*. (2006) showed that aqueous methanolic extract of *Asparagus africanus* Lam aerial parts had some form of activity against *C. albicans* with an inhibition zone of less than 4 mm. In the same study, a number of plants belonging to different families were demonstrated to be active against *C. albicans*. *Biden pilosa*, young shoots of *D. minutiflora*, *Sida acuta* and *P. umbellate* showed activity against *C. albicans* (Kamanzi *et al*., 2003).

Only three of the antibiotics used in this study showed activity against *C. albicans* with gentamicin showing the highest activity followed by streptomycin and tetracycline with moderate activity. In general, among the tested microbial strains, *C. albicans* was found to be more resistant to many plant extracts as compared to bacterial strains.

The active components usually interfere with growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration. Most of the extracts had a minimum inhibitory concentration ranging from 3.2mg/ml to 50mg/ml and as can be discerned from figures 4.1, 4.2 and 4.3, some of the selected extracts had the ability to

interfere with the growth of some of the bacteria considerably slowing their growth rates. Taken together these results suggest that the extracts were mostly bacteriostatic.

In extract treated rats, percent survival rate was high throughout the experimental period compared to the control group exposed to *S. aureus*. Higher doses of the methanolic, ethanolic and aqueous extracts were found to be particularly active in protecting the rats against *S. aureus* infection. It is not clear to what extent the antibacterial activity of the extracts would play a role in lowering the mortality given the weak or lack of activity seen. It is more probable that the extracts stimulated the innate immune system enabling the host to mount an immediate response to invading pathogens thereby protecting the rats and increasing their percent survival rate.

In recent years, different factors such as AIDS, cancers, chemotherapy, corticosteroid therapy and other underlying diseases have been known to compromise immune defenses, predisposing individuals to serious and fatal infections. Regarding the above mentioned points, immunomodulators that enhance specific and non specific components of immune system may represent a reasonable approach to protect humans and animals exposed to pathogens (Shokri *et al*., 2006). Scientific literature is continuously reporting plant drugs having immunomodulatory activity and most of these leads for this activity are from traditional medicines from different parts of the world (Thakur *et al*., 2006). Numerous medicinal plants have been identified and developed as chemotherapeutic agents and as modulators of the immune system

(Auttachoat *et al*., 2004). The active constituents of the plants may strengthen or stimulate the immune responses by interacting with various cells of the immune system. Immune responses can be either innate (nonspecific) mediated by macrophages, natural killer cells and polymorphonuclear leukocytes (neutrophils) or adaptive (specific) mediated by B or T lymphocytes. Plants and plant derived compounds are capable of increasing macrophage activation and enhancing the functions of T and B- cells and NK cells. Examples of plants with reported immunomodulatory activity include *E. purpurea*, *E. pallida*, *Eleutherococcus senticoccus* and *Panax ginseng* (Wagner, 1989; Auttachoat *et al*., 2004; Scaglione, 2006). Compounds reported to possess immunomodulatory activity include fatty acids, flavonoids, tannins, alkaloids, polysaccharides (Guerra *et al*., 2003).

For many generations, extracts of *A. setaceous* and *C. volkensii* have been used in Kenya to treat various infections (Kokwaro, 1976; Njoroge and Bussmann, 2006a). However prior to this work, there have been no published studies on the pharmacological and immunological effects of these two plant species from Kenya. This particular study has revealed that most of the organic and aqueous extracts of aerial parts, roots of *A. setaceous* and leaves, stem and roots of *C. volkensii* possess weak antibacterial or antifungal activity. It is interesting to note that most of the aqueous extracts were devoid of antibacterial and antifungal activity. This was intriguing given that in the traditional method of treating bacterial infection was by administering a decotion of the plant or part there of by boiling it in water.

Indeed, decotion of *A. racemosus* has been reported to be devoid of antibacterial activity (Sagrawat and Khan, 2006). This may be taken as indicator that although these plants extracts may not be able to exert a direct physiological antimicrobial effect, it may be that their clinical efficiency lies in their associated activity in the stimulation of other systems, such as macrophages or nitric oxide. Indeed, several plant secondary metabolites have been found to interfere with different immune system functions, including the activation of cell mediated immunity (Wagner *et al*., 1987).

Immune activation is an effective as well as protective approach against emerging infectious diseases. Basic research on natural substances with immunomodulating properties are performed by stimulating cells of the immune system including neutrophils, macrophages, T and B cell, NK cell (Srikumar *et al*., 2005). Circulation of immune cells is essential for maintaining an effective immune defense. Total WBCs were observed to have increased following the administration of the dichloromethane *C. volkensii* leaves, methanol, ethanol and aqueous extracts of all the plant parts to rats. The increase in total WBCs count observed may have resulted from stimulation of leucocytosis by the extracts and enhanced production in the bone marrow (Okokon *et al*., 2004). Thatte *et al*. (1987) demonstrated that extracts of *A. racemosus* were able to cause leucocytosis in mice. It may be possible that the extracts contain some bioactive agents that enhance production of WBCs. It has been reported that GM-CSF, M-CSF, interleukins IL-2, IL-4 and IL-5 regulate

the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBCs (Adebayo *et al*., 2005). It may be that some components of these extracts enhance the production of these regulatory factors or increased the sensitivity of the committed stem cells responsible for the production of WBC to these factors. Thus administration of the extracts may protect against infection.

Leucocytes are known to increase sharply when infection occurs, as one of the first line of defense of the body. The increase in total WBC count following oral administration confirms the antiinfective properties of these plants (Valigra, 1994; Iranloye, 2002).

Geidam *et al*. (2004) demonstrated that aqueous stem extract of Momordica balsamina linn elevated WBC counts in normal rats but did not affect other hematological parameters. Administration of *Rubia cordifolia* was demonstrated to increase total WBC count in immunocompromised albino rats indicating that the extract was able to stimulate hemotopoetic system. Some of the chemical constituents isolated from *Rubia cordifolia* included alkaloids, cardiac glycosides, tannins, flavanoids and phenols which have been reported to possess immunomodulatory activity (Kannan *et al*., 2009). n- butanol soluble and ethyl acetate soluble fractions of methanolic extract of *Lagenaria siceraria* fruits significantly increased the WBC and lymphocyte counts but had insignificant changes in monocyte, eosinophil and basophil counts. Significant increase in WBC count was observed in *Withania*

somnifera treated mice compared to untreated control (Mishra *et al*., 2005). In a review by Sagrawat and Khan (2007), *A. racemosus* was reported to protect rat and mice against experimentally induced abdominal sepsis while oral administration of decotion of powdered roots of *A. racemosus* produced leucocytosis along with enhanced phagocytic activity of the macrophages and polymorphs. Sheeja and Kuttan (2006) demonstrated that intraperitoneal administration of the extracts of *Andrographis paniculata* significantly increased the total WBC count and bone marrow cellularity.

In the present study, DCVL, MCVL, MCVR, MASA, MASR, ECVL, ECVS, ECVR, EASA, EASR, ACVL, ACVS, ACVR, AASA and AASR were found to be able to affect differential WBC counts in treated rats. It elevated neutrophil numbers while causing a decrease in lymphocyte as well as monocytes. The ability of the extract to alter the distribution and occurrence of lymphocytes, neutrophils, eosinophils, basophils and monocytes suggests the potentiality of the extract acting as an immunostimulant (Oladunmoye and Osho, 2007). n- butanol soluble and ethyl acetate soluble fractions of methanolic extract of *Lagenaria siceraria* fruits significantly increased the WBC and lymphocyte count but had insignificant changes in monocyte, eosinophil and basophil counts. Both fractions significantly increased antibody titers in a dose dependent fashion showing augmentation of humoral response but not CMI (Gangwal *et al*., 2008). Quercetin (flavanoid) did not significantly influence the hematological parameter and had a tendency to decrease eosinophils and

basophil counts in Quercetin- supplemented group of mice. It also increased the spleen population of NK cells (Dringeliene *et al*., 2005).

Cyclophosphamide is probably the most common antineoplastic used in cancer chemotherapy and is an essential component of several effective chemotherapeutic formulae. However, cyclophosphamide shows potent immunosuppressing properties (Thatte *et al*. 1987; Morris *et al*., 2003) having suppressive and cytotoxic activity. Cyclophosphamide administration induces acute and transient myelosuppression, primarily through damage to rapidly proliferating hematopoietic progenitors and their mature progeny leading to decline in the number of peripheral blood cells (Sheeja and Kuttan, 2006). Cyclophosphamide is an alkylating agent resulting in cross linking of DNA and causes inhibition of DNA synthesis. It has a number of side effects in long term treatment. The major side effect of this drug is on immune system and haematological suppression (Joshi and Mishra, 2008; Nayak and Abhilash, 2008). As expected, cyclophosphamide caused a significant drop in WBC in rats, but upon treatment of the rats with the methanol, ethanol and aqueous extracts of *C. volkensii* (leaf, stem and root) and *A. setaceous* (aerial part and root), an increase in total WBC was observed, when compared to the negative control treated with cyclophosphamide. Lithium carbonate was superior to extracts in reversing the effects of cyclophosphamide induced leucopenia.

In pretreated rats, methanol, ethanol and aqueous extracts of *C. volkensii* (leaf,

stem and root) and *A. setaceous* (aerial part and root), were found to have an active protective effect in rats, when administered before cytostatic. Injection of a single dose of 200mg/kg cyclophosphamide produced a fall in total WBC on the third day. A rebound increase in total WBC count occurred on the 9th day. The leucopenia which occurred in control animals following cyclophosphamide was less marked in the treated animals. A point to note is that WBC counts in all treated animals never went below 6000-8000 cells/mm after injection of cyclophosphamide. Thatte *et al*. (1987) reported that A. racemosus could be producing leucocytosis probably by activating macrophages. Activated macrophages are known to secrete a large number of colony stimulating factors and IL-1 which in turn stimulate other immunocytes like neutrophils (Joshi and Mishra, 2008). IL-1 and CSF are substances known to cause leucocytosis and it appeared that activation of macrophage produced protection of the rats against cyclophosphamide induced leucopenia. It may be that the extracts of *A. setaceous* and *C. volkensii* also act in this manner to confer protection. The methanolic, ethanolic and aqueous extracts showed an increase in WBC and percent neutrophils and the extracts at a dose of 500mg/kg body weight was more effective. The study has demonstrated that extracts of *A. setaceous* and *C. volkensii* protected mice against cyclophosphamide induced leucopenia. It showed that the extracts offer protection against cyclophosphamide induced myelosuppression and hence holds promise as candidates to overcome this problem. These results open up exciting possibilities for the use of plant products in the prevention of adverse bone marrow effects associated with cyclophosphamide.

These results are also in agreement with other studies. Administration of the ethanolic extract of *A. paniculata* in cyclophosphamide treated mice was found to enhance total WBC count which was drastically reduced in the cyclophosphamide alone treated control animals suggesting that cyclophosphamide induced myelosuppression was reversed or inhibited by extract administration through its immunomodulating activity (Sheeja and Kuttan, 2006). Preclinical studies investigating the effect of *A. racemosus* against the myelosuppressive effects of single and multiple doses in mice showed that it prevented to a significant degree, leucopenia produced by cyclophosphamide. It is now accepted that *A. racemosus* is a potent immunostimulant with effects comparable to lithium and glucan producing leucocytosis with predominant neutrophilia (Thatte and Dahanukar, 1988). Nayak and Abhilash (2008) demonstrated that alcoholic extract of *Pimenta dioica* leaves increased WBC counts in mice treated with cyclophosphamide after 10 days of treatment. The increase in WBC was attributed to the immune stimulant properties of the *P. dioica*. In another study whose result conflicts with the result of this study, Khoo and Ang (1995) demonstrated that the aqueous extracts of *Astragalus membraneceus* and *Ligustrum* did not prevent cyclophosphamide induced myelosuppression. It did not delay the onset, hasten the recovery or shorten the duration of leucopenia.

Neutrophils are short lived phagocytic cells and are the most abundant circulating granulocytes. Their granules contain powerful microbicidal molecules

(Olandunmoye and Osho, 2007). A number of functions carried out by neutrophils and macrophages were examined. To carry out their activity effectively, neutrophils need to move from the bloodstream to areas of infections in the tissue. Margination of neutrophils from the bloodstream requires a firm adhesion, which is mediated through the interactions of the β2 integrins present on the neutrophils. The β2 integrin is stored in the cell granules and are up regulated for a firm adherence. Integrins also regulate the vascular development and angiogenesis by promoting proliferation, migration and adhesion of endothelial cells (Bang *et al.*, 2007). The adhesion of neutrophils to nylon fibers correlates to the process of migration of cells in blood vessels (Radzan and Roy, 2008). Adherence to nylon column was increased in groups treated with DCVL, DCVS, MCVL, MCVS, MCVR, MASA, MASR, ECVL, ECVS, ECVR, EASA and EASR. All the aqueous extracts were also observed to be effective in increasing percent neutrophil adhesion. This may have been due to the up regulation of β2 integrins (Srikumar *et al.*, 2005). It has also been suggested that the main attractive substances for neutrophils are pro-inflammatory cytokines, such as IL-Iβ, that regulate endothelial molecule expression on vascular endothelial cells and promote neutrophil adherence to these cells (Reis *et al.*, 2009). The plant extracts were also found to be more active at higher doses tested. A number of extracts were found to significantly depress neutrophil adhesion including DCVR, HCVL, HCVS, HCVR, HASA and HASR. It is possible that compounds present in these extracts may have caused shedding or internalization of the β2 integrins resulting in lack of firm adherence by neutrophils.

A polyherbal formulation Guard Sansar was demonstrated to have significant immunomodulatory activity by increasing the rate of carbon clearance and the percent neutrophil adherence to nylon fibers (Meera *et al*., 2008). A polyherbal formulation NRZTCD which contains extracts of *Withania somnifera*, *Emblica officinalis* and *Ocimum sanctum*, was demonstrated to increase the number of peritoneal macrophages, rate of carbon clearance, and the percentage neutrophil adhesion to nylon fibers (Radzan and Roy, 2008). Oral administration of *Haridadi ghrita* was demonstrated to significantly increase neutrophil adhesion and delayed type hypersensitivity following antigenic challenge by SRBCs. Thus *Haridadi ghrita* potentiated the CMI by facilitating the footpad thickness response by SRBCs in sensitized rats. It showed no effect on the humoral immunity (Fulzele *et al*., 2003)

Phagocytosis by neutrophils constitutes an essential arm of the host defense against foreign antigens. Neutrophils have receptors for fragment crystallizable (Fc) and complement component (C36) which are involved in uptake of foreign antigen. Phagocytic index was enhanced in groups treated with MCVL, MCVS, MCVR, MASA, MASR, ECVL, ECVS, ECVR, EASA, EASR, ACVL, ACVS, ACVR, AASA and AASR. The ability of the extracts to modulate phagocyte functions might offer obvious therapeutic benefits for bacterial infections since phagocytes play an essential role in the host's defense against infection by ingesting invading microorganisms and by mediating inflammatory process (Lin and Hsieh,

2005). Phagocytic index was seen to be depressed in group of animals treated with HCVL, HCVS, HCVR, HASA, HASR and ACVS.

NBT reduction was found to be significantly enhanced in groups treated with DCVL, MCVL, MCVS, ECVL, ECVS, ECVR, EASA, EASR, ACVL, ACVS, ACVR, AASA and AASR. This enhancement was seen with higher concentrations of the extracts. NBT reduction was found to be depressed in animals treated with DVCS, DCVR, DASA, DASR, MCVR, MASA, MASR, HCVL, HCVS, HCVR, HASA and HASR. The capacity to reduce NBT to formazon indicated that the neutrophils are producing oxygen radicals, which are important microbicidal mechanism of these cells that could cause pathogen death (Muniz Junqueira *et al*., 2004). During the process of phagocytosis by the polymorphonuclear leucocytes, there occur significant increases in oxygen consumption, hexose monophosphate shunt activity and hydrogen peroxide formation. Nitroblue tetrazolium is yellow or white, water soluble, nitro substituted aromatic tetrazolium compound that reacts with cellular superoxide ions to form formazon derivative (Esfandiari *et al*., 2003). Superoxide is generated as a by product in aerobic organisms from a number of physiological reactions such as the electron flow in the chloroplasts and mitochondria and from some redox reactions in cells. It can react with hydrogen peroxide to produce hydroxyl radical ($0H^-$), one of the most reactive molecules in the living cells. Hydroxyl radical can cause the peroxidation of membrane lipids, breakage of DNA strands and inactivation of enzymes in cells (Chen and Pen, 1996). The cytoplasmic NADPH which is

produced by oxidation of glucose through the hexose monophosphate shunt, serve as an electron donor. The oxidase system, available in the cytoplasm, helps transfer electrons from NADPH to NBT and reduces NBT into formazon (Esfandiari *et al*., 2003).

The higher reduction in NBT dye reduction assay, represent higher activity of oxidase enzyme reflecting phagocytosis stimulation in proportion to intracellular killing (Saraphanchottiwithaya *et al*., 2007). NBT reduction test relies on the generation of bacterial enzymes like NADPH-oxidase in neutrophils during intracellular killing. These enzymes are necessary for normal intracellular killing against foreign antigens (Srikumar *et al*, 2005). NBT reaction also indirectly reflects the ROS generating activity in the cytoplasm of cells as ROS are formed during phagocytosis by neutrophils (Esfandiari *et al*., 2003). It is known that neutrophils are capable of producing 100- fold higher amounts of ROS than non activated neutrophils, and activated neutrophils increase NADPH production via the hexose monophosphate shunt. The Myeloperoxidase system of both PMNL and macrophages also is activated, which leads to respiratory burst and production of high levels of ROS (Blake *et al*., 1987; Esfandiari *et al*., 2003). Such an oxidative burst is an early and effective defense mechanism in cases of infection for killing microbes. It would seem that these plant extracts have the ability to enhance the activity of neutrophils which means they should be able to respond to and destroy pathogenic agent more quickly.

The extracts could possibly be useful for treating patients suffering from neutrophil function deficiency.

The seeds and leaves of Urtica dioica were found to increase NBT reduction (Basaran *et al*., 1997). Dhillon and Bhatia (2008) demonstrated that aqueous extracts of leaves and seeds of oat possessed in vivo immunomodulatory activity in Swiss albino mice by enhancing NBT reduction and phagocytosis. Aqueous extracts of *Nerium oleander* produced a significant increase in the percentage of NBT positive cells in extract treated group in comparison to control. It also had immunopotentiating activity on cellular mediated immunity by enhancing DTH response (Farwachi, 2007).

Macrophage phagocytosis was also found to be enhanced in groups treated with methanol, ethanol and aqueous extracts of *C. volkensii* (leaf, stem and root) and *A. setaceous* (aerial part and root). This enhancement was seen with higher concentrations of the extracts. Macrophages constitute a major part of the host defense system against infection and cancer. Macrophages carry out their bactericidal and tumoricidal activities by the oxygen-dependent killing via products of oxidative metabolism such as H_20_2, superoxide anion, and hydroxyl radical, and the oxygen-independent killing via cytokines and hydrolytic enzymes (Okokon *et al*., 2004). Macrophages are stimulated by foreign agents to produce cytokines and reactive oxygen and nitrogen species (Toshkova *et al*., 2006).

The present study showed that cell mediated immunity as evaluated by DTH to sheep red blood cells, can be potentiated by the injection of the methanol, ethanol and aqueous extracts of *C. volkensii* (leaf, stem and root) and *A. setaceous* (aerial part and root). DTH reactions observed on the skin of the experimental animals treated with extracts 48h post inoculation expressed the existence of cellular mediated immune response. The cutaneous reaction is attributed to liberation of lymphokines, skin reactive factor and monocyte chemotactic factor from sensitized T-cells. The thickening and reddening of skin in immunized animals are attributed to vasodilatation that cause increased capillary permeability and local influx of mononuclear cells at the site of inoculation (Farwachi, 2007). The immunopotentiating action of the extracts on the DTH could be due to its effect on the number of specifically committed lymphocytes and the availability of blood monocytes that could be recruited locally at the site of interaction (Amirghofran *et al*., 2008). The mechanism(s) whereby the plant extracts can enhance the activity of these components is unclear, but it may be due to the increased secretion of cytokines or the increased chemotactic response of the immune cells (Amirghofran *et al*., 2008). It is known that DTH is the result of macrophage-mediated tissue inflammatory response driven by Th1 cells (Chan *et al*., 2007). In general, DTH reactions are mediated by $CD4^+$ cells and down regulated by $CD8^+$ cells. Cells involved in DTH include langerhans and other cutaneous dendritic cells that transport inducers from the skin to regional lymph nodes where it is presented to T-lymphocytes. Before this can happen, however, protein antigens must be broken down into small peptides

by the antigen presenting cells (APCs) such as dendritic cells, macrophages and monocytes. The peptide fragments are presented on the surface of the APCs and bound to major histocompatibility complex (MHC) molecules (Rios *et al*., 2005). Once the skin is exposed to a new contact either in the same location or elsewhere an immune response causes a considerable reaction at the point of contact and this reaction is characterized by the infiltration of T-lymphocytes into challenged skin sites and the development of a cutaneous inflammation (Rios *et al*., 2005). The DTH response which is a direct correlate of CMI was found to be statistically increased at doses of 500 and 1000mg/kg body weight of the extracts. During CMI responses, sensitized T- lymphocytes when challenged are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. In this study, the footpad thickness was enhanced after extract treatment suggesting CMI enhancement. Increase in the DTH responses indicates that the extracts had a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction (Fulzele *et al*., 2003).

Sagrawat and Khan (2007) in their review reported that an alcoholic extract of *A. racemosus* was found to enhance both humoral and cell mediated immunity of albino mice injected with sheep red blood cells as particulate agents. Lower concentration of thyme essential oil was found to enhance murine DTH reaction and this was correlated with the known immunomodulatory effects of carvacrol

which is one of the main substances in thyme oil. It is reported that carvacrol selectively activates the extracellularly- responsive kinase subgroup in Jurkat T- cells and stimulates the JNK (c- Jun N- terminal kinase) subgroup in THP-1 monocytic cells (Human acute monocytic leukemia cell line) and so may act as an effective agent to modulate the functions of immuno- responsive cell via intracellular pathways (Juhas *et al*., 2008). n- butanol soluble and ethyl acetate soluble fractions of methanolic extract of *Lagenaria siceraria* fruits significantly reduced SRBC induced DTH reaction in rats but significantly increased the WBC and lymphocyte count but had insignificant changes in monocyte, eosinophil and basophil counts. Both fractions significantly increased antibody titers in a dose dependent fashion showing augmentation of humoral response but not CMI (Gangwal *et al*., 2008).

It is not clear to what extent the phytochemicals identified in this study played a role in enhancing some of the immune functions investigated. Flavonoids, a group of low molecular weight phenylbenzopyrones have been reported to have various pharmacological properties including antioxidant activity, anticancer, bactericidal, anti- malarial and immunomodulatory effects (Lyu and Park, 2005). Flavonoids can be classified into several chemical classes with the most common being anthocyanidins, catechins, flavones, flavonols, flavonones and isoflavonoids (Lyu and Park, 2005). Some immunomodulatory properties of certain flavonoids have been reported, catechin, epigallocatechin gallate, epicatechin, luteolin, chrysin, quercetin and galangin increased IL-2 secretion while epigallocatechin, apigenin and

fisetin inhibited the secretion (Lyu and Park, 2005). These results indicated that flavonoids have the capacity to modulate the immune response. Tannins, saponins and carbohydrate have also been reported to modulate immune functions (Wagner, 1987; Holderness *et al*., 2007).

These findings demonstrate the *A. setaceous* and *C. volkensii* can be added to the ever growing list of herbal medicines that have the potential to enhance immune responses.

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

From the results it can be concluded that:-

1. Both organic and aqueous extracts of *A. setaceous* and *C. volkensii* have antibacterial and antifungal activity with organic extracts showing more antimicrobial activity than the aqueous extracts.

2. Organic extracts of *A. setaceous* and *C. volkensii* have great potential as antimicrobial compounds against *S. faecalis*, *P. aeruginosa*, *S. aureus* and that they can be used in the treatment of infectious diseases, caused by some of these microbes. The dichloromethane and hexane extracts showed maximum antibacterial and antifungal activity and so these can be used to discover bioactive natural products that serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs.

3. The aqueous extracts of *A. setaceous* and *C. volkensii* had limited antibacterial and antifungal properties against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. faecalis* and *C. albicans*. The result of the study would suggest that traditional cures and remedies solely reliant on the antimicrobial properties of aqueous extracts of these plants would have little or no microbicidal activity.

4. The studies indicate that the aqueous, methanol and ethanol extracts of the two plants possess a potential of significant immunomodulatory and adaptogenic activity. The methanol, ethanol and aqueous extracts of *A. setaceous* aerial parts and roots, and *C. volkensii* leaves, stem and roots have the ability to increase the number of total WBC counts and specifically the neutrophils in albino rats.

5. The methanol, ethanol and aqueous extracts of *A. setaceous* aerial parts and roots, and *C. volkensii* leaves, stem and roots were effective in activating and enhancing neutrophil adhesion, phagocytosis and formation of reactive oxygen species by neutrophils. It also enhanced phagocytosis by macrophages. These plant extracts have the ability to enhance the activity of neutrophils and macrophage which means they should be able to respond to and destroy pathogenic agents more quickly.

6. The study has demonstrated that aqueous, ethanolic and methanolic extracts of *A. setaceous* and *C. volkensii* were effective in protecting rats against cyclophosphamide induced leucopenia. These results open up exciting possibilities for the use of plant products in the prevention of adverse bone marrow effects associated with cyclophosphamide treatment.

7. The traditional use of the plants to treat infectious diseases has

been supported by laboratory results from this study which has demonstrated that the main mode of action of the plant extracts of *A. setaceous* and *C. volkensii* would be through modulation of the immune system and functions. It would be difficult to postulate the extent to which antimicrobial property of the plant extracts play a role in preventing and controlling diseases in an *in vivo* environment given the weak *In vitro* antimicrobial activity they have demonstrated in this study.

6.2 RECOMMENDATIONS

From the results and conclusions of the study the following recommendations can be made:-

The present study demonstrated the immunomodulatory activity of the methanolic, ethanolic, and aqueous extracts in albino rats. These extracts could be considered as good candidates for developing immunomodulating agents. However the mechanism of plant extracts induced immunomodulation is not known and requires further comprehensive studies if these are to be included in the list of therapeutic agents.

Future research should concentrate on fractionating the crude extracts using the available chromatographic method. The isolated fractions could then be subjected to some of the tests conducted in this study as well as other experiments. This would give an indication of the activity of these purified fractions in comparison to the mother fractions.

There is also need to carry out chemical characterization of the plant extracts in order to elucidate the structure of the most active compounds present in the extracts.

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