Nutri-medicinal plants used in the management of HIV/AIDS opportunistic infections in western Uganda: documentation, phytochemistry and bioactivity evaluation

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Cover: *Plectranthus amboinicus* (Lour.) Spreng and one of its major chemical compound, carvacrol (photo by Asiimwe Savina)
Abstract

As a result of the AIDS epidemic, many people are immunocompromised and opportunistic infections are common. Medicinal plants constitute one of the fundaments of HIV treatment and are commonly used in management of HIV – related ailments, and also to counteract the side effects of antiretroviral therapy. This study documents and evaluates nutri-medicinal plants traditionally used in the management of opportunistic infections associated with HIV/AIDS in western Uganda. A six-stage process of documentation, evaluation and analysis of results was conducted: (1) ethnobotanical studies leading to identification and documentation of medicinal and nutritional plants most frequently used in the treatment of opportunistic infections of HIV/AIDS (2) Collection of plant samples and preparation of the extracts of each of the selected plants needed for bioactivity evaluation; (3) Phytochemical analysis of crude plant extracts (qualitative and GC/MS analysis); (4) pharmacological evaluation of the crude plant extracts (antimicrobial, antioxidant and mineral nutrient evaluation); (5) safety evaluation of the active extracts using animal models, and (6) Statistical analysis of the results.

The study recorded 324 plant species distributed in 75 families, with potential to treat ailments associated with immuno-compromised people living with HIV/AIDS in western Uganda. The study revealed that folk medicine is still widely practiced. Fidelity level values indicated the most preferred plant species for particular ailments. The high consensus values indicated that there was high agreement in the use of plants for various ailments. The selected preferred plant species were subjected to chemical screening to ascertain their pharmacological activities and they could be prioritized for conservation. The study allows for identifying high value medicinal plants indicating high potential for economic development.
Phytochemical screening of the aqueous and ethanol extracts of selected twenty plant species revealed the presence of tannins, saponins, flavonoids, anthocyanins, coumarins and steroid glycosides. Some of the major chemical compounds identified by gas chromatography mass spectrometry of the essential oils include α- phellandrene, linalool, carvacrol, geraniol, β-eudesmene, β-cubebe, α-caryophyllene, 1-8 cineole and caryophyllene oxide. The essential oils of *Plectranthus amboinicus, Erlangea tomentosa, Plunchea ovalis* and *Crassocephalum vitellinum* were highly active against *Candida albicans* and *Cryptococcus neoformans*. One of the essential oil fractions of *Crassocephalum vitellinum* (1.56 mg/ml) was highly active against *Cryptococcus neoformans*. Antioxidant activities of the plant species were also tested. The antioxidant activity of *Pseudarthria hookeri* (43.68%) and the ferric reducing power of *Symphytum officinale* (10.48 Mm/L) were the highest values. The ability of the plant extracts to scavenge free radicals may partly justify the traditional use of these plants to boost immunity in HIV/AIDS patients. Mineral nutrient analysis revealed high amounts of iron in *Plectranthus amboinicus* (5.8 mg/kg dry weight), zinc in *Pseudarthria hookeri* (6.9 mg/kg dry weight) and selenium in *Plunchea ovalis* (1.14 mg/kg dry weight). These elements are essential in maintenance of the immune system. Hematological analysis of the aqueous extract of *Plectranthus amboinicus* showed that the plant has immunostimulating properties by increasing the number of lymphocytes in the test animals. Further ethnopharmacological studies are needed for the documented plants particularly the most active ones.

**Key words:** Ethnobotanical study, Medicinal plants, HIV, AIDS, opportunistic infections, bacteria, fungi, GC-MS, phytochemistry, antioxidant, histopathology, biochemistry, hematology, western Uganda.
List of Publications and Manuscripts

This thesis is based on the following papers and manuscripts which will be referred to in the text by Roman numerals as follows:

*Journal of Ethnopharmacology 150(2013)639–648*

II. Documentation and consensus of indigenous knowledge on medicinal plants used by the local communities of western Uganda. Savina Asiimwe, Agnes Namutebi, Anna Karin Borg-Karlson, Maud Kamatenesi Mugisha and Hannington Oryem-Origa. 

III. Ethnobotanical study of indigenous knowledge on medicinal and nutritious plants used to manage opportunistic infections associated with HIV/AIDS in western Uganda. Maud Kamatenesi Mugisha, Savina Asiimwe, Agnes Namutebi, Anna Karin Borg-Karlson, and Esezah Kyomugisha Kakudidi. 
*Journal of Ethnopharmacology 155(2014)194–202*

IV. Chemical composition and Toxicological evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* Lour. Spreng, Savina Asiimwe, Anna Karin Borg-Karlson, Muhammad Azeem, Kamatenesi Maud Mugisha, Agnes Namutebi and Ndukui James Gakunga. 

V. Chemical composition and antimicrobial evaluation of the essential oil and fractions obtained from *Plectranthus amboinicus* (Lour.) Spreng traditionally used in the management of HIV/AIDS opportunistic infections. Savina Asiimwe, Anna Karin Borg Karlson, Muhammad Azeem, Abier Hamed Sofrata, Robert Byamukama, Maud Kamatenesi- Mugisha and Agnes Namutebi. 
*Manuscript for Phytochemistry*

*Manuscript for Nutrition*
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
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<tr>
<td>DPPH</td>
<td>1,1- Diphenyl -2 - picrylhydrazyl</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
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<tr>
<td>FRAP</td>
<td>Ferric reducing antioxidant power</td>
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<tr>
<td>GC-MS</td>
<td>Gas Chromatography Mass Spectrometry</td>
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<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HS-SPME</td>
<td>Head space solid phase microextraction</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>IK</td>
<td>Indigenous Knowledge</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Dose that causes mortality in 50 % in organisms tested</td>
</tr>
<tr>
<td>MBC/MFC</td>
<td>Minimum bacterial concentration and minimum fungicidal concentration</td>
</tr>
<tr>
<td>MPLC</td>
<td>Medium pressure liquid chromatography</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>United Nations Programme on HIV/AIDS</td>
</tr>
<tr>
<td>THETA</td>
<td>Traditional and Modern Health Practitioners Together Against AIDS</td>
</tr>
<tr>
<td>TBAs</td>
<td>Traditional Birth Attendants</td>
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<tr>
<td>TM</td>
<td>Traditional Medicine</td>
</tr>
<tr>
<td>TK</td>
<td>Traditional Knowledge</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>OECD</td>
<td>Organization for Economic Corporation Development</td>
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<tr>
<td>PLWHA</td>
<td>People living with HIV/AIDS</td>
</tr>
<tr>
<td>UNGASS</td>
<td>United Nations General Assembly Special Session</td>
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Thesis outline

This thesis is composed of chapters that have been published in various peer accredited reviewed journals or are in preparation. Chapter one gives an overview of the problem of HIV/AIDS in Uganda and a worldwide perspective. It also gives a general overview of how nutri-medicinal plants are traditionally used in the management opportunistic infections of HIV/AIDS; objectives, research questions, problem statement and significance of the study. Chapter two describes the methods used in data collection during the study which include ethnobotanical survey of plants used, investigations of the phytochemical constituents and antimicrobial activities of plant extracts. The isolation of the active compounds in the plant extracts through bioassay – guided fractionation is also discussed therein. The antioxidant activities and toxicity evaluation of the plant extracts is presented in chapter three. Chapter four presents results and general discussion as well as published articles and manuscripts which are in preparation. Chapter 5 outlines conclusions and recommendations from the study in the perspective of contributing knowledge to this pertinent subject.

Dedication

I dedicate this piece of work to my beloved daughter Asiimwe Josephine Byarugaba for her endurance and support during the times I would be away from home. To my husband Dominic Byarugaba, my sons Derrick Taremwa and Davis Twiine who always support my endeavours. To my friends for helping me achieve such an honored accomplishment and to many individuals that are continuously striving for an integrated health care delivery system in Uganda.
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1. Introduction

The work undertaken in this thesis is part of a broader project aimed at integration of nutri-medicinal plants as therapeutics in health care delivery in the western region of Uganda. The main goal of the project is to identify, upgrade and integrate nutri-medicinal plants used in the management of opportunistic ailments including HIV/AIDS and contribute to improved health in Uganda. The project involves documenting and validating of indigenous plants that are used for medicinal as well as nutritional values, by conducting ethnopharmacological and nutritional screening of selected plants. The project also aims at initiating propagation and domestication of key, rare and endangered nutri-medicinal plants for wide scale usage in communities for targeted ailments in immuno-compromised groups of people and to train local communities on techniques of multiplication of indigenous medicinal plants used in the management of HIV/AIDS and other opportunistic infections. Cultivation and domestication are central in this project so as to add value to African nutri-medicinal plants for easy accessibility to the wider community. An assortment of key plant species used in the management of HIV/AIDS clustered as backyard orchard plants will go a long way in the conservation efforts of these key species.

1.1 The origin of HIV and the AIDS pandemic

Acquired immunodeficiency syndrome (AIDS) is a chronic and potentially fatal disease of the immune system caused by the human immunodeficiency virus (HIV). The virus attacks a specific type of white blood cells known as T-lymphocytes which are critical to the normal functioning of the human immune system that defends the body against all types of illnesses. These cells are measured in the blood as CD4 count, and the lower the CD4 count, the weaker the immune system. Healthy people have between 600-1200 cells/mm$^3$ of blood. When CD4 cells count is less than 200 cells/mm$^3$, this is considered an advanced stage of HIV called AIDS. Therefore, CD4 cells act to control and direct the immune response and are used to monitor the progress of HIV infection. The consequences of a weak immune system in HIV/AIDS include the manifestations of opportunistic infections of which skin diseases form a large portion (Filberto et al., 2011). Opportunistic infections involve multiple systems of the body such as immune, gastrointestinal, genitourinary, endocrine, dermatological and nervous system.
HIV belongs to the lentivirus, subfamily of the retrovirdae and is a single-stranded RNA virus (Armbruster, 2008). AIDS in humans is caused by two lentiviruses, HIV-1 and HIV-2, identified in 1983 and 1986 respectively. HIV-1 has long been suspected to be of chimpanzee origin (Gao et al., 1999). Both HIVs are a result of multiple cross species transmissions of simian immunodeficiency virus (SVI) naturally infecting African primates (Sharp and Hahn, 2011; Sharp et al., 2005). However, one transmission event, involving SIVcpz from chimpanzees in southeastern Cameroon gave rise to HIV-1 group M; the principle cause of the AIDS pandemic, which has infected millions of people worldwide and is found in virtually every country on the globe (Reeves and Doms, 2002; Sharp and Hahn, 2011). In humans, HIV can be transmitted through infected blood, semen, vaginal secretions, breast milk, and during pregnancy or delivery of a new born.

The first AIDS case was diagnosed in 1981 in the United States, when cases of rare diseases caused by unknown virus among homosexual men were reported. Such cases include pneumonia caused by *pneumocytis carinii* and Karposi sarcoma, a rare skin cancer caused by a virus (Gottlieb et al., 1981). Since then, the disease has spread to epidemic proportions around the world. HIV/AIDS affects mankind globally, but the highest prevalence is in Sub-Saharan Africa (with 68% of all cases of HIV/AIDS and a prevalence above 15% in some regions), and in some areas of South-East and Central Asia. In 2013, about 35 million people globally were living with the HIV infection, 2.1 million people became newly infected with HIV, while 1.5 million people died from AIDS-related illnesses (UNAIDS, 2014). However, treatment is not available globally and UNAIDS still estimates that there are currently 5000 AIDS-related deaths worldwide per day (UNAIDS, 2013).

In Uganda, the first case was identified in 1982 along the shores of Lake Victoria (Mugerwa et al., 1996). Consequently, the epidemic spread very fast to all parts of the country initially concentrating in urban and semi-urban centers (Mugerwa et al., 1996). By the end of 1992, the national prevalence rate was estimated at 18.3% with some centers registering rates above 30% (Mugerwa et al., 1996). This was followed by a steady decline in prevalence rates from the mid-1990s to 2000 to around 6% attributable to favourable prevention policies. Uganda’s HIV sero-prevalence rate of 7.3% among adults and 0.7% among children is among the world’s highest
UNAIDS, 2013), with an estimated 1.3 million people living with HIV (Lamorde et al., 2010). About 43% of new infections occur among married people in monogamous heterosexual relationships. The HIV pandemic is currently the most socio-economic challenge that faces the most economic productive sectors of society – the young and women. The western region of Uganda has the most marked variability and antenatal HIV prevalence, with Mbarara district recording the highest HIV prevalence rate of 13.7% in the region (UNGASS, 2010). Poverty, underdevelopment and illiteracy contribute to the spread of HIV in the developing world, yet HIV/AIDS is also observed to aggravate the poverty situation thus hindering development efforts. This calls for a fast mechanism of intervention by applying all forms of remedy, to combat the alarming rates.

1.2 Traditional medicine in the management of opportunistic infections of HIV/AIDS

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2002b). Traditional knowledge (TK) of medicinal plants and their use by indigenous cultures is not only useful for conservation of cultural traditions and biodiversity, but also for community healthcare and drug development for present and future generations (Pei, 2001). In Africa and Asia, 80% of the population still uses traditional remedies rather than modern medicine for primary health care (WHO, 2002a).

The pharmacological treatment of disease began long ago with the use of herbs, and methods of folk healing throughout the world used herbs as part of their tradition (Schulz et al., 2001). Traditional medicine has demonstrated its contribution to health through reduction of excessive mortality, morbidity and disability due to diseases such as HIV/AIDS, malaria, tuberculosis, diabetes, sickle cell anemia and mental disorders. The devastating effect of HIV/AIDS pandemic coupled with the severe shortage of health personnel has compelled patients to develop coping mechanisms by adopting alternative sources of primary health care. One of the sources is the use of herbal therapies because they are easily accessible and affordable especially in rural settings.

In Africa, herbal medicines are used as primary treatment for HIV/AIDS and related infections including insomnia, nausea, dermatological disorders and weakness (Hodgson and Rachanis,
2002; Mills et al., 2005). Studies have shown that majority of people living with HIV and receiving antiretroviral drugs use herbal medicine after HIV diagnosis mainly to offset the side effects from antiretroviral treatment (Kazhila and Marius, 2010) and to treat opportunistic infections associated with the disease (Kisangau et al., 2007; Langlois-Klassen et al., 2007; Peltzer et al., 2008). This is because patients on highly active antiretroviral therapy (HAART) suffer side effects like poor appetite and nausea due to interference with absorption and utilization of nutrients (Ridder, 2003).

However, research shows that traditional knowledge (TK) and plant diversity are currently being lost at an accelerated rate by such forces as climate change, habitat destruction and globalization (Thomas et al., 2009). Due to continuous destruction of habitats, numerous undiscovered species are lost from the wild unrecorded. TK systems are also fading away along with cultures they once kept alive. Hence, the aim of this study was to document and validate the medicinal plants that double as medicine and food supplements in health care delivery especially in the management of opportunistic infections associated with HIV/AIDS in western Uganda. It is therefore important to document the uses of medicinal plants because such information can help preserve the medicinal plant resources and the knowledge associated with them. The work presented in this thesis contributes to the understanding of herbal medicine in managing opportunistic ailments associated with HIV/AIDS in western Uganda.

1.3 Major groups of antimicrobial compounds from plants
Infectious diseases caused by bacteria, fungi, insects and arthropods are major threats to public health despite tremendous progress that has been made in the medicinal sciences (Cos et al., 2006). Microbial infections are the most frequent opportunistic diseases occurring during HIV/AIDS which affect many people in Africa (Couidiati et al., 2011). The search for substances with antimicrobial activity from plants have been considered interesting by researchers since they are frequently used in traditional remedies for many infectious diseases. A number of modern drugs have been isolated from natural sources as remedies for human and animal diseases as they contain phytochemicals of therapeutic value (de Melol et al., 2011; Edeoga et al., 2005; Raginee et al., 2013). Different plant parts (root, leaves, stem bark, flowers and seeds) are traditionally used to cure various ailments and diseases such as malaria, cough,
skin infections, asthma, diarrhoea and cancer among others. The plant parts produce a wide
range of chemical compounds such as alkaloids, saponins, tannins, flavonoids, terpenes, phenols
and essential oils that can be used to treat various chronic and infectious diseases (Cowan, 1999;
de Melol et al., 2011; Murakami et al., 2000; Periyanayagam et al., 2013; Reichling, 2010;
Sukirtha and Growther, 2012). For instance, tannins have been reported to hinder development of
microorganisms by their ability to precipitate and inactivate microbial adhesions enzymes and
cell envelops proteins (Cowan, 1999). Steroidal saponins have been reported to exhibit
antimicrobial activities against such organisms as Cryptococcus neoformans and Candida
species (Abbasolu and Türköz, 1995). Analysis of essential oils (mixtures of chemical
compounds obtained from aromatic herbs, flowers and fruits) shows that they contain different
compounds of which terpenoids in form of mono-, sesqui- and hemiterpenes are the most
abundant (Loza-Tava, 1999; Trombetta et al., 2005). This abundance is equally related to the
soil type and general ecological and climatic setting of their location.

According to research, it takes years for a new drug to get through the research and development
pipeline to manufacture and the cost is quite enormous. In addition to this, drug resistance in part
caused by the misuse of medications, has rendered several antibiotics and other life-saving drugs
useless. Both these trends mean that scientists and pharmaceutical companies are urgently
looking for new drug sources and are increasingly turning their eyes to traditional medicine.
Research shows that almost a quarter of all modern medicines are derived from natural products,
many of which were first used in traditional remedies centuries ago. This study focused on
natural plant products as a useful source of antimicrobial molecules, active in particular, on
bacteria and fungi, as the main causes of infection in HIV/AIDS people. Studies exploiting the
mechanism of action and the structure- activity aspects of these natural compounds may provide
both additional antimicrobial leads and drugs, and also significant insight into potential
possibilities to overcome the antimicrobial resistance. Hence, the aim of this study was to
provide insights regarding the possibilities of the most important natural antimicrobial
compounds derived from plant sources containing a wide variety of secondary metabolites,
which are useful as alternative strategies to control microbial infections in HIV/AIDS patients.
As a result, many herbalists stand to set up more therapy centers sometimes to receive greater
numbers from time to time.
1.4 Common opportunistic bacterial and fungal organisms in HIV-infected patients

Opportunistic infections (OIs) are infections that take advantage of a weak immune system. When the immune system is weakened by HIV disease, bacteria and fungi can get out of control and cause a wide range of illnesses resulting in mild to life threatening illnesses such as diarrhoea, bacterial meningitis, respiratory infections, fungal infections of the skin, headache, weight loss, night sweats, fever, cryptococcal meningitis, herpes zoster, candidiasis (fungal infection of the oral cavity, throat and vagina), tuberculosis (a bacterial infection that attacks the lungs), *Pneumocystis carinii* pneumonia and *Mycobacterium avium* complex which causes recurring fevers (CDC, 2013; Murray and Pizzorno, 1999; Vermani and Garg, 2002). Patients with HIV infection are particularly prone to infections with organisms such as *Candida albicans*, *Cryptococcus neoformans*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (methicillin-susceptible and -resistant strains) are some of the most common pathogens of immunocompromised individuals (Zgoda and Porter, 2001). *Klebsiella pneumonia* is a causative organism of pneumonia. *Pseudomonas aeruginosa* are residents of the intestinal tract and in moist areas of the human skin. They cause a variety of systemic infections notably finger, toe gaps, thigh and armpits in patients with cancer, immunocompromised patients with AIDS, respiratory tract infections (*Pseudomonas pneumonia*), urinary tract, eye infections and wounds. *P. aeruginosa* is notorious for its resistance to several antibiotics (Cooper et al., 2011; Philip et al., 2009). *Candida albicans* is a causal agent of opportunistic oral and genital infections in humans. *S. aureus* causes eye infections and is frequently found in the human respiratory tract and on the skin.

Acute bacterial and fungal infections have been important causes of morbidity and mortality especially among patients immunosuppressed by HIV/AIDS. *Pneumococcal pneumoniae* is recognized as one of the leading early HIV-related problem and susceptibility increases with progressive immunosuppression (Schuchat et al., 1991). According to world health organization, increasing access to antiretroviral therapy (ART) has transformed the prognosis of HIV infected patients. However, treatment coverage still remains low. As a result, many patients continue to die of HIV-related opportunistic infections (OIs). Cryptococcal meningitis is one of the most
important opportunistic infection that causes more than 500,000 deaths in sub Saharan Africa (Park et al., 2009).

1.5 Nutrition and antioxidants in the pathogenesis of HIV/AIDS: A healthy immune system

HIV has been considered the main cause in the progression of AIDS and it weakens the defensive role of the human immune system (Anthony and Ashok, 2011). Therefore, the human body requires a balanced diet that provides nutrients, minerals and vitamins for a functional and effective immune system. The major immunity boosting components in herbs, fruits and vegetables include flavonoids, phenols and carotenoids which are antioxidants that protect cells from oxidative damage. Flavonoids have been reported to stimulate the immune system, reduce platelet aggregation, reduce blood pressure and have antioxidant and antimicrobial effects (Craig, 1999). Carotenoids boost the immune system to fight bacteria by increasing the number of white blood cells.

Antioxidants are molecules that can delay or prevent an oxidative reaction catalyzed by free radicals. It has been observed that perturbations in the antioxidant defense systems and consequently redox imbalance are present in many tissues of HIV infected patients (Pham-Huy et al., 2008). Free radicals and antioxidants are produced either from normal cell metabolism or from external sources such as cigarette smoking, pollution, radiation or medication, and they play a dual role as both toxic and beneficial compounds (Phum-Huy and Pham, 2008; Saikat et al., 2010). However, accumulation of free radicals in the body results into a phenomenon called oxidative stress, which leads to chronic and degenerative illnesses such as autoimmune disorders, cancer, cardiovascular and neurodegenerative diseases (Pham-Huy et al., 2008). Hence, antioxidants act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species, thereby enhancing the immune defense system and lowering the risk of degenerative diseases. Some of the vital phytochemical metabolites include poly-phenols, quinones, flavonoids, catechins, coumarins, terpenoids and in addition to the smaller molecules like ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E). Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds (Sies et al., 1992). Nutrition in terms of antioxidants and trace minerals is integral to the care of all patients infected with human immunodeficiency virus (HIV) by supporting their natural immunity (Nkengfack et
al., 2012). Minerals like selenium, zinc, iron, copper, manganese etc are well known antioxidants (Shirwaikar et al., 2004) and are necessary to strengthen the body's own antioxidant protection / immune system. Many studies link low levels of serum micronutrients with worsened HIV disease status and mortality, for instance, low selenium (Kupka et al., 2004), low zinc (Lai et al., 2001). Research shows that combining selenium with antiretroviral therapy (ART) suppresses the progression of viral load in HIV and boosts numbers of immune cells (Stephen, 2008b). It has also been reported that zinc affects a large number of HIV individuals (Jones et al., 2006) and is a cofactor in more than 300 enzymes that influence different organ functions having a secondary effect on the human immune system (Lothar and Philip, 2000). For instance, daily intake of zinc reduces diarrhoea and increases weight gain.

Plants used traditionally as sources of food and medicine constitute a potentially useful resource for new and safe drugs for the treatment of opportunistic infections. According to World Health Organization (WHO, 2003), nutritional support is an integral part of a comprehensive response to HIV/AIDS, helping to maintain the immune system and sustain healthy levels of physical activity. It has been reported that HIV infection can jeopardize the body’s efficiency to utilize food nutrients and can lead to malnutrition; which in turn can contribute to increased immunocompromised state (Hecker and Kotler, 1990). In this way, proper nutrition enables HIV patients to take medication, manage side effects from antiretroviral drugs and maintain adequate nourishment by restoring intestinal function and weight gain (Guarino et al., 2002; Tinnerello, 1998). The beneficial effects of plant materials result from the combinations of secondary products (phytochemicals) present in the plant. These phytochemicals can be used in treating multiple illnesses such as malaria, HIV/AIDS and cancer among others. Studies have suggested that the mechanisms responsible for progression of AIDS could be reversed through administration of antioxidant reducing agents. Hence, this study was set out to determine the nutritive values of selected plants in terms of total antioxidant capacity and mineral nutrient composition.
1.6 Safety evaluation of nutri-medicinal plants: the need for toxicity testing of herbal extracts

Toxicity studies show how potent a chemical is by measuring the concentration that will affect the test organisms, thereby assessing the potential health risk in humans caused by intrinsic adverse effects of chemical compounds present in plant extracts. Plants have been used for medicinal purposes for centuries and are usually promoted as being ‘natural’ and ‘safe alternatives’ to conventional medicines, but many contain useful as well as toxic constituents (Adewunmi and Ojewole, 2004). Traditionally, people think that medicinal herbs being natural are safe and free from undesirable effects failing to recognize that herbs are composed of chemical compounds some of which may be toxic. For instance, *Amaranthus* species contain high levels of oxalic acid, which has the ability to bind metals like calcium and magnesium thereby interfering with their metabolism (Soetan and Aiyelaagbe, 2009). Although more than 80% of people today depend on herbal medication as a component of their primary health care according to world health organization, there is still great concern about the safety and efficacy of herbal use. Care must be taken not to consume harmful plants or high doses of plant extracts that could have deleterious effects on vital body organs either in short or long term. Apart from efficacy, safety of herbal medicines is of paramount importance as there is limited scientific evidence to establish the safety and efficacy of most herbal products used in traditional medicine. Although there are no systemic side-effects reported for humans in the literature (Jon and Ted, 2008), many different side effects have been reported owing to active ingredients, contaminants or interactions with drugs (Stephen, 2008a). Herbal medicines have stood a test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. This scenario withstanding, it is important to determine phyto-extract toxicological effects for safety.

Biochemical tests have immense benefits in the diagnosis and monitoring of liver diseases (Vasudevan and Sreekumari., 2007). The liver being the major organ responsible for metabolism of toxic substances entering the body, its functions can be altered by liver injury following acute or chronic exposure of toxicants (Alisi and Onyeze, 2008). Damage to the liver is associated with cellular necrosis and increase in serum levels of biochemical parameters like Alanine
aminotransferase, bilirubin and aspartate aminotransferase (Wolf, 1999). Evaluation of hematological parameters can also be used to determine the extent of deleterious effect of xenobiotics (such as saponins, alkaloids and tannins) on the blood of an animal. Such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for humans when the data are translated from animal studies. The adverse effects may manifest in form of alterations in levels of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs (Ashafa et al., 2009). Hence, there is need to study the effects of these plants which have potential therapeutic benefits to ascertain their safety in animals using biochemical and haematogical indices.

Several studies have indicated the possibility that using plant extracts in high doses could lead to toxic injury to the kidneys which interfere with renal tubular functions and induce acute renal failure (Bwititi et al., 2000; Ijeh and Agbo, 2006). Research shows that most of the existing data on traditional medicine in Africa deal only with medicinal plants and their uses, ignoring chemical and pharmacological studies. The administration of herbal preparations without any standard dosage coupled with inadequate scientific studies of their safety has raised concerns on their toxicity. Hence, this study was done to evaluate the toxicological effects of five selected plants used in traditional medicine to manage opportunistic ailments associated with HIV/AIDS and other immunocompromised groups of people, such as the children and pregnant mothers.

1.7 Problem statement
The last two decades have witnessed dramatic rise in the incidence of life threatening opportunistic infections due to the growth in the immunocompromised population especially HIV/AIDS patients. The challenge has been to develop effective strategies for the management of these infections in HIV patients. Despite the introduction of antiretroviral therapy (ART) to arrest progression of immunodeficiency, less than 10% of HIV infected people have access to any therapy for HIV infection. The development of drug resistance, lack of curative effect, high cost, toxicity and unavailability are some of the shortcomings associated with the use of ART. These shortcomings have resulted in increased efforts to search for better alternatives from natural products. The use of herbal remedies is widespread among those living with HIV in Uganda and more than 80% of Ugandans depend on traditional medicine for primary health care
needs. Even with the few plants documented through ethnobotanical surveys, little work has been done on the screening of these plants to prove the validity of the claimed medicinal uses as described by traditional healers. Coupled to this shortcoming is the secrecy traditional medicine practitioners attach to their knowledge of herbal medicine.

Plants have not received their share of research especially validation in terms of safety, much as organizations like THETA have promoted them in management of HIV/AIDS in Uganda. Above all, health issues are continually challenging as pathogens evolve and the environment changes from time to time; a situation that calls for constant research and testing of remedies. Plants are widely used but their effects are still largely unknown in science studies, and nutrition which is a key ingredient in medical treatment is ignored in most scientific research. The pharmacological activities and toxicity profiles of most herbal drugs is unknown, and hence, the need to develop cheaper, safer and effective antimicrobial drugs through research.

1.8 Significance of the study

Documentation of plant species with active compounds against HIV/AIDS opportunistic infections helps to preserve or keep important indigenous knowledge safe. The key to medicinal plants research revolves around the detection, isolation and characterization of active compounds as therapeutic agents. Scientific validation of herbal medicines provides basic understanding of a plant’s efficacy and this may lend further support to the widespread use of TM in health care systems, provided toxicological investigations are carried out. Several herbs have been attributed to improve immune response and could reduce symptoms of HIV/AIDS; however, this needs scientific validation in terms of safety and efficacy. Information on safety and toxic levels of the plants can be a starting point for the formulation of dosage levels of the herbal remedies.

Bioactivity analysis may lead to the identification and isolation of novel chemical compounds and may confirm which plants are useful as nutraceutical and pharmaceutical products in the management of HIV/AIDS related infections. With increasing antibiotic resistance, it is important to find effective treatments for microbial infections. However, routine testing for chemical composition, biological activity and toxicity of plant derived natural products are all critical attributes to be considered for a complete understanding of the effect of medicinal plants on human health care. The rationale of the study was to address the health burden in terms of
both medicine and nutrition intervention. This was done stemming from the first principles of use which is indigenous knowledge information. This is why this study was set out to document and validate the herbal and nutritive plants; and their practices in the management of HIV/AIDS-related infections. Efforts geared towards improving the quality of herbal medicines will in turn improve the quality of health, nutritional status and economic empowerment of the people involved in the conservation, collection, preparation and administration of these plant products. Hence the value of this research lies not only in promoting the value of nutri-medicinal plants in the context of HIV/AIDS epidemic, but also in the need for improved primary health care. This research will also create new knowledge in the area of pharmacology and nutrition.

1.9 Objectives

1.9.1 Main objective

The overall objective of the study was to document and validate the efficacy of nutri-medicinal plants used to manage HIV/AIDS opportunistic ailments.

1.9.2 Specific objectives

The specific objectives of the study were to:

1. Identify and document plant species with nutritional and medicinal properties.
2. Determine the antioxidant and antimicrobial properties of the selected plant species.
3. Identify the bioactive molecules in the most active plant extracts.
4. Determine the toxic levels of the most active plant extracts using animal models.

1.9.3 Research questions

The study was guided by the following research questions

1. What medicinal plants are used to treat/manage HIV/AIDS immune compromised ailments?
2. What are the nutritive and medicinal values of the used plants?
3. What are the active chemical compounds responsible for bioactivity in the screened plant extracts?
4. Are the plants raw materials safe for use as functional foods and pharmaceuticals?
2. Materials and methods

2.1 Description of study sites
This study was carried out in ‘Greater Mbarara’ which has been sub-divided into four districts of Ibanda, Kiruhura, Isingiro and Mbarara. The districts are situated in western Uganda on coordinates 00° 07’S 30° 30E; 00° 12’S, 31° 00E; 00° 50’S 30° 50E and 00° 36’S 30° 36E respectively (Fig. 1). The study areas are similar in climate, vegetation, population and economic activities. They are in the same geographical range (U2), and have a mean annual rainfall ranging from 1,100–1,200 mm and temperatures ranging from 17°C and 30°C.

The districts are predominantly occupied by Banyankore, Batagwenda, Bahima and other immigrants; Bakiga, Baganda, Itesot, Banyarwanda, Congolese, and Bafumbira. Mbarara district, the largest in the region, is bordered by Ibanda district in the north, Kiruhura district to the east, Isingiro district to the south east, Ntungamo district to the south west, Sheema district to the west and Buhweju district to the North West. Agriculture, which includes mixed farming, cultivation of crops and grazing of cattle and goats, is the main economic activity in the districts and accounts for more than 50% of the Gross domestic product. Crops grown include bananas, sweet potatoes, Irish potatoes, maize, cassava and ground nuts. Health service provision is a key priority of the districts. The districts have established health center facilities from health centre II at Parish level, health center III at sub county level and Health Center IV at county level.

This study was done in western Uganda due to the highest burden of HIV/AIDS in the region with a prevalence rate of 13.7% (UNGASS, 2010), and also due to a lot of biodiversity which is beneficial to human health and has been used overtime by the local communities (Anoka et al., 2008; Anywar et al., 2014; Asiimwe et al., 2013; Bunalema et al., 2014; Byarugaba et al., 2007; Kakudidi et al., 2000; Kamatenesi-Mugisha and Oryem-Origa, 2007; Kamatenesi et al., 2008; Katuura et al., 2007; Namukobe et al., 2011; Tabuti et al., 2003). Therefore, the rich biodiversity forms a basis for the investigation on the biological active substances of natural medicines that can pave way for the development of new alternatives and environment friendly medicines that can be used in the management of HIV/AIDS opportunistic infections and other diseases, such as, malaria, respiratory, cardiac and gastrointestinal ailments.
Figure 1. Location of study areas in western Uganda
2.2. Ethnobotanical field survey (Papers I, II, III)
An ethnobotanical study was conducted in four districts of western Uganda; Mbarara, Ibanda, Kiruhura and Isingiro from December 2010 to May 2011. Key informants were selected purposively (Ma Dolores, 2007; Martin, 1995) based on the skills, knowledge and practices in medicinal plant usage. The key informants were renowned traditional medical practitioners such as herbalists, traditional birth attendants and other local herbal medicine users like the elderly women who were identified and chosen from the different villages with the assistance of local administrators and community elders through visiting homesteads (Fig 2).

Fig 2. Methods used in field data collection

Subsequent interviewees were found with snow ball sampling (Salganik and Heckathorn, 2004). In all the study areas, semi structured interviews with the aid of a questionnaire, focused group discussions (FGDs) and field visits (Silverman, 2005) were used to document indigenous knowledge on local names of plants used, parts and growth forms of the plants, conservation status, mode of preparation and application of the herbal remedies. Interviews were conducted in
the local language of the area, Runyankore, except for a few cases where respondents were not natives and translators were hired. Interviews were made with each traditional healer about the knowledge and use of medicinal plant species used to treat human diseases in the study area, with emphasis on opportunistic ailments associated with HIV/AIDS. The healers were professional practitioners who medicate the local people by using crude extracts from plant materials (leaves, bark, roots, seeds, flowers, stem and fruits). In order to comply with internationally accepted ethical standards and ensure the protection of participants the proposal for this study was approved by the Uganda National Council for Science and Technology. Before conducting interviews, the aim of the study was explained clearly and informants were asked for their consent. The purpose and benefits of the study were explained to the participants. The questionnaire also included questions name of respondent, age, gender, religion, education, tribe and occupation. The village, Parish, Sub-county and County where respondents came from were also recorded.

The HIV/AIDS opportunistic conditions considered during the study were tuberculosis, herpes zoster (shingles) and oral candidiasis. Other symptomatic but undefined conditions considered were malaria, fungal infections of the skin, cough, diarrhoea, syphilis, hiccups, fever and headache (CDC, 2013). Nutrition-related conditions such as anaemia, appetite and immunity boosting were also considered. Plant specimens were identified in the field while those that could not be identified were collected, pressed and deposited at Makerere University Herbarium for identification. The specimens were identified by comparing with the already existing specimens and using taxonomic literature such as (Katende et al., 1999; Maundu et al., 1999; Tallantire and Lind., 1962). Scientific names of plant species were identified basing on the International Plant Name Index (IPNI).

2.3 Selection of plants and extract preparation for bioactivity evaluation

One of the standard approaches for selecting plants for drug discovery is the use of ethnomedical information about the traditional uses of the plants against various disease conditions (Fabricant and Farnsworth, 2001). For this study, twenty plants that were frequently mentioned in all the study areas were selected for bioactivity analysis. Secondly, there was little or no ethnobotanical and ethnopharmacological information about the selected plants. One of the plants was selected
for curiosity purposes basing on its utilization by both humans and chicken believed to be a good vegetable and changes the egg yolk to yellow. Lastly, some of the species from the same genera as the selected plants elsewhere in the world have shown significant antioxidant and antimicrobial activities.

Fresh plant materials (leaves and stem barks) were collected from peoples’ herbal medicine gardens and from the wild in the various villages in Mbarara district. The plant materials were air dried and ground into powder using a mortar and pestle, and finally stored in polythene bags before analysis. The powders (100g each) were first soaked in ethanol for 48 hours, filtered and dried using a rotary evaporator. The powders were also soaked in water and the extracts were concentrated to dryness using an oven at 40°C. The process was repeated for all the samples and the extracts stored under room temperature for further analysis. Dried leaves and bark samples (100g) were also subjected to steam distillation for isolation of essential oils using hexane which was then evaporated using a rotary evaporator. The essential oils were transferred to clean vials and stored in a freezer until analysis with Gas Chromatography – Mass Spectrometry.

2.4 Qualitative phytochemical analysis of the crude extracts
Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemical compounds. In this study, phytochemical screening was done to determine the groups of chemical compounds present in the water and ethanol plant extracts. Chemical qualitative analysis for major active constituents such as alkaloids, tannins, steroids, reducing sugars, coumarins, anthraquinones and carotenoids, was undertaken using standard qualitative methods of analysis as described by (Harbone, 1993; Trease and Evans., 2002).

2.4.1 The use of water and ethanol in phytochemistry
Since herbal medicine research aims at identifying bioactive phytocompounds in medicinal plant extracts used by local people to treat diseases based on indigenous knowledge, the solvent chosen must be the same as that used by local communities. Hence, water and ethanol are the commonly used solvents. Water is a universal solvent used to extract plant products with antimicrobial activity (Prashanti et al., 2011) and ethanol (at a concentration of 70-96%) is more effective in isolating bioactive phytocompounds than water. Ethanol is used by local
communities in form of fermented porridge or local brew (made out of banana juice and fermented sorghum – locally known as ‘tonto’ in Runyankore dialect) to extract and administer the herbal remedies. Since this study aims at validating indigenous knowledge, water and ethanol extracts were prepared for ethnopharmacological screening. Nearly all identified components from plants active against microorganisms are aromatic and saturated organic compounds, and are most often obtained through ethanol and water extraction (Lewis and Elvin-Lewis, 1995).

2.4.2 Chemical qualitative analysis of the aqueous and ethanol extracts

Identification of steroids / triterpenoids
The aqueous leaf extract (3 ml) was evaporated to dryness in a water bath; acetic anhydride (0.5 ml) and chloroform (0.5 ml) were added. Concentrated sulphuric acid (1 ml) was then added at the bottom of the test tube containing the sample. The contact zone of the two liquids was observed for a violet or brownish red ring formation.

Identification of basic alkaloids
The aqueous leaf extract (10 ml) was evaporated to dryness and hydrochloric acid (1.5 ml, 2%) was added to the residue. The solution was then divided into three equal volumes in the test tubes. Three drops of Mayer’s reagent was added to one test tube. Basic alkaloids were indicated by a yellowish-white precipitate or coloration of the solution. The other test tube acted as a control.

Identification of flavones aglycones
The aqueous leaf extract (3 ml) was evaporated to dryness in a water bath, the residue dissolved in methanol (2 ml, 50%) and the solution heated on a water bath for 3 minutes. The solution was then divided into equal volumes. A piece of magnesium ribbon (0.1g) and concentrated hydrochloric acid (4 drops) were added to one test tube. Flavone aglycones were indicated by change in colour of the sample to red or orange. The other acted as a control.

Identification of anthraquinones (Anthracenocides aglycones)
The aqueous leaf extract (3 ml) was transferred into a test tube and ammonia solution (1 ml, 25%) added. The solution was shaken thoroughly. Anthraquinones were identified by the presence of red colour in the aqueous or ethanolic organic layer.
Identification of coumarins
The aqueous leaf extract (5 ml) was evaporated to dryness in a water bath. Distilled water (3 ml) was added and the mixture heated on a water bath to boil and the mixture cooled under running water. The solution (0.5 ml, 10%) was added. Both test tubes were observed under Ultra Violet light and presence of coumarins was indicated by (blue or green) fluorescence in test tube containing ammonia solution.

Identification of tannins
The ethanol leaf extract (1 ml) was diluted with distilled water (2 ml) and 3% solution of ferric chloride (3 drops) was added. Tannins were identified by occurrence of a blackish-blue colour for gallic tannins and green blackish for catechol tannins.

Identification of reducing compounds
The ethanol leaf extract (1 ml) was diluted with distilled water (2 ml), and Fehling’s solutions 1 and 2 (1 ml) were added to the mixture. The solution was heated on a water bath. Reducing compounds were identified by the presence of a brick red precipitate at the bottom of the test tube.

Identification of flavonoids
The organic layer obtained after hydrolysis (5 ml) was evaporated to dryness. The procedure was then followed as earlier reported in identification of flavone aglycones in the water extracts as indicated above.

Identification of sterol glycosides
The organic layer obtained after hydrolysis (5 ml) was evaporated to dryness. The procedure was then followed as earlier reported in identification of sterols/ triterpenoids in the water extracts as indicated above.

Identification of anthocyanins
Ammonia solution (3 ml, 10%) was added to acidic solution (3 ml) obtained after extraction of the hydrolyzed solution drop wise. Change in colour of the solution from red to either violet or greenish blue is due to presence of anthocyanins in the extracts.
2.5 Collection of headspace volatiles, Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is a method used to collect headspace volatiles from plants and insects (Borg-Karlson and Mozuraitis, 1996; Kännaste et al., 2008; Kännaste et al., 2013). Fresh leaves (attached to the plant) and dry leaves were enclosed in bottles and the volatiles were extracted by headspace (HS) SPME using a Polydimethylsiloxae/divinylbenzene 65µm- fiber (PDMS/DBV), equilibration time was 15-60 minutes. During collection of volatiles, leaves were kept in a glass beaker that was sealed with aluminium foil (Fig 3). The extracted materials were subsequently desorbed and analyzed in a gas chromatograph mass spectrometer (GC-MS). In addition, the leaves were macerated in cold water for 1 hour and volatiles collected for 1 hr.

![Figure 3](image)

**Figure 3.** SPME of volatiles: (A) fresh leaves; (B) dry leaves

2.6 Isolation of essential oil

Fresh leaves, dry leaves and dry bark samples (100g) were each subjected to steam distillation for 4-5 hours to isolate essential oils (Figure 4). The distillate was collected and extracted three times with 100 ml of HPLC grade hexane (Carlo Erba Reagents, France). The hexane extracted essential oil was dried using anhydrous magnesium sulphate (Alfa Aesar, United Kingdom), filtered and the solvent was evaporated on rotary evaporator at 20º C under reduced pressure. The essential oils were weighed and stored in tightly closed glass vials in a freezer at -20 ºC for further analysis with GC-MS.
2.7 Fractionation of essential oil using Medium pressure liquid chromatography

In order to separate compounds in the essential oil for antimicrobial activity testing, the oil of the dry leaves of four plants (plants that showed significant antimicrobial activity) was subjected to preparative medium pressure liquid chromatography (MPLC), equipment (Baeckstroem SEPARO AB, Lidingö, Sweden) (fig 5). 20 g silica gel (60 Å, 40-63 µm, Carlo Erba Reagents, France) was packed in 15mm internal diameter glass column fitted with pistons that could be adjusted to the desired bed length of silica gel. An FMI lab pump, model QD (Fluid metering Inc., Oyster bay, NY) was used to maintain constant flow and gradient of mobile phase. The gradient elution was employed starting from 100% hexane till 20% ethyl acetate in hexane running through 2.5%, 5% and 10% respectively. 100 ml of each binary solvent was used. The eluent was collected in 10 ml fraction tubes that were analyzed by thin layer chromatography (TLC) using pre-coated TLC plates (Silica gel 60 Å, 0.2 mm coating on aluminum plates with F254). Based on TLC results, different fraction tubes were pooled together giving rise to 5 fractions. The TLC plates were visualized using ultra violet light and vanillin staining agent (3 g vanillin, 0.5 ml sulphuric acid in 100 ml absolute ethanol).
2.8 Chemical analyses of essential oils

Separation and identification of volatile compounds of essential oils and fractions were carried out on gas chromatography–mass spectrometry (GCMS) using a Varian 3400 GC connected to a Finnigan SSQ 7000 quadrupole mass spectrometer. The GC was equipped with a split/splitless injector (splitless mode, 30 sec); the carrier gas was helium, with a constant pressure of 10 psi and a flow rate of 1 ml/min. The GC was equipped with a DB-WAX capillary column (30 m, 0.25 mm ID) and 25 µm film thickness, J & W Agilent USA). The temperature program of the GC oven was 40 °C for 1 min, then increasing at a rate of 3 °C/min till 235 °C and maintained for 9 min. The injector temperature was isothermally set at 230° C and the transfer line connecting to the MS was isothermally set at 235 °C. The MS ion source temperature was 150 °C; mass spectra were obtained at 70 eV with a mass range of 30 - 400 m/z. Initial identification of unknown compounds was made by comparing their mass spectra with NIST-08 (National
Institute of Standard and Technology, USA) MS library, Mass Finder 3, or comparing retention index with published literature (Adams, 2007) or determined with reference to a homologous series of normal alkanes. The final authentication was made by analyzing standard compounds (purchased from Sigma-Aldrich) on GC-MS using same parameters that were used for essential oil and fractions. One microliter aliquot containing 3µg/µl of essential oil or fraction was injected into the GC injector for analysis.

2.9 Microorganisms and preparation of sample extracts used for antimicrobial assay

The antimicrobial activity of the plant extracts was tested against four standard human pathogenic bacteria of the American Type Culture Collection (ATCC) viz, gram positive *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49619); gram negative *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), and two yeasts: *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 90112). Ciprofloxacin (30 µg) and Nystatin (100 units) were used as positive standard controls for bacteria and fungi, respectively. The organisms were obtained from MBN Clinical Laboratories, Kampala, Uganda. The organisms were cultured on Mueller Hinton Agar for 24 hours at 37 °C and the fresh inoculums were used for the test against the leaf and bark extracts. Preliminary screening was done on 20 selected plant species (Appendix C) and later bioassay guided fractionation was done and four most active plants were selected for further screening; *Plectranthus amboinicus*, *Crassocephalum vitellinum*, *Erlangea tomentosa* and *Ipomea hildebrandtii* respectively (Appendix D).

2.10 Antimicrobial assay of the crude extracts (manuscript V)

The antimicrobial activity was measured by the agar well diffusion method (NCCLS, 2002; Perez et al., 1990). Sterile nutrient agar was prepared and placed in labelled Petri dishes and allowed to gel. The antifungal culture was prepared using the test organism on Sabourad dextrose agar (SDA). Four wells were bored into the nutrient agar using a 7 mm sterile cork borer and inoculums containing 10⁵ CFU/ml of bacteria spread on the solid media with sterile cotton swab moistened with bacterial suspension. Fifty microlitres (50 mg/ml) of essential oils and fractions were dispensed in each well. The dried solvent extracts (0.2 g) were reconstituted in 2 ml of normal saline and distilled water respectively, to make a stock solution with a
concentration of 100 mg/ml. Fifty microlitres (50 μl) of solvent extract of each plant was dispensed into each well and allowed to diffuse for 30 minutes. Also 50 μl of normal saline was placed in the wells separately as a control. Inoculation was done for 1 hour to ensure diffusion of the antimicrobial agent into the medium. The extracts were screened in comparison with standard Nystatin (100 units /disc) as a positive antifungal agent. Ciprofloxacin (30μg) was used as a positive standard antibacterial agent while normal saline served as a negative control. Each test was performed in duplicates and the inoculated petri dishes were incubated at 37 °C for 24 hours. Antimicrobial activities were evaluated by measuring diameters of the inhibition zone of microbial growth in millimeters (mm). Results are shown as mean diameters of minimum two observations ± S.E.M.

2.10.1 Determination of Minimum Inhibitory Concentrations (MICs)
Minimum inhibitory concentration was carried out to determine the lowest concentrations of the extract that will inhibit the visible growth of a microorganism under test after overnight incubation. The estimate of minimum inhibitory concentration (MIC) was carried out by broth dilution method. Half dilutions of plant extracts from 50 mg/ml to 1.56 mg/ml were used. Serial dilution was carried out on the extracts using a 2-fold dilution factor, such that any dilution to the right reduces the concentration by a factor of 2. The tubes were inoculated with microorganisms suspension at a density of 10^5 CFU/ml. MIC values were taken as the lowest concentration of extract inhibiting visible growth of test organisms after 24 hr of incubation at 37 °C. The petri dishes were incubated at 37 °C for 24 hours.

2.10.2 Determination of Minimum Bactericidal (MBC)/ Fungicidal Concentrations (MFC)
The Minimum Bactericidal / Fungicidal Concentrations were carried out to determine the lowest concentration of the extract required to kill a particular bacterium or fungus. For determination of MBC/MFC, suspensions from the MIC studies were used. Using a standard loop of 6 mm, samples from the MIC plates with visible growth were removed for subculture in Muller Hinton Agar (MHA) and Sabaurouds dextrose agar. The isolated organism on the agar was incubated at 37 °C for 18-24 hrs. After incubation, the plates were observed; the concentration that exhibited no bacterial growth was considered as the MBC/MFC value (Williams and Wilkins, 2007).
2.10.3 Growth curve studies using Bioscreen method

The bacterial strains used in this experiment are: *Staphylococcus aureus* ATCC 25923 (+ve), *Streptococcus pneumonia*, *Pseudomonas aeruginosa* (PA0-1) (-ve), *Klebsiella pneumonia* (-ve). *Klebsiella* and *P. aeroginosa* were incubated overnight in 5% CO₂ on Luria Agar Base, Miller (Difco™) and they were suspended in Luria Broth (Difco™). *S. aureus* was incubated overnight in normal atmosphere on Tryptic Soy Broth (TSB), Y agar plates (1.5% Bacteriological agar No 1 in Tryptic Soy Broth), (Bacto™, Becton Dickinson, Sweden) supplemented with 0.5% yeast extract (BBL™), and was suspended in (TSB) (Bacto™) supplemented with 0.5% yeast extract. *S. pneumonia* (T4) was incubated on Colombia base agar (Acumedia, Baltimore, MD, USA) supplemented with 0.01% tryptophan (Merck, VWR International, Sweden) and citrated horse blood (5%) in a 5% CO₂ atmosphere, and it was suspended in C+Y media.

Bacterial strains were streaked on their corresponding agar plates and incubated overnight in an incubator with the required atmosphere. After 24 hours one loop full from each bacterial strain was suspended in a tube with 10 ml of corresponding suspension media. The tubes were placed in a water bath at 37 ºC until an optical density (OD) of 0.5, measured at 600nm was reached. Using fresh tubes of media the cultures were diluted to an OD₆₀₀nm = 0.05 and 400 µl was transferred to a Bioscreen C MBR multi-well plate (Oy Growth Curve AB Ltd). The growth kinetics were monitored by changes in the OD₆₀₀nm at 37 ºC using a Bioscreen C (lab systems) plate reader. When the OD₆₀₀nm reached approximately 0.3 this was considered time zero and 4 µl of the compounds of interest were added. Bacterial growth was monitored and measurements were made every 20 min with 5 sec shake and 5sec rest cycle before every reading. The growth was measured and recorded over a 12 hours period.

Viable counts:
The bacterial cultures were prepared as described before then at time point of interest 20 µl of the bacterial culture was collected and added to 180 µl of 1xPBS so to achieve a ten-fold series of seven concentrations. Using a multi-channel pipette, 10 µl of each of the seven concentrations was plated onto a single agar plate. The plate was tilted at 45 degree angle whilst pipetting in order to increase the surface area covered by each concentration. The agar plate was then
incubated over night at 37 °C in the required atmosphere. Growth of bacteria was recorded in the form of colony forming units (CFU).

3. Analysis of the plants for nutritive values (manuscript VI)

3.1 Evaluation of total antioxidant activity (TAC) by FRAP method

FRAP method depends on the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH. The ferrous complex has an intensive blue color which can be monitored at 593 nm (Benzie and Strain, 1996).

Reagents: acetate buffer, 300mM/L, pH 3.6 (3.1g sodium acetate 3H2O and 16 mL conc.; Acetic acid per 1L of buffer solution); 10 mM/L TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM/L HCl; 20 mM/L FeCl36H2O in distilled water. FRAP working solution: 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl3 solution. Aqueous solution of known Fe (II) concentration was used for calibration, in a range of 0.1- 0.8 mM/L. For the preparation of calibration curve 0.1, 0.2, 0.4, 0.6, 0.8 μM/mL aqueous Fe(II) as Mohr salts solution (1mM) were mixed with 2.5 mL FRAP working solution; FRAP reagent was used as blank. A concentration of 50 mg/ml of extract was used. The absorption was read after 10 minutes at 25 °C and 593 nm. All determinations were repeated three times. Total antioxidant capacity in Fe (II) equivalents was calculated. Correlation coefficient (r²) for calibration curve was 0.9994.

3.2 Evaluation of total antioxidant activity (TAC) by DPPH method

The total antioxidant capacities (TAC) or the amount of specific antioxidants in different plant extracts was measured using the DPPH (2, 2 – Diphenyl 1-1 picryl hydrazyl) assay which is associated with electron and radical scavenging activity. The assay makes use of a stable free radical, (1, 1 – diphenyl -2- picryl hydrazyl). DPPH shows a strong absorption maximum at 517nm (purple). The reaction involves colour change from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. The degree of colour change will be correlated with the samples’ antioxidant concentrations. Total Antioxidant activity (TAC) was determined using Ultra Violet spectrophotometric method (Habila et al., 2010; Nickavar et al., 2006).

Hydrogen atom – or electron-donation ability of the corresponding medicinal herbs was measured from the bleaching of the purple-colored ethanol solution of DPPH. 0.5 mL of various
ethanol extracts diluted 1/10 were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 40 minute incubation at room temperature the absorbance was read against a blank at 517 nm. Antioxidant capacity expressed as percentage inhibition of the DPPH free radical was calculated in the following way (Burits and Bucar, 2000; Cuendet et al., 1997).

\[
TAC_{DPPH} (\%) = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100
\]

where A is absorbance.

3.3 Mineral nutrient analysis using atomic absorption spectrophotometry

Minerals are important for maintaining good health. Ten plants, selected on the basis of use in traditional medicine for improving nutritional status, were analyzed for the presence of mineral elements like zinc, iron and selenium, which are particularly important for HIV infected people for regeneration of CD4 T cells and maintaining the sero status of patients (Mehta and Fawzi, 2010). About 1-2 grams of plant leaves was weighed into a clean dry silica dish/ crucible. Then 1.5ml of 15% magnesium acetate was added and the mixture heated on a hot plate to completely char the sample. The crucible was placed in a muffle furnace maintained at 450 °C and allowed to ash for 4 -6 hrs. The crucible was removed from muffle and placed in a desiccator and allowed to cool at room temperature. The sample was diluted to get the mineral into the linear concentration range of instrument using deionized water. The concentrations of iron, zinc and selenium were determined by using atomic absorption spectrophotometer (Schimadzu – AA-6300) according to AOAC official methods (AOAC, 2000). The reference standards were prepared by suitable dilutions of the stock standard solutions of each micronutrient standard solution.

3.4 Safety evaluation of selected plants (Paper IV)

3.4.1. Acute toxicity studies

Toxicity tests show how potent a chemical or substance is by measuring the concentration that will affect the test organism. The aim of acute toxicity test is to establish a starting dose in humans and the therapeutic index, i.e the ratio between therapeutically effective dose and the lethal dose (LD_{50}/ED_{50}) (Gosh, 1984). The higher the index the safer the compound. LD_{50} test is used to evaluate toxicity by determining the dose required to kill 50% of the test organisms. The acute oral toxicity tests on plant extracts were carried out using female and male albino mice
and were conducted according to the Organization for Economic Cooperation Development (OECD, 2002) guidelines 423 where the limit test dose of 5000 mg/kg was used.

Dose is the amount of test substance administered at one time, and is expressed as weight of test substance per unit weight of test animal (mg/kg). Healthy young animals were acclimatized to the laboratory conditions for at least 10 days prior to the test before being assigned to the treatment groups. This was to ensure that the micro-environment of the laboratory was not a factor to affect the tests. The animals were fasted overnight (18 hours) and divided into one control group and three treated groups, each group consisting of two animals (male and female) labeled I – IV. The different groups of mice were treated with different doses of the extracts using the up and down procedure (OECD, 2008a). The mice were carefully observed for any behavioral changes and toxicity manifestations at different time intervals. Control animals received 1ml of distilled water through the same oral route using intragastric syringe. Extracts that did not kill any mice were considered non-toxic. The volume of extract administered to the mice was calculated as follows (Gosh, 1984): Volume of extract (ml) = weight of mice (kg) x dose rate (mg/kg) / Stock solution (mg/ml)

3.4.2. Sub acute toxicity studies (Paper IV)
The sub acute toxicity test was performed following the Repeated-dose oral toxicity protocol described by the OECD guideline 407 for testing of chemicals (OECD, 2008b). Twenty four adult albino wistar rats were divided into four groups of 6 rats per group. Having determined that the LD$_{50}$ was above the limit test dose of 5000 mg/kg, the extract doses for sub acute test were determined from $1/2$, $1/4$ and $1/8$ of the limit test dose of 5000 mg/kg. Groups I, II and III received 625, 1250 and 2500 mg/kg body weight respectively, of the aqueous extract once daily for 28 days. Group IV (control) received 1 ml of distilled water. The body weights of individual animals were evaluated weekly and recorded. The body weight changes were monitored throughout the experimental period on weekly basis. Similarly, the animals were observed for any manifestation of toxicity and mortality. At the end of 28 days, the rats were sacrificed by choloform anesthesia. The liver, kidney, intestines and lungs were removed and preserved in 10% formalin. Tissue organs were sectioned in paraffin wax and stained with hematoxylin and eosin for assessment of tissue morphology. Histopathological, hematological and biochemical
studies were performed to determine if there was any deleterious effect of the plant extracts on the tissues and organs after extract administration for 28 days period of observation.

3.5 Statistical analysis of data

3.5.1 Quantitative analysis of ethnobotanical data (Papers I, II, III)

The therapeutic indications cited during ethnobotanical field interviews were grouped by body systems according to the disease categories proposed by the International Classification of Diseases (CDC, 2013; WHO, 2006). By means of the consensus of the informants, the importance of a species for a determined purpose and the categories that present greater importance to the people was obtained by the calculation of the Fidelity level (FL) and from the Informant Consensus Factor (FIC) indices respectively (Friedman et al., 1986; Heinrich et al., 1998). To test the consistency or homogeneity of informant’s knowledge in treating a particular illness category, calculation of consensus factor (Fic) was followed (Heinrich et al., 1998; Trotter and Logan, 1986). This factor is given as: 

\[
\text{Fic} = \frac{(N_{ur} - N_t)}{(N_{ur} - 1)}
\]

Where \(N_{ur}\) is the number of use reports of informants for particular ailment category, \(N_t\) is the number of species used for a particular ailment category by all informants. This factor ranges from zero to one, where increasing values indicate high rate of informant consensus. To assess the importance of individual species in each group, fidelity level was calculated (Friedman et al., 1986). This shows the percentage of informants claiming the use of a plant species for the same major purpose. Fidelity level index, FL = \(I_p/N\) x100, where \(I_p\) is number of informants who indicate use of a species for the same major ailment, \(N\) is the total number of informants who mentioned the plant for any other use (Friedman et al., 1986). The calculation of FL was limited to plants with at least more than 5 citations for a particular illness category. Increasing values of FL for a species indicate its uniqueness to treat a particular illness (Pandikumar et al., 2011).

3.5.2 Analysis of toxicity results

The data for toxicity tests was subjected to one-way analysis of variance (ANOVA) test and differences between the control group and extract treated groups were determined by post hoc Dunnett’s multiple comparison tests, using Graph Pad Prism (Version 5) software to obtain the toxicity levels of the different plant extracts. Results were presented as mean ± SEM and considered to be statistically significant at \(p \leq 0.05\) with 95% confidence interval.
4. Results and discussion

4.1 Ethnobotanical studies - documentation of nutri-medicinal plants used in the management of HIV/AIDS opportunistic ailments in western Uganda (Papers I, II, III)

The purpose of this study was to document traditional botanical knowledge of 4 study areas and to assess the quantitative comparison of the medicinal plant knowledge of the local communities using informant consensus factor (ICF), Fidelity Level (FL) and Use values (Heinrich et al., 1998; Trotter and Logan, 1986). The results presented in this chapter are the outcome of a series of interviews conducted between December 2010 and May 2011 with local communities of Mbarara, Ibanda, Isingiro and Kiruhura districts in western Uganda. This chapter attempts to provide answers to the following research questions; what medicinal plants are used to treat/manage HIV/AIDS opportunistic infections? What are the most important plant use categories? What are the most useful species per use category? Which plant families contain the highest number of useful species in each use category? In order to evaluate the usefulness of different plant species to the local communities, different plant species and their uses are discussed in two broad categories; medicine and food (Papers I, II, III). In the context of this study, a response or citation is defined as an answer from a participant with regard to the use of particular plant species. A plant use is a well defined use of a particular plant species for one particular goal by one or more participants. Apparently plant use and ethnobotanical relationships continue to increase with increase in biodiversity inventories and associated attributes to particular taxa up to species level.

After getting prior informed consent, interviews were conducted in order to make informants mention the medicinal plants that they use in the management of opportunistic infections of HIV/AIDS. A total of 143 informants, including 114 females and 29 males aged between 23 and 85 years participated in the study. Forty nine (34.3%) traditional medical practitioners (traditional healers and traditional birth attendants) were interviewed. The selected respondents especially traditional healers were well known in the community due to their long practice in service provision related to traditional health care. This study recorded 324 nutri-medicinal plants used in the management of opportunistic ailments of HIV/AIDS and other diseases, 51 of which were not properly identified. The plants are distributed in 75 Families. Based on the
information obtained, the reported HIV related diseases were grouped into 5 major categories (Paper I, II). The categories with high number of plants were respiratory infections (cough, tuberculosis), appetite and immunity boosting as well as bacterial and fungal infections (candida, syphilis, UTIs etc). Therefore, the results of the ICF point to these medicinal categories as the ones that informants are most confident about. High ICF values are obtained when only one or few plant species are reported to be used by a high proportion of informants to treat a particular ailment. Hence, high ICF values are used to select interesting species for the search of bioactive compounds (Canales et al., 2005; Heinrich et al., 1998). Research shows that plant species with higher frequencies of use among cultural groups contain higher levels of secondary alkaloids than those with lower frequency of use (Quinlan et al., 2002). The ICF results could be useful in prioritizing medicinal plants for scientific validation of plants and plant products as pharmacologically effective remedies are expected from plants with higher ICF values.

However, the study also recorded plants used for other disease conditions other than HIV/AIDS opportunistic infections. The ailments were grouped into 14 categories, with the highest number of plants being used for gastrointestinal and reproductive health disorders (Paper III). Most of the plants used had more than a single therapeutic use, for instance Albizia coriaria was used for herpes zoster, cough, skin infections, tuberculosis, syphilis, headache, diarrhoea and uterine pains. On the other hand, one ailment would be treated using many plants, for instance, cough was treated using Warburgia ugandensis, Albizia coriaria, Basella alba, Cajanus cajan, Tetradenia riparia, Abutilon guineense and Acacia hockii.

The healing potential of the most frequently reported plants was determined using fidelity level (FL) (Friedman et al., 1986) (Papers I, II, III). An increasing value of fidelity level for a species indicates its uniqueness to treat a particular illness. Families Asteraceae, Fabaceae, Euphorbiaceae and Lamiaceae were the most dominant throughout the study areas (Fig 6). Family Asteraceae had the highest number of plants used (44), followed by Fabaceae (20), Lamiaceae (19) and Euphorbiaceae (18) respectively.
Majority of herbal remedies were prepared by boiling, grinding /pounding, soaking in hot or cold water of fresh or dry plant materials (leaves, roots, seeds, fruits and flowers). Some herbal preparations were taken by mixing with food, honey, milk or tea, especially for oral administration. Other drugs were applied eternally for conditions like fungal infections of the skin. It was reported that dosages were given by estimation depending on age and type of sickness. About 61% of herbs are used for remedy preparation, followed by trees and shrubs. Throughout the study areas, more than half of nutri-medicinal plants grow in natural environments including forested areas, fallow and farm lands. This puts the key plant species at a risk since they are threatened by human activities. Hence, there is a need for in-situ conservation and domestication of key plant species. The only constraint remains with plant species that require a unique micro-climate if they are shade or non-shade plant species.

This study follows a quantitative approach because it quantifies useful plant species and is based on participant consensus. Quantitative Ethnobotany has been defined as the application of quantitative techniques to the direct analysis of plant use data (Phillips and Gentry, 1993a). Ethnobotanical research is currently experiencing a revival as evidenced by numerous recent publications (Abbasi et al., 2013; Anywar et al., 2014; Asiimwe et al., 2013; Bunalema et al., 2014; Byarugaba et al., 2007; Claudio et al., 2013; Guimbo et al., 2011; Gurib-Fakim, 2006; Hardon et al., 2008; Hernández et al., 2003; Kakudidi et al., 2000; Kamatenesi, 2010; Kazhila and Marius, 2010; Padhi and Panda, 2013; Senthilkumar et al., 2013; Traore et al., 2013; Wu et
al., 2001; Xi-long et al., 2013). Plants used in traditional medicine therefore have an important role to play in the maintenance of health in all parts of the world and in the introduction of new treatments. This study revealed a rich diversity of nutri-medicinal plants used to treat various disease conditions.

4.2 Phytochemistry, antimicrobial and antioxidant activities of nutri-medicinal plants

(Manuscripts V & VI)

4.2.1 Chemical analyses of essential oils

The importance of ethnomedicinal plants lies not only in their chemotherapeutic value in traditional health care but also in their potential as sources of biologically active entities. Phytochemistry is the core of phytomedicines because therapeutic efficiency correlates directly with the presence of various phytochemicals. The aim of this study was to evaluate the phytochemical profiles of selected nutri-medicinal plants, and their biological (antibacterial, antifungal and antioxidant) activities. Ten plants were selected for bioactivity testing based on ethnomedical information provided by local communities and traditional medical practitioners, and how frequently they are used to treat opportunistic infections of HIV/AIDS (Paper I and II).

Qualitative chemical analysis revealed the presence of secondary metabolites: tannins, saponins, alkaloids, flavonoids, terpenes and terpenoids, coumarins, reducing sugars and steroids. The pharmacological properties of these groups of chemical compounds are reported in literature (Ango et al., 2012; Cowan, 1999; Teke et al., 2013). For instance, tannins and flavonoids have antioxidant, anticancer, antifungal, antiviral, antiallergic and detoxification activities (Adedapo, 2013). Their presence in the leaves of the studied plants justifies their use in traditional medicine to treat candida, syphilis, boost immunity and treat many other infectious diseases. The water extracts were rich in phytochemical compounds especially tannins, saponins and steroid glycosides. This justifies the use of water as the main media for herbal remedy preparation. Some of the main compounds identified by GC-MS include linalool, linalyl acetate, β-cubebene, carvacrol, α- terpineol and γ-terpinene. The biological and medicinal properties of the same compounds have also been reported by other researchers (Paduch et al., 2007; Skočibušić and Bezić, 2004; Thanighairassu and Sivamani, 2013).
It is well known that herbal medicinal products usually contain more than one plant or active constituents and their therapeutic efficacy is not provided by a single compound. Some of these compounds act synergistically to modify the bioavailability and efficacy of the active constituent. For instance, tannins have been considered in traditional medicine to treat various diseases, and their synergistic effects with various antibiotics, such as carbenicillin and tetracycline, have been proved beneficial against antibiotic resistance bacteria (Dusane et al., 2015; Okuda and Ito, 2011).

4.2.2 Major groups of volatile compounds identified in the essential oils

Medicinal plants have been a source of a wide variety of biologically active compounds for centuries and used extensively as crude material or as pure compounds for treating various disease conditions. There is ongoing research to explore the diverse sources that may provide more effective and less toxic antimicrobial compounds (Saleem et al., 2010). Figures 7-10 show the major compounds present in the essential oils of the studied plants.

Fig 7. Major compounds in the essential oil of *Plectranthus amboinicus*
Fig 8. Major compounds in the essential oil of *Crassocephalum vitellinum*

Fig 9. Major compounds in the essential oil of *Erlangea tomentosa*

Fig 10. Major compounds in the essential oil of *Ipomea hildebrandti*
4.2.3 Antibacterial and antifungal testing of plant extracts (*Manuscript V*)

Microbial infections are the most frequent opportunistic diseases occurring during HIV/AIDS which affect many people in Africa. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. In response to this, there is an increasing interest in the use of essential oils and plant extracts as sources of natural products which have been screened for potential uses as alternative remedies for the treatment of many infectious diseases (Tepe et al., 2004). Studies have shown that essential oils possess antibacterial, antifungal and antioxidant properties (Burt, 2004). The number of higher plant species on this planet is estimated to be between 250,000 – 500,000 of which only 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically (Verpoorte, 2000). Plant extracts have been of great interest due to their potential uses as alternative remedies for the treatment of many infectious diseases particularly as antibiotics. Plant-based anti-microbials represent a vast untapped source of medicines with therapeutic potential (Cowan, 1999). In the present study, four plants that are traditionally used to treat various ailments including HIV/AIDS – related infections were selected for antimicrobial testing (Table 1).

**Table 1. Traditional uses of the plants tested for antimicrobial activity**

<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific name</th>
<th>Local name (Runyankore)</th>
<th>Ailment(s) treated</th>
<th>Parts used</th>
<th>Family name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Plectranthus amboinicus</em> (Lour) Spreng.</td>
<td>Akacuncu akakye</td>
<td>Cough, immunity boosting, colic and stomach infections, skin infections</td>
<td>Leaves</td>
<td>Lamiaceae</td>
</tr>
<tr>
<td>2</td>
<td><em>Crassocephalum vitellinum</em> S. Moore</td>
<td>Esunuunu</td>
<td>Herpes zoster, fever, diarrhoea, oral candidiasis, syphilis, energy boosting, tumors, uterine pains, headache</td>
<td>Leaves</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>3</td>
<td><em>Erlandea tomentosa</em> S. Moore</td>
<td>Ekyoganyanja</td>
<td>Skin infections, diarrhoea, syphilis, appetite boosting, anaemia,</td>
<td>Leaves</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>4</td>
<td><em>Ipomea hildebrandti</em> Vatke</td>
<td>Bingirebita</td>
<td>Tinea capitis and corporis and other fungal infections of the skin, tumors, herpes zoster and syphilis,</td>
<td>Leaves</td>
<td>Convolvulaceae</td>
</tr>
</tbody>
</table>
Two tests were used to evaluate the antimicrobial activities of essential oils. In the first test, the agar well diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. The activity of essential oils and fractions, water and ethanol extracts of the four plants were tested against 2 gram negative, and 2 gram positive opportunistic human bacteria, and 2 fungal pathogens using agar well diffusion method. All the tested extracts were active against one or more of the organisms tested (Appendix C). The findings of antimicrobial screening showed that some of the essential oil fractions were more active than the pure oils. This is because fractionation of the crude extracts allows the distribution of these metabolites in the fractions according to polarity (Houghton and Raman, 1998). Hence, the phytocompounds can easily be separated and the associated activity might be due to the presence of different compounds in the individual fraction.

The essential oil from dry and fresh leaves from *Plectranthus amboinicus* was screened to compare the activities of compounds from both forms of leaves. The essential oil from the fresh and dry leaves of *Plectranthus amboinicus* was found to have strong antifungal activity against *Candida albicans* (MIC = 1.56 mg/ml) and *Cryptococcus neoformans* (Manuscript V). Previous studies on the methanolic extract of *P. amboinicus* have indicated that the plant has antifungal activities against *Candida albicans* and *Cryptococcus neoformans* (Nagalakshmi et al., 2012).

The essential oils and fractions, water and ethanol extracts of other plants were also tested against the same organisms (Appendix C & D). The activity of the water extracts was very poor. This is also reported by other researchers that organic solvents and essential oils give more consistent biological activity compared to the water extracts (Prashanti et al., 2011). In this study, only the bark aqueous extracts of *Albizia coriaria* and *Maytenus senegalensis* showed mild activity against *Streptococcus pneumoniae* and *Staphylococcus aureus*. The essential oil and fractions of *Crassocephalum vitellinum* also showed very strong activity against *Cryptococcus neoformans* and *Candida albicans*. The fractions were more active on the yeasts than the pure oil. The study showed that on further fractionation of the essential oils of *Crassocephalum vitellinum* the hexane soluble fractions demonstrated the highest sensitivity towards *Cryptococcus neoformans*. However, the water and ethanol extracts showed weak activity against the tested bacterial strains. Similar findings on *C. vitellinum* showed that the
ethanolic extract exhibited weak antibacterial activity (Moshi et al., 2014). Hence, the plant has displayed good antifungal activity. Similar studies on other species, such as, *Crassocephalum bauchiense* showed that the ethyl acetate extract was active on *Candida albicans* and *Cryptococcus neoformans* than the fractions (Mouokeu et al., 2014). The antifungal properties of *C. vitellinum* can be attributed to the presence of tannins, terpenes, flavonoids, phenols and sterols and which have been reported in literature (Cowan, 1999; Paduch et al., 2007). These results support its traditional use in treatment of oral Candidiasis (Table 1).

The pathogen *Cryptococcus neoformans* is an environmental fungal pathogen afflicting immunocompromised patients as well as immune competent individuals. Infection occurs in the upper respiratory tract where pathogenesis presents as cryptococcal meningitis, causing life threatening serious opportunistic infection meningoencephalitis (Samie et al., 2010; Tripathi et al., 2012). Research shows that *C. neoformans* is a major threat to AIDS patients in Sub Saharan Africa, causing illness to about 1 million people annually, despite major developments in HIV treatment (Park, 2009; Warkentien and Crum-Cianflone, 2010). About 5-8% of HIV positive patients experience the disease during the course of AIDS. Cryptococcosis is observed in advanced stage when HIV patients have CD4+ T cell count of < 50 cells/µl of blood (Sandhu and Samra, 2013). Research also indicates that *C. albicans* and *C. neoformans* are some of the causes of diarhoea in HIV positive children (Ukaropol, 2003). *Candida albicans* is a yeast infection, which in immunocompromised conditions may affect the skin, oral mucosa or genital areas.

The results obtained from this study support the traditional uses of the plants for treating opportunistic infections in HIV/AIDS patients. These results are relevant since *C. albicans* and *C. neoformans* are the leading causes of fetal fungal infections in immunocompromised patients. The essential oil from the fresh leaves of *P. amboinicus* was also active against *Klebsiella pneumoniae* compared to the standard ciprofloxacin (Appendix D). Figure 11 shows the activity of the essential oil extracts of the plants against different organisms tested. *P. amboinicus* was active against all the test organisms, while *Ipomea hildebrandtii* was only active against *Candida albicans* and *Cryptococcus neoformans*. This confirms its use in traditional medicine to treat
fungal infections especially tinea infections (Table 1). Results also indicate that all the essential oils were active against *C. albicans* and *C. neoformans* (Fig 11).

**Fig 11. Activity of the essential oils against the tested organisms**

### 4.2.4 Growth curve studies using Bioscreen method

In the second test, the Bioscreen method was used to measure microbial growth (Johnston, 1998; Wu et al., 2000). Growth pattern of the test organisms was studied on the basis of optical density (OD) and log_{10} cfu/ml. From the study results, only the crude essential oil of *Plectranthus amboinicus* showed activity against *Klebsiella pneumoniae* (Fig 12) and *Pseudomonas aeruginosa* (Fig 13). With an increase in the antibiotic resistance over the past decade there has been a renewed interest in alternatives to antibiotic therapy. For instance, *Pseudomonas aeruginosa*, an opportunistic human pathogen that causes cardiac and respiratory infections in cystis fibrosis patients and in patients with compromised defense mechanisms, is resistant to various antibiotics. This has led to the search for new therapeutic strategies (Cooper et al., 2011). In this study, *P. amboinicus* was found to be active against *P. aeruginosa*. This could be due to the high amount of carvacrol present in the essential oil. This may be supported by research which shows that carvacrol inhibits the growth of several bacterial strains including *P. aeruginosa* by causing damage to the cell membrane of the bacteria and inhibiting their proliferation (Di Pasqua et al., 2007). The studied plants showed promising results as antibacterial and antifungal potential drugs.
Many plant-derived compounds such as terpenes have been found to be active against a variety of microorganisms, including gram positive and gram negative bacteria (Paduch et al., 2007). Linalool, one of the major components in this study has been found to have antimicrobial activity against various microbes and inhibits spore germination and fungal growth (Josphat et al., 2007). A mixture of α-terpineol, 1, 8-cineole and linalool has been shown to possess antibacterial activity against gram negative and gram positive bacteria of the oral cavity, skin and respiratory tract (Paduch et al., 2007). The presence of similar compounds in this study may support the use of the plants under study to treat candidiasis and other fungal infections of the skin. Structures of some terpene compounds with antibacterial and antifungal activity are listed in Appendix B.
4.2.5 Antioxidant capacity and mineral nutrient composition (Manuscript VI)

Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress, whereas the micro- and macro minerals are the key components for overall body growth and development. Natural antioxidants present in plants are responsible for inhibiting or preventing deleterious consequences of oxidative stress, which play a role in the pathogenesis of opportunistic infections of HIV/AIDS (Otang et al., 2012). For instance, terpenoids have been found to have immunomodulatory properties (Theis and Lerdau, 2003).

Ten plants were screened for antioxidant activities and mineral nutrient composition. The antioxidant activity was determined using DPPH and FRAP assay tests. Reducing power assay is used to evaluate the ability of natural antioxidant to donate an electron and DPPH assay is a measure of the presence in the medicinal plants of the compounds with radical scavenging capacity. The extracts expressed strong antioxidant power as measured by DPPH (free radical scavenging) compared to FRAP (reducing power). According to their antioxidant capacity, the 10 medicinal plant extracts were divided into 3 groups; a) very low FRAP (< 1 mM), n= 3; low FRAP (1-5 mM), n= 5; good FRAP (>5 mM), n=2. Symphytum officinale and Erlangea tomentosa showed promising antioxidant capacity as reducing power. The highest TAC_DPPH values were obtained for Pseudarthria hookeri, Plunchea ovalis and Ipomea hildebrandti. The antioxidant activity of other Plunchea species has been reported (Hidayat et al., 2013). A survey on the genus Plunchea revealed the presence of eudesmane – type of sesquiterpenoids, monoterpenes and flavonoids (Goyal and Aggarwal, 2013; Hidayat et al., 2013). Similary, several compounds belonging to the groups of monoterpenes and flavonoids have shown anticancer activity, which is due to the antioxidant activity. This study showed that P. ovalis had 38% scavenging activity. This could be attributed to the presence of high amounts of flavonoids and tannins in the plant extracts.

Plant antioxidants are composed of a number of different substances like ascorbic acid, terpenoids and polyphenolic compounds such as flavonoids. Therefore, the presence of these phytochemicals could support the herbal medicine uses of the plants as antioxidants whose antioxidant activity is well known (Gonzalez-Burgos and Gomez-Serranillos, 2012; Graßmann,
2005; Ruberto et al., 2002). Since reactive oxygen species are thought to be associated with the pathogenesis of AIDS, and HIV-infected individuals often have impaired antioxidant defenses, the inhibitory effect of the extracts on free radicals may partially justify the traditional use of these plants in the management of opportunistic infections in HIV/AIDS patients. Many plants produce a variety of compounds including terpenes and their derivatives which have been found to be useful in the prevention and therapy of several diseases including cancer and to have antioxidant and immunomodulatory properties (Gonzalez-Burgos and Gomez-Serranillos, 2012; Ruberto and Baratta, 2000; Ştef et al., 2009). Such compounds protect body cells against damaging effects of reactive oxygen species by scavenging the free radicals in the body.

The plants were analyzed for the presence of zinc, iron and selenium, which are particularly important for HIV infected people, mainly for regeneration of CD4 T cells, reducing diarrhoeal morbidity and maintaining the sero status of patients. The results for mineral nutrient analysis of the plants are given in manuscript VI. The plants were selected on the basis of use in traditional medicine for improving nutritional status in terms of immunity and energy boosting. The mineral contents in the plant samples were found at different levels, ranging from 0.03 – 6.9 mg/kg dry weight. According to research, the amounts of minerals present in the studied plant species meet the average dietary intake level that is sufficient to meet the nutrient requirements needed for daily maintenance of the immune system (Manuscript VI).

4.3 Safety evaluation of nutri-medicinal plants (Paper IV)

4.3.1 Acute toxicity study

Toxicity is the ability of a chemical to damage an organ system, such as liver or kidneys; to disrupt a biochemical process such as the blood-forming mechanism, or disturb an enzyme system at some site in the body. Toxicity studies help to assess the potential health risks caused by intrinsic adverse effects of chemical compounds present in plant extracts. However, all substances have some level of toxicity above the allowable limit which is dosage. There are two types of toxicity, acute and chronic. Acute toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hrs (OECD, 2002). A chemical is considered to be extremely toxic if it has LD$_{50}$ of 1mg/kg and practically non toxic if it has an LD$_{50}$ of 1500 mg/ kg and above (Gosh, 1984).
Toxicity tests were done in mice and rats to determine the safety of the plant extracts. The oral route of administration of the extracts to the test animals was used, which is in line with the route of administration of medicines by humans. The study was conducted to determine whether crude plant extracts of selected nutri-medicinal plants are safe for human consumption, since most of the remedies are administered orally. Toxicological assessment of the water, ethanol and essential oil extracts of Plectranthus amboinicus, Pseudarthria hookeri, Tarenna pavetoides, Symphytum officinale and Plunchea ovalis was carried out in albino mice and rats. Acute toxicity was tested in mice starting with doses of 2000 mg/kg up to a high concentration of 10,000 mg/kg body weight, when no toxicity signs or death occurred and dose was increased sequentially. As the dose increased, signs of acute toxicity became more obvious especially with essential oils where several deaths were recorded (Appendix E). The water and ethanol extracts did not show any adverse signs of toxicity or cause any mortality, but there was increased urination, defecation, drowsiness, twitching of gut muscles and hypoactivity. The lethal dose at 50 % (LD$_{50}$) for some of the essential oils (Table 2 and Fig 14), water and ethanol extracts was greater than 5000 mg/kg for a single extract. Probit analysis was used to estimate LD$_{50}$ value (Finney, 1952). The extracts could therefore be classified as being safe following the OECD guidelines for acute oral toxicity (OECD, 2002).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Log dose</th>
<th>No: dead</th>
<th>% Dead</th>
<th>Probits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3,500</td>
<td>3.54</td>
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<td>0</td>
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<td>2</td>
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<td>3.87</td>
<td>6/6</td>
<td>100.0</td>
<td>6.96</td>
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</tbody>
</table>

Log LD$_{50}$ = 3.74, antilog of 3.74= 5506 (Fig 14). Hence, LD$_{50}$ is 5506 mg/kg. According to OECD guidelines and Gosh, (1984) classification of toxic doses, whereby LD$_{50}$ above 5000mg/kg is considered to be experimentally safe.
4.3.2 Sub acute toxicity studies (Paper IV)

Sub-acute toxicity test was done in rats using standard procedures (Gosh, 1984; Lorke, 1983; OECD, 2002). The purpose of this test was to determine the maximum tolerated dose and nature of toxic reactions. In sub acute toxicity tests, the adverse effects may manifest in form of alterations in levels of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs (Ashafa et al., 2009). Results of such tests in animals can then be extrapolated onto human beings. However, man is generally six times as sensitive as the dog, and ten times as sensitive as the rat to the toxic effects of drugs (Gosh, 1984). However, metabolic variations have to be put into account. Special tests may be done to determine teratogenic effects of the drug and also whether the chemical is carcinogenic or not (Gosh, 1984).

The sub acute toxicity test was evaluated through biochemical, hematological and histopathological parameters. Daily doses of 2500 mg/kg, 1250 mg/kg and 625 mg/kg of the aqueous extract were administered for 28 days and signs of toxicity were recorded. Histopathological examination was done on the liver, kidneys, lungs and intestines. Body weight changes were measured weekly for 28 days of daily single dose of extract administration (Paper IV). The results of sub acute test showed that *Plectranthus amboinicus* is not toxic, LD$_{50}$ > 10,000mg/kg, which is above the limit test dose of 5000 mg/kg. There was a significant increase
in lymphocytes (WBCs), neutrophils and platelets. Studies have reported that infections induce decrease in immune response through the inhibition of CD4+ and CD8+ cells, mainly the lymphocytes and neutrophils (Yapo et al., 2011). Hence, the results of this study indicate that the extract has immune stimulating properties, which is essential for HIV positive patients. The extract may also be a supportive treatment for thrombocytopenic (bleeding) disorders which are sometimes caused by viral or bacterial infections like Hepatitis C, where the blood does not clot.

There was a significant increase in serum urea and creatinine, which is an indication of functional damage to the kidneys. Some plants increased urination, a property that has a significant diuretic effect in rats. Diuretics can be used to treat conditions like hypertension, kidney, liver diseases and urinary tract infections. Although there was no incidence of mortality from the toxicity tests implying that the water extracts are relatively safe with low risk of acute intoxication, histological examinations of the internal organs of rats showed severe toxic effects after a month’s administration of the extract. However, toxic effects were observed as the concentration of extracts increased. This means that the extract is toxic at high doses. Hence, proper formulation of the herbal extract is needed for better treatment results. Data from such studies is important for any future in vivo and clinical studies of these plants. Chronic toxicity studies are necessary to support the safe use of these plants. The results support the traditional use of the plant in boosting immunity.

From the results of acute toxicity tests, it was observed that essential oils showed lower lethal doses (more toxic) than the water extracts. The weak acute toxicity levels of the water extracts could be the reason why these plants have long been used for medicine. Many essential oils being complex mixtures of various compounds could be the reason why they are more toxic. Many essential oil components are known to be toxic when taken orally (Shaaya et al., 1999).
5. Conclusions

Traditional knowledge can lead to useful medicinal plants used to manage and treat various disease conditions including infections associated with HIV/AIDS. The study recorded a rich diversity of plant species with potential to treat various ailments including infections associated with immuno-compromised people living with HIV/AIDS in western Uganda. Such studies can help stimulate confidence in traditional medicine and enhance appreciation of herbal medicine among the people and to appreciate the value of the plant resources and therefore enhance conservation efforts of the plant species. The high consensus values obtained from the results means the majority of informants agree on the use of plant species and this reflects the importance of the nutri-medicinal plants to the people. There is urgent need to document key plant species with medicinal and nutritional values because a number of them are becoming rare and others are threatened with extinction in their natural habitats. Ethnopharmacological studies are needed to validate the use of the documented plants (Papers I, II, III).

This study has shown the presence of phytochemical compounds and antimicrobial activities of plant extracts. Phenolic compounds, alkaloids and terpenes are highly potent bioactive compounds and could perhaps be responsible for most activities shown by the studied plants. It is concluded that the plant extracts possess antimicrobial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different constituents of the plants on the target organisms. All the four screened plants were active against one or two microbial organisms. *Plectranthus amboinicus* was active against all the tested organisms and was the only plant that showed activity against *Pseudomonas aeruginosa*. The results of the present study seem to be promising and may enhance the natural products uses, showing the potential of these plants in the treatment of infectious diseases caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Candida albicans*, *Cryptococcus neoformans* and *Pseudomonas aeruginosa*. The results show that *Ipomea hilderbrandtii* expressed only antifungal properties. This is in support of its use in traditional medicine to treat fungal infections of the skin (*Tinea capitis* and *Tinea corporis*). The study forms a basis for further phytochemical and pharmacological
studies to isolate and characterize the bioactive principles necessary for the development of new antimicrobial drugs. Further studies are needed to carry out bioassay-guided fractionation of the essential oil in order to isolate the active compounds that can be useful in management of these fungal infections.

- All the ten screened plants showed antioxidant activity in terms of reducing power and radical scavenging capacity. This study shows that all the plant species contain sufficient amounts of minerals required daily for maintenance of the immune system. These results may be useful for the evaluation of dietary information and we conclude that these plants have the potential to be sources of natural antioxidants and mineral nutrients. The inhibitory effect of the extracts on free radicals may justify the traditional use of some of the plants as immunity boosters in the management of opportunistic infections in HIV/AIDS patients.

- No mortalities were observed during acute and subacute toxicity studies. However, results indicate that some of the extracts caused treatment-related toxicological abnormalities which increased with dosage. The aqueous extract of *P. amboinicus* is safe to use as indicated by the high LD50 value, but should be used with caution at high doses. Therefore, this forms the basis for chronic toxicity studies in order to formulate a dosage that is safe for human consumption.

- Further research is also needed to isolate the plants’ active compounds in order to understand their modes of action. The significant activity observed in this study could be attributed to the interaction of one or more identified metabolites against the test organisms.
Challenges and future directions of herbal medicine research: Integrating modern and traditional medicine

Traditional and modern medicines have much to offer each other despite the differences. For millennia, people around the world have healed the sick with herbal or animal-based remedies handed down through generations. Herbal medicine is becoming increasingly used to enhance general health and well-being, and it is also used alone for specific ailments or with modern medicine. Traditional and modern practices can be integrated as two branches of medical science with the ultimate incorporation of elements of both to form a new branch. This incorporation has been demonstrated to be practicable in many countries particularly in Asian countries such as China, Japan, Korea and India among others (WHO, 2001). Traditional/herbal medicine can be incorporated as an integral part of a country’s formal health care system with each being separately recognized as legitimate forms of health care within the same framework.

However, one of the most important factors affecting the quality of herbal medicines is the lack of effective policies on quality assurance in the processing and manufacturing of herbal products under good manufacturing practices. In addition to this, proof of efficacy and safety for the vast majority of herbal medicine has not been fully established through an evidence-based approach.

Research and development to isolate therapeutically active ingredients is of critical importance to the development of medical science. There is need to formulate and develop a national policy on traditional medicine (TM) to ensure adequate regulatory mechanisms are in place for promoting and maintaining good practice, safety, efficacy and quality of traditional medicines.

Secrecy of traditional healers is a big problem to research on good remedies. There is need to coin a suitable patent and royalty system policy conducive to support all parties involved in novel drug discovery.
6. Acknowledgements

All Praises are for the Almighty God, the only to be Praised, whose Blessing and exaltations flourished my thoughts and enabled me to improve my knowledge up to this level. I offer my humble and sincerest words of thanks to our Lord Jesus Christ who is forever a torch of knowledge, wisdom and guidance for humanity. I would like to take this opportunity to thank all who have contributed in different ways to bring this study to a successful completion.

My heartfelt appreciation goes to the Swedish government through the Swedish International Development Agency (SIDA) for funding this research. Sincere gratitude to Dr Peter Sundin, Mr Hossein Aminaey and Pravina Gajjar, coordinators at the International Science Programme, Sweden. The experiments were conducted at the Royal Institute of Technology, KTH, School of Chemical Science Engineering, Stockholm, Sweden; at Makerere University and MBN Clinical Laboratories, Kampala, Uganda. The support and cooperation received from these institutions is highly appreciated.

My deepest thanks go to my supervisors; Professor Anna-Karin Borg-Karlson, Associate Professor Maud Kamatenesi Mugisha and Dr Agnes Namutebi for accepting me as their student, for sharing their vast knowledge with me, for their unending enthusiasm and ability to always encourage and give me support when needed. I am thankful to the Doctoral Committee Members; Professor Hannington Oryem-Origa, Associate Professor Esezah Kakudidi and Associate Professor Robert Byamukama for guidance and encouragement. Very special thanks go to Professor J. Y. T Mugisha and Associate Professor John Mango who never got tired of signing my vouchers whenever I needed funds. It was a pleasure to work with you all.

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Professor Peter Baeckstroem for introducing and explaining MPLC technique. To all the technical group at Makerere University: Nakibuuka Mary, Catherine Twesiime, Atuhaire Collins, Musisi Lubowa, Korugyendo Emily, Kavuma Peter, Jesca Boonabaana, Ndyanabo Susan and Portase Rwaburindore; Dr Fred Bwanga, Emmanuel Aboce and Irene Najjingo at MBN clinical laboratories, Kampala. The moral support from my PhD colleagues is highly acknowledged; Mubiru Edward, Bualfu Collins, Kakuba Christian, Jamil Ssenku, Addisalem Abathun, Peace Musiimenta, Christine Kyarimpa, Namukobe Jane, and Madrine Adia Madina. Thank you all.

I cannot ignore my appreciation and deep sense of gratitude from my family, especially my dear husband Associate Professor Byarugaba Dominic for the love and support; my children for their patience during the times I was away from home. Taremwa Derrick, Twiine Davis and Asiimwe Josephine, I love you all. You are wonderful may God bless you. My sincere gratitude to my dearest parents, Mr and Mrs Apollinari Tirikwendera who remembered me in their prayers. I am thankful to my sister Helen Musiime who shared my responsibilities during my field work research. I thank my brothers Alex Mugisha, Emmanuel Mbuga and Agaba Godfrey for supporting me.

Finally, I want to thank all the local communities of Mbarara, Ibanda, Isingiro and Kiruhura districts especially Medius Namanya, Jenipher Kabona, Maurice Nturanabo, Nyamwija J, Kwetegyereza Fred, Paulina Tumuheirwe and all the Local Council Chairpersons for identifying all the respondents that shared their knowledge with me which has made this project a great success.
7. References


Appendix A: the author’s contribution to the publications

Papers I, II, III: I participated in planning and collecting ethnobotanical data from the study areas and writing the manuscripts.

Paper IV: I participated in planning of experiments, performed chemical analyses and writing the manuscript.

Manuscripts V and VI: I participated in planning of experiments, performed chemical analyses and writing manuscripts.
Appendix B: Chemical structures of major compounds in plants with antimicrobial and antioxidant activities.

Limonene  α-phellanderene  α-terpinene

1,8-cineole  carvacrol  geraniol

nerol  linalool  α-caryophyllene

β-eudesmene  caryophyllene oxide  cis, cis- farnesol
Appendix C. Preliminary antimicrobial screening of the twenty plant extracts
Antifungal activity of ethanol extracts against *Candida albicans*

![Bar chart showing antifungal activity of various plants against *Candida albicans*.](chart)

Antibacterial activity of essential oils *against* *Klebsiella pneumoniae*

![Bar chart showing antibacterial activity of various plants against *Klebsiella pneumoniae*.](chart)

Antibacterial activity of essential oils *against* *Streptococcus pneumoniae*

![Bar chart showing antibacterial activity of various plants against *Streptococcus pneumoniae*.](chart)
Appendix D: Antimicrobial screening of the four active plant extracts

Table 1. Zone of inhibition, MIC and MBC of essential oil (dry leaf) of *Plectranthus amboinicus* on selected bacterial and fungal species

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Essential oil</th>
<th>Ethanol extract</th>
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<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition</td>
<td>Control</td>
<td>MIC (mg/ml)</td>
<td>MBC /MFC (mg/ml)</td>
<td>Inhibition</td>
<td>MIC (mg/ml)</td>
<td>MBC (mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>38.5 ± 0.5</td>
<td>31.5 ± 0.5</td>
<td>25</td>
<td>50</td>
<td>Na</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>32.0 ± 0.5</td>
<td>25 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>32 ± 0</td>
<td>35 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
<td>-</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td><em>Candida albicans</em></td>
<td>54.4 ± 4.0</td>
<td>15 ± 0</td>
<td>1.56</td>
<td>25</td>
<td>Na</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>25.0 ± 0</td>
<td>15 ± 0</td>
<td>25</td>
<td>25</td>
<td>Na</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
</tbody>
</table>

Inhibition zone diameter (mm) mean ± SEM, n=2; MBC – Minimum Bactericidal activity; MFC – Minimum fungicidal activity; Na- no activity

Table 2. Zone of inhibition, MIC and MBC of essential oil (fresh leaf) of *Plectranthus amboinicus* on selected bacterial and fungal species

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Essential oil</th>
<th>Water extract</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Inhibition</td>
<td>Control</td>
<td>MIC (mg/ml)</td>
<td>MBC /MFC (mg/ml)</td>
<td>Inhibition</td>
<td>MIC (mg/ml)</td>
<td>MBC /MFC (mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>50.0 ± 0</td>
<td>31.5 ± 0.5</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Na</td>
<td>25 ± 0</td>
<td>-</td>
<td>-</td>
<td>13.0 ± 0</td>
<td>50</td>
<td>50</td>
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<td></td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Na</td>
<td>35 ± 0</td>
<td>-</td>
<td>-</td>
<td>Na</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
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<tr>
<td><em>Candida albicans</em></td>
<td>26.0 ± 0</td>
<td>15 ± 0</td>
<td>25</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>17.0 ± 0</td>
<td>15 ± 0</td>
<td>25</td>
<td>50</td>
<td>Na</td>
<td>-</td>
<td>-</td>
<td></td>
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</table>

Inhibition zone diameter (mm) mean ± SEM, n=2; MBC – Minimum Bactericidal activity; MFC – Minimum fungicidal activity; Na- no activity
Table 3. Zone of inhibition, MIC and MBC of essential oil of *Crassocephalum vitellinum* on selected bacterial and fungal species

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Essential oil &amp; fractions</th>
<th>Water extract</th>
<th>MIC (mg/ml)</th>
<th>MBC /MFC (mg/ml)</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition (mm)</td>
<td>Control</td>
<td></td>
<td></td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>10 ± 0</td>
<td>31.5 ± 0.5</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>14 ± 0</td>
<td>25 ± 0</td>
<td>25</td>
<td>50</td>
<td>15.0 ± 0</td>
<td>25</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>25.0 ± 0</td>
<td>35 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Candida albicans</em></td>
<td>20 ± 0</td>
<td>15 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<td>34.5 ± 2.5</td>
<td>15 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
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<td>Fr 3 (51 ± 2)</td>
<td>15 ± 0</td>
<td>3.12</td>
<td>50</td>
<td>Na</td>
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<td><em>Cryptococcus neoformans</em></td>
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<td>15 ± 0</td>
<td>25</td>
<td>50</td>
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<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>Fr 5 (37 ± 3)</td>
<td>15 ± 0</td>
<td>25</td>
<td>50</td>
<td>Na</td>
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Table 4. Zone of inhibition, MIC and MBC of essential oil of *Erlangea tomentosa* on selected bacterial and fungal species

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Essential oil &amp; fractions</th>
<th>Ethanol extract</th>
<th>MIC (mg/ml)</th>
<th>MBC /MFC (mg/ml)</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition (mm)</td>
<td>Control</td>
<td></td>
<td></td>
<td>Na</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>15.0 ± 0</td>
<td>31.5 ± 0.5</td>
<td>25</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Fr 5 (16 ± 1)</td>
<td>31.5 ± 0.5</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>16.0 ± 0</td>
<td>25 ± 0</td>
<td>25</td>
<td>50</td>
<td>15 ± 0</td>
<td>50</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Fr 2 (15 ± 0)</td>
<td>25 ± 0</td>
<td>25</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Fr 3 (15.5 ± 0.5)</td>
<td>25 ± 0</td>
<td>50</td>
<td>50</td>
<td>Na</td>
<td>-</td>
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<td><em>Staphylococcus aureus</em></td>
<td>Fr 4 (11 ± 0)</td>
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<td><em>Candida albicans</em></td>
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<td>15 ± 0</td>
<td>25</td>
<td>50</td>
<td>10 ± 0</td>
<td>50</td>
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<tr>
<td>Test organism</td>
<td>Essential oil &amp; fractions</td>
<td>Inhibition (mm)</td>
<td>Control</td>
<td>MIC (mg/ml)</td>
<td>MBC /MFC (mg/ml)</td>
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<tr>
<td>Klebsiella pneumoniae</td>
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<td>31.5 ± 0.5</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>Na</td>
<td>25 ± 0</td>
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<tr>
<td>Streptococcus pneumoniae</td>
<td>Na</td>
<td>35 ± 0</td>
<td>-</td>
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<tr>
<td>Candida albicans</td>
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<td>12.5</td>
<td>25</td>
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<td>Fr 2 (28 ± 0)</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>10.0 ± 0</td>
<td>15 ± 0</td>
<td>50</td>
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</tbody>
</table>

Inhibition zone diameter (mm) mean ± SEM, n=2; MBC – Minimum Bactericidal activity; MFC – Minimum fungicidal activity; Na- no activity

Table 5. Zone of inhibition, MIC and MBC of essential oil of *Ipomea hildebrandtii* on selected bacterial and fungal species
Appendix E: toxicity signs observed in mice during acute toxicity study of the essential oils of the plant leaves (treatment-related changes)

<table>
<thead>
<tr>
<th>Plant species name</th>
<th>Dose (mg/kg)</th>
<th>Observed signs of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plectranthus amboinicus</td>
<td>5000</td>
<td>drowsiness, ataxia, paralysis, numbness, irritation and hyperurination</td>
</tr>
<tr>
<td></td>
<td>6500</td>
<td>Hypoactivity, drowsiness, ataxia, paralysis, numbness, irritation and hyperurination</td>
</tr>
<tr>
<td>Pseudarthria hookeri</td>
<td>4500</td>
<td>Paralysis of hind limbs</td>
</tr>
<tr>
<td></td>
<td>5500</td>
<td>Circular movements</td>
</tr>
<tr>
<td></td>
<td>6500</td>
<td>Died in 24 hours</td>
</tr>
<tr>
<td>Plunchea ovalis</td>
<td>5000</td>
<td>Hyperurination, hypoactivity</td>
</tr>
<tr>
<td></td>
<td>6500</td>
<td>Paralysis</td>
</tr>
<tr>
<td></td>
<td>7500</td>
<td>Paralysis, died in 4 hours</td>
</tr>
<tr>
<td></td>
<td>8500</td>
<td>Convulsions, lameness, paralysis, died in 10 minutes</td>
</tr>
<tr>
<td>Symphytum officinale</td>
<td>2000</td>
<td>Irritation</td>
</tr>
<tr>
<td></td>
<td>3500</td>
<td>Paralysis, died in 2 hrs</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>Died in 2 hrs</td>
</tr>
<tr>
<td></td>
<td>4500</td>
<td>Severe paralysis, died in 2 hrs</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>Weak with facial swollenness, died in 1 hr</td>
</tr>
<tr>
<td>Tarenna pavettoides</td>
<td>5000</td>
<td>Circling movements</td>
</tr>
<tr>
<td></td>
<td>5500</td>
<td>Circling movements, died in 2 hrs</td>
</tr>
<tr>
<td></td>
<td>7500</td>
<td>Paralysis, increased heartbeat, irritation, died in 1 hr</td>
</tr>
</tbody>
</table>
Appendix F. Chromatograms and mass spectra of essential oils of some active plant species

Chromatogram of the essential oil of *Plectranthus amboinicus* showing carvacrol (14.3%) as the abundant compound.
Chromatogram and mass spectra of the essential oil of *Crassocephalum vitellinum* showing caryophyllene oxide (9.5%) as the abundant compound.