ASSESSMENT OF BIOACTIVITY OF SELECTED PLANTS AGAINST PESTS AND MICROBES FROM AGRO-PASTORAL COMMUNITIES IN MBULU DISTRICT, TANZANIA

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY OF THE OPEN UNIVERSTY OF TANZANIA

2015

CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by the Open University of Tanzania, a thesis titled **"*Assessment of Bioactivity of Selected Plants against Pests and Microbes from Agro-pastoral Communities in Mbulu District, Tanzania*"** in fulfilments of the requirements for the degree of Master of Science (Chemistry) by Thesis of the Open University of Tanzania.

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DECLARATION

I, **Michael Qwarse**, do hereby declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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Date

DEDICATION

This work is dedicated to my parents Xwatsal Margwet and Maria D. Deeng'w, my wife Antonia Onna and my childrens Christopher Onna and Christopher M. Qwarse for always being there for me all the time.

ACKNOWLEDGEMENTS

I have no words to thanks God Almighty who bestow me this opportunity to complete my study. I wish to express my profound gratitude to all who read the thesis and in particular my supervisors, Dr. Matobola J. Mihale and Dr. Joseph Sempombe for introducing me to this area and providing excellent scientific and personal advice, guidance, critical comments, suggestions and encouragement in this study. They always supported me, had time for questions and discussions. I acknowledge the logical and critical criticism, comments, suggestions and encouragement provided by Professor Veronica Mugoyela, Dr. Leonia Henry, and Dr. Fortunatus Sungwa.

I sincerely acknowledge the financial support I got from the Commission for Science and Technology. I am highly indebted to the staff of Faculty of Science and Environmental Studies, Open University of Tanzania (OUT), School of Pharmacy and Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences for their logistic and moral support.

I wish to express my sincere appreciation to beloved mum, Mrs Antonia Onna, My children, Christopher Michael and Christopher Onna for their support and encouragements during my studies. I would like to express my gratitude to my colleagues at MUHAS: Nelson Masota, Pius Ipagala and my friends all over Tanzania amongst others: J. Siayi, Paschal P. Onna, Simon Vitalis, Reginald Barhe, Bahati Bryson, Roger, and Zaituni Msegwa for their social and moral support.

ABSTRACT

This study intended to have an ethnobotanical inventory of medicinal and pesticidal plant species and their bioactivities and toxicity against selected bacteria, fungi and storage pests. Bioactivities (antimicrobial and antioxidant activities) against disease causing organisms, toxicity against brine shrmps, first filial generation (F1) progeny, feeding deterrence and repellence against selected storage pests and quality grains evaluation as well as phytochemicals profiles were determined using standard procedures. A total of 66 dentifies plant species mainly from *Minosoidaceae, Solanaceae,* and *Euphobiaceae* families were used for pesticidal and medicinal purposes with oral route as the main route of administration and leaves as commonly used plant parts. Antibacterial activities of selected plants were between 25% and 75% of the activity of gentamycin sactivity. Antifungal activities of selected plant species varied from 0% to 55 % of the fluconazole activity. Antioxidant activity of *Phytolacca dodecandra* roots measured by percent absorbance was 90% as compared to 98% of the Butylated Hydroxy Toluene (BHT) activity. Brine shrimp cytotoxicities ranges of *P. dodecandra,* (LC_{50} = 4.6 – 34.7 µg/mL), *Cynoglossum geometrium (*LC_{50} = 71.09 µg/mL) and *Ocimum filamentosum* (LC_{50} = 28.08 µg/mL) are indicate potential cytotoxicity of plants. *P. dodecandra* leaf extracts killed 98% of *Sitophilus zeamais* and 99% of *Tribollium castaneum* at concentration of 150 mg/mL on day 3. Similarly, *P. dodecandra* leaf extracts reduced grain damage to 0% and had moderate repellence of 57% and 66% to *Sitophilus zeamais* and *Tribollium castaneum,* respectively. Little effects of leaf extracts on the grain quality (seed damage, colour and odour) was observed. Seed viability decreased to about 30% after 90 days. Isolation and structure elucidation of bioactive ingredients in selected plant species is recommended.

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LIST OF ABBREVATIONS AND ACRONYMS

µg/mL	Microgram per millilitre
µl	Microlitre
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Variance Analysis
AS	Absorbance
ATCC	American Type and Culture Collection
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
BST	Brine shrimp test
cm	Centimetre
COSTECH	Commission for Science and Technology
CRD	Controlled Randomized Design
DAT	Day after Treatment
DMSO	Dimethly Sulfoxide
DNA	Deoxynuclei acid
DPPH	2, 2-diphenyl-1-pictryhydrazyl
EPA	Environmental Protection Agency
EtOH	Ethanol
FAO	Food and Agriculture Organisation
FI	First fillian generation
HIV	Human Immunodeficiency Virus
IC_{50}	Inhibitory Concentration with 50% Inhibition
IK	Indigenous Knowledge
IPM	Integrated Pest Management
IR	Inhibition Rate

ITM	Institute of Traditional Medicine
IZ	Inhibition Zone
l	Litre
L12:D12	Light: Darkness
MeOH	Methanol
mg/mL	Milligram per mililitre
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
min.	Minute
mm	Millimetre
MUHAS	Muhimbili University of Health and Allied Sciences.
ND	Number of Dead
nm	Nanometer
No	Number
^{O}C	Centigrade
OUT	Open University of Tanzania
PR	Percentage Repellence
R.H	Relative humidity
RNA	Ribonucleic acid
RSA	Radical Scavenging Activity
S.D	Standard Deviation
SDA	Sabourand Dextrose Agar
SOD	Superoxide Dismutase
spp	Species
TBH	Tertiary Butylated Hydroxy
TBHQ	Tert-Butyl- Butylated Hydroquinone

TLC	Thin Layer Chromatography
TM	Trade Mark
USA	United State of America
UV	Ultra Violet
w/w	Weight per weight
WHO	World Health Organisation

CHAPTER ONE

GENERAL INTRODUCTION

This chapter introduces the definition of the problem, statement of the problem, general objective, specific objectives, research questions and significance of the study.

1.1 Background of the Problem

Biologically active compounds derived from natural sources have been an important part of our therapeutic strategies due to their chemical diversity and various bioactivities against diseases and pests. The low cost, environmentally friendly, less toxic, biodegradability, availability and broad spectrum of crude natural products have provided the potential for improving the health conditions of plant and animal communities in developing countries by providing the needed treatment at affordable costs (Akerele, 1984; Dahlin, 2009).

Therapeutic use of medicinal and pesticidal plants goes back to 400 years before the era of Sumerian civilization when *Hippocrates* used approximately 400 different plants species for medicinal and pesticidal purposes (Cragg *et al*., 1997). The common plants used by traditional healers in different parts of the world include antimalarials such as *Cinchona suceiruba,* (*Rubiaceae*), *Artemisia annua, Dichrona febrifuga, Azadirachta indica,* (neem) and *Cassia occidentalis* (Willcon *et al*., 2004) and antimicrobial plants like *Dodonaea viscosa, Quercus baloot, Achillea conferta, Aglaia edulis, Ajuga lupulina, juga remoata, Allium cepa, Aloe barbadensis* and *Curcuma longa* (Khurram, 2010).

Nowadays, traditional healers use marine products such as coral, algae, fungi, bacteria and sponges to treat infectious diseases like malaria, *trypanosomiasis* and *leishmaniasis*,

autoimmune diseases such as *rheumatoid arthritis*, cancer and amoebiasis as well as pests control in field and store (Nkunya, 2002).

The potential for plants as sources for new biologically active compounds for diseases and pests management still remains largely unexplored. Out of the estimated 400,000 to 500,000 plant species worldwide, only a small percentage (1%) has been investigated phytochemically and even smaller fraction has been submitted to biological or pharmacological screening (Reddy, 2009). In Africa, an estimated 5,400 wild harvested medicinal plant species are used in traditional medicine (Neuwinger, 2000).Tanzania alone is endowed with a great abundance of floral diversity, which is estimated to constitute about 10,000 vascular plant species, of which at least 25 % are considered to be indigenous to the country and among them, about 1,200 species so far have been reported to occur exclusively in Tanzania (Nkunya, 2005; ITM, 2012).

The wild medicinal plant species are derived from five phytogeographical regions in Tanzania, which are: the Afro–montane region including the Eastern Arc mountains amongst others, Lake basin regions such as Lake Tanganyika and Lake Victoria, the Masaai region in Central and Northern part of Tanzania, Zambezi region, which is covered by the Miombo woodlands in the Western and Southern part of the country, and the Zanzibar – Inhambane region consisting of coastal, thickets, forests and woodlands (FAO, 2010; Nahashon, 2013). About 60% of the Tanzanian population in both rural and urban areas depend on traditional medicine and herbs as their primary health care, and as a means of generating income (Nahashon, 2013). In addition, 75% of people with HIV/AIDS as well as 60% of children suffering from fevers rely on traditional medicine (WHO, 2002).

Lack of technical resources and assistance to the majority of subsistence farmers undermine their efforts in diseases and pests management at family level; as a result most of bioactive plants used traditionally have not been tested scientifically for efficacy and toxicity in both humans and animals. Under these conditions, losses from crops and animal products can often be significant affecting the livelihoods and food security of many poor communities (Mugisha-Kamatenesi *et al.*, 2008 and Mihale *et al.*, 2009).

In Tanzania, like in any other tropical countries, most crops are affected by a wide range of fungal, bacterial, viral, arthropod pests, and weeds. To combat them, indigenous people in Mbulu district (dominated by agro-pastoralists) traditionally use wild and exotic medicinal and pesticidal plants species for pests and diseases management. They exploit the properties of secondary metabolites in plants as decoctions, infusions, fluid extracts, tinctures, pillars (semisolid) extracts, fermentation and powdered extracts (Handa *et al*., 2008).

The common plant families used in Mbulu include *Mimosoideae, Bombacaceae, Annonaceae, Meliaceae, Malvaceae, Balanitaceae, Euphorbiaceae, Caricaceae, Celastraceae, Papilionoideae, Dracaenaceae, Tiliaceae, Anacardiaceae, Salvadoraceae, Polygalaceae, Myrtaceae, Combretaceae, Olacaceae, Rutaceae* and *Rhamnaceae* (Mbuya *et al*., 1994). *R*oots, leaves, barks, fruits, shoots, juice and exudates of these plant families are used to treat various human diseases as well as animals' diseases and pests in field and stored crops (Mbuya *et al*., 1994).

1.2 Statement of the Problem

Mbulu district particularly in rural area is characterized by high poverty levels and food insecurity prompted by, among others, food grains pests as well as human and animal

diseases. Poverty rate in Mbulu district is 62 percent of the population living under the basic needs poverty line. The literacy in study area is 68 percent, health access is 44 percent and 22 percent of children under age of 5 years suffer from chronic malnutrion (stunting) (CDI, 2005).

Since the available pests and disease control measures are expensive, subsistence farmers do rely heavily on the indigenous knowledge and skills to protect themselves, their animals and field crops and stored crop products (Ogendo *et al.*, (2005). Microbial infections have a major impact on human and animal health especially in Mbulu rural areas where antibiotics and other drugs are not freely available or are too expensive.

Mbulu rural area is endowed with very rich plant resources commonly used traditionally as potential sources of medicines and pesticides. The known medicinal plants have for long been used to children and adults, pregnant woman and breast feeding women as well as people with chronic diseases regardless of the possible adverse effects that may be anticipated. This may lead to adverse outcomes like sudden death or prolonged long term effects in organisms that use the plants or plants' products.

Apparently, a documented scientific study on the plants used traditionally by agro-pastoral in Mbulu district for pests and disease management is very little. This study, therefore, intends to scientifically assess their bioactivity (medicinal and pesticidal potencies), efficacy, toxicity and phytochemical profile on selected plant species used in Mbulu district. Hence, the study will form a baseline for further research on the isolation and chemical characterization of the active ingredients in the plants.

1.3. Objectives

1.3.1 General Objective

The main objective of this study is to assess the bioactivity of selected plants from agro-pastoral communities in Mbulu district against pests and microbes.

1.3.2 Specific Objectives

i. To develop an inventory (ethno-botanical survey) of medicinal and pesticidal plant species used by agro-pastoral societies in Mbulu district.

ii. To conduct bioassay-guided bioactivity studies of the selected bioactive plant species against grains storage pests, bacterial and fungal.

iii. To assess the cytotoxicity of selected plant species against brine shrimp and anti-oxidant by 2, 2-diphenyl-1-picryhydrazyl (DPPH).

iv. To determine the phytochemical profiles of selected medicinal and pesticidal herb species used in Mbulu district according to ethno-botanical uses.

1.4. Research Questions

i. What types of medicinal and pesticidal plant species are commonly used by agro-pastorals in Mbulu district?

ii. What are the biological (antibacterial, antifungal, and anti-pest) activities of selected bioactive plant species available in Mbulu district?

iii. To what extent are the selected plant species cytotoxic to brine shrimp and active antioxidant?

iv. What are the phytochemical profiles of the selected plant species commonly used in Mbulu district according to ethno-botanical uses?

1.5. Significance of the Study

In Tanzania, like in any other tropical countries, particularly in Mbulu rural area, most livestock and crops are affected by a wide range of fungal, bacterial, viral, arthropod pests and weeds. These are compounded by the lack of technical resources and assistance to the majority of subsistence farmers. Under these conditions, losses from crops and animal products can often be significant or even fatal, affecting the livelihoods and food security of thousand of poor communities in Mbulu district. In view of this situation, the application of improved and intensive control measures would contribute to the sustainability and enhancement of food production and hence food security. Most diseases in Mbulu district are malaria, fever diarrhoea, chronic condition and neglected diseases including typhoid, *trypanosomiasis*, plague (zoonotic disease) are both causes and consequence of poverty (CDI, 2005), and this is more pronounced when there is food insecurity in subsistence farmers. Eliminating or reducing their impact should witness a rise in productivity and human health condition. Provision of cheap and safe drugs and environmentally friendly agrochemicals are crucial to achieve this goal in the study area.

In order to spur positive economic and social development in health and subsistence agriculture, the existing Indigenous Knowledge (IK) base and practices that have served mankind for generations need to be understood, scientifically rationalized and packaged accordingly in the study area. Since disease, pest management and food grain production forms an integral component of rural farmer's food security (lifeline) as a source of family savings and incomes in Mbulu rural area. This implies that any efforts that lead

to substantial reductions in hospital expenditures as well as food grain losses (qualitative and quantitative) through alternative disease management and improved crop husbandry practices (particularly insect pest management) will definitely contribute towards improved health, food security and improved household incomes. Any significant reduction in grain losses will considerably boost the local grain supply, household incomes and will indirectly improve health people and animals.

The localized efficient harnessing and packaging of indigenous plant based products for use by the target farming communities will provide the necessary acceptability and sustainability indices. Poor documentation, lack of rationalization and standardization hamper the efficient utilization of indigenous knowledge of plant based bioactive products in Mbulu rural area. Hence, the utilization of indigenous pest and disease management technologies will stimulate investment with a promising multiplier economic development.

CHAPTER TWO

LITERATURE REVIEW

The chapter reviews plants as sources of bioactive natural products, global development of biomedicines and biopesticides worldwide and Tanzania in particular. Ethno - botanical uses and distribution of selected plant species including *Gymnema, sylverstre, Phytolacca dodecandra, Cynoglossum geometrium, Leonotis nepetifolia* and *Ocimum filamentosum* are described. Furthermore, biopesticidal properties such as antifeedant, pest's repellence and toxicity activities are reviewed. Moreover, pharmacological activities such as antioxidant activities and antimicrobial activity are assessed. Likewise, the phytochemical profiles of phenols, alkaloids, terpenoids, saponins, tannins, glycosides and flavanoids are described.

2.1 Plants as Sources of Bioactive Natural Products

Natural products are compounds of plants, microorganisms and animals origin that have not been subjected to any kind of processing or treatment other than a simple process of preservation (Samuelsson, 1999). In most cases, natural products may refer to primary and secondary metabolites from plants and animals. Primary metabolites are photosynthetic products that are essential to the livelihood of the organisms in which they occur. Secondary metabolites are molecules produced by an organism that are not strictly necessary for the survival of the organism (Kurt, 1993). The secondary metabolites are the ones commonly employed as pharmaceuticals, agrochemicals, food additives, poisons, hormones, and ingredients in cosmetics, acaricides, pesticides, fragrances, flavouring agent and allelochemicals (Kurt, 1993).

Bioactive natural products form an important part in pharmacological research and drug development as starting materials for the synthesis of drugs or as model for pharmaco-

logically active compounds (Karlovsky, 2008; African Union, 2013). Screenings of medicinal and pesticidal plants for various biological activities have shown very promising results. Plant species demonstrated antibacterial, antifungal and anti-pesticidal activity against measles, gonorrhoea, syphilis and skin fungus as well as crop storage treatment. Natural products from various sources have proven to be bioactive against malaria (Reddy, 2009) and cancer cells (Sempombe *et al*., 2014).

Plants have served as sources of the bioactive compounds because each plant is a unique chemical factory capable of synthesizing large numbers of highly complex and unusual chemical substances. The biologically active substances from plants form templates for synthetic compounds. Since about 80% of individuals from developing countries rely on plants for survival, such plants should be investigated to better understand their properties, safety and efficacy (Ellof, 1998). To have their bioactivity profile of each plants, antibacterial, antioxidant and cytotoxicity activities to disease causing microorganisms and pesticidal activities against crop storage pests and crop field pests need to be assessed.

2.2 Global Uses of Biomedicines and Biopesticides

Medicinal and pesticidal plants have been used for thousands of years as traditional treatments for numerous human diseases and pests in developed countries (China, Mexico, India, Japan, Brazil and USA) and developing countries, like Egypt, South Africa, Nigeria, Ethiopia,Kenya, Uganda and Tanzania (Saker *et al*., 2006). Most drugs and pesticides currently in use were derived from plants, marine animals, fungi, bacteria and to a lesser extent from animal organs. For example, over 40% of the medicaments used today were originally isolated from secondary metabolites of plant species (Cragg *et al*., 1997). In addition, about 50% of approved drugs between 1981 and 2010 were of natu-

ral products origin (Newman and Cragg, 2010). Similarly, the corresponding 40% of new pesticides used worldwide during the same period originated from natural products (Cantrell *et al*., 2012). Despite of the increasing modern health, 80% of the world population still rely on plants based options for the primary health care (Cragg *et al*., 1997; Swai, 2003; Mahmoud, 2004; Kisangau *et al*., 2007).

2.2.1 Botanical Biomedicines in Tanzania

In Tanzania, traditional and allopathic or convectional health care systems have coexisted since colonial times but operating in parallel circles although their main objective is to serve the same people (Mbwambo, 2007). A small Island of the coast of Tanzania, Zanzibar has traditional medicine embeded in its culture. The mostly reported medicinal plants used in Zanzibar are *Azadirachta indica, Solanum incanum, Ocimum suave, Ocimum canum, Lippia asperifolia, suregads zazibariensis and aurantifolia*. (Baylor, 2015).

In Tanzania traditional medicine is a thriving trade (commercialization) in both rural and urban areas, for example in Dar es Salaam there are 70 vendors and 50 vendors in Tanga, but the actual numbers are probably higher. The most commercialized medicinal plants reported in Dar es Salaam and Tanga used by Zigua, Makonde, Pare, Kwere and Maasai are *Zanthoxylum chalybeum*, *Cassia abbreviata* Oliv, *Albizia anthelmintica*, *Zanha africana* (Radk) exel, *Rapanea melanophloeos* (L) Mez, *Acacia nilotica* (L.) Wild, *Bonamia mossambicensis* (Klotzsch) Hallier, *Hymenaea verrucosa* Gaerth, *Keetia venosa* (Oliv) Bridson, *Warburgia elongate* Verdc, *Ximenia aegyptia* L, *Sclerocarya birrea* (A.Rich). Hochst and *Combretum fragrans* F.Hoffm. (Otieno, 2015).

Tanzania, particularly in Babati district the most used plant species as medicine by subsistence farmers and traditional healers are *Azadirachta indica* (locally called Mwarobaini) to repellent mosquito, to treated malaria, thyphoid, ulcer and chicken pox. *Moringa oleifera* (locally called Mlonge) to treated intestinal worms. Morever, *Spirostachys africana* (locally called Ndulele) and Albiza *anthemintical* are used to treat ear diseases, pneumonia and stomach ache. However, *Warburgia salutaris* is used to treated mouth sores and spots in the lung. *Aloe vera* is used to treat malaria, thyphoid and all problems with intestinal. Also, *Zanthoxyllum chalybeum* (locally called Mlungulungu) is used to treat asthma, heart burns and headaches.(Lupala 2009; Iancu 2011).

Exploration of medicinal properties of plants or plants extracts and fungi has created through observation, trial and errors, a heritage of skill/ knowledge and from expertise in different ethnic cultures and civilizations through ages and by oral tradition in urban and rural areas of the country. Currently, it's estimated that there are about 75,000 traditional health practitioner/ peoples population as 1:400, while that of doctors/ patients is1:20,000. Although, traditional medicine plays a great potential in the primary health care, the efforts to link their knowledge with the formal health sector have not yet fruitful. The growing recognition of traditional medicine in health care system facilitates the change in consciousness with regarding to the political economy (Mhame, 2000).

2.2.2 Botanical Pesticides in Tanzania

Botanical pesticides have been used in agriculture for pest management in the field, in storage, seed protection or for veterinarian treatment (Dahlin, 2009). The risk of getting cancer and other illness due to the use of chemical pesticides has made many farmers shift to use the botanical pesticides in Tanzania. Many farmers have testified that there has been an improvement in the respiratory health of their children and the parents since

they reduce the use of chemical pesticides (Dahlin, 2009). Commonly used plants in Tanzania include *Azandirachta indica, Capsicum* spp, *Tagets minuta, Nicotiana* sp, C*arica papaya, Tanacetum cinerariefolium, Croton dichogamus, Galinsoga parviflora, Cassia absus* as well as *Tephrosia vogelii. Azadirachta indica* or neem is a widely cultivated tree in many areas in Tanzania due to its ability to adapt to the local climate and great variety of uses. For example, leaves or the seeds of the plant are commonly used in Babati for management of pests in the field and in store. *Capiscum genus,* though commonly are mostly for culinary purposes and as an ornamental plant, it is used to control crop pest in field and *Tagetes minuta* or Mexican marigold is used as an ornamental plant and as a repellent in stored crops pests (Dahlin, 2009)

Figure 2.1:Neem leaves
Source: Dahlin, (2009)

Figure 2.2: *Tagetes* spp
Source: Dahlin, (2009)

Figure 2.3: *Capiscum spp*.
Source: Dahlin, (2009)

Euphorbia species are a common sight throughout Tanzania which is mainly used in veterinary, field and storage pesticide management. *Carica papaya*, commonly known as papaya, is widely cultivated throughout the tropical world and Tanzania which providing humans with nutritious fruit, the leaves and seeds have applications as a botanical pesticide in field crops. Tobacco, species of the *genus Nicotiana*, is often cultivated in some parts of Tanzania, in Babati district is used as a botanical pesticide against field and storage pests, also are used to treat bloat in livestock animals, its main active compound is nicotine (Isman 2000; Dahlin, 2009). Pyrethrum is extracted from

plants of the species *Tanacetum cinerariaefolium* (Isman, 2000). It is one of the world's most common plant derived pesticides use to control ticks and pest in field crops.

Gallant soldier or *Galinsoga parviflora* is used in field and storage crops management. *Croton dichogamus* (locally called *Girigirimo)* and *Cassia absus (*locally called *Mlutulutu)* are used against many pests in Babati. T*ephrosia vogelii*, which is very potent in fish poisoning and toxic to aquatic animals (Isman, 2000; Dahlin, 2009) is used in ticks control, as well as field and storage crop management (Abate, 2000).

Figure 2.4: ***Nicotiana***
Source: Dahlin, (2009)

Figure 2.5: ***Carica papay***
Source: Dahlin, (2009)

Figure 2.6: *Tanacetum*
Source: Isman (2000)

Figure 2.7:*Tephosia* spp
Source: Dahlin, (2009)

Figure 2.8: *G. parviflora*
Source:Stadler *et al*., (1998)

Figure 2.9:*Cassia absus*
Source:Chlabra (1987)

Even where synthetic pesticides are affordable to growers (for example, through government subsidies), limited literacy and a lack of protective equipment result in thousands of accidental poisonings annually (Forget *et al*. 1993; Isman 2000 & 2006). Many

pesticidal plants are well distributed in the tropical region. However, efficacy against pests is the limiting factor in the adoption of their use (Morse *et al*. 2002).

2.3 Ethno-botanical Uses of Selected Plant Species

2.3.1 Gymnema Sylverstre

2.3.1.1 Plant Description and Distribution

Gymnema sylverstre is a medicinal and pesticidal herb of the *Asclepiadaceae* family. It is a slow growing large perennial climber that can reach up to 600 m in height (Kesha-vamurthy and Yoganarasimhan, 1990; Kritikar and Basu, 1998). Their leaves are green, simple, petiolate which are opposite, acute apex and reculate venation with the length of 2 -6cm and width of 1-4 cm. Their stem is hairly and light brown and pubescent on both surfaces (Sudhanshu *at el*., 2012). The herb has a characteristic odour and a slightly bitter taste that can inhibit the perception of sweet taste for a few hours. Their powdered material is slightly yellowish-green with a pleasant aromatic odour (Kritikar and Basu, 1998; Tandon and Girohi, 2010; Saneja *et al*., 2010). The flowers of herb are small, yellow, in axillary and lateral umbel like cymes. The lobes of the calyces are long, ovate obtuse and pubescent. The coralla is pale yellow, campanulate, valvate with a single corona and five fleshy scales.

Figure 2. 10: ***Gymnema syverstre***
Source: Author, (2014)

The plant is distributed throughout the world, predominantly in tropical countries like India, Indonesia, Japan, Malaysia, Sri Lanka, Vietnam, Australia, Southwestern China, Ethiopia, South Africa, Saudi Arabia, Phillippines, Kenya, Uganda and Tanzania (Kritikar and Basu, 1998; Saneja *et al*., 2010).

2.3.1.2 Traditional Uses of *G. sylverstre*

G. sylverstre possesses beneficial digestive, anti-inflammatory, hypoglycemic, anti-helminthes effects. The leaves and stems of the herb are widely used for the treatment of diabetes, diuretic in proprietary medicines, stomachache, diarrhoea, urinary infections, liver disease, snake bite, dental caries, anti–obesity, corneal opacity, and constipation. In addition, it is used in the treatment of jaundice, hemorrhoids, cardiopathy, asthma, bronchitis, and leucoderma (Saneja *et al*., 2010; Sadhanshu *et al*., 2012; Di Fabio *et al*., 2013). Furthermore, it has larvicidal properties against vectors of filariasis and ovian malaria. It has also been used for control of pests of the *Lepidoptera* and *Coleoptera* orders especially *Sitophilus oryzae (*Tandon and Girohi, 2010; Ahalya and Mikunthan, 2012). Inhabitants of Mbulu particularly the Harzabe use *G. sylverstre* roots for treatment of gonorrhea and measless.

2.3.2 Phytolacca dodecandra

2.3.2.1 Plant Description and Distribution

Phytolacca dodecandra (L' Herit) (synonyms: *P. abyssinica* Hoffin, *Pircunia abyssinica* Moq.) is perennial herb of *Phytolaccaceae* family, which is a climber plant growing rapidly with hanging branches. Plants have average height of 2 to 3 meters, although it can reach a height of up to 10 meters. Under favourable climatic conditions, the plant bears fruit twice a year usually in December to January and June to July. The leaves of *P. dodecandra* are pinnate, opposite appearing whorled, ovate with 10-15cm long, 4-

12cm broad, margins entire and often undulated. Flowers of the plant are inflorescences stand axillary, in racemes, rapidly symmetric and hypogynic, the sepals are rounded and fruit are reddish. *P. dodecandra* is distributed in East, West, Central and Southern Africa and some parts of South America and Asia (Lemma, 1970, 1975).

Figure 2. 11: ***Phytolacca dodecandra***
Source: Author, (2014)

2.3.2.2 Traditional Uses of *P. dodecandra*

P. dodecandra are used in Ethiopia and other countries in the world to protect grain in the store, control fresh water snails (as molluscicides), schistosomiasis, and powders are used to produce laundry detergents, to control larvicidal effect of mosquitoes, housefly, and parasite such as mice (Karunamoorthi *et al*., 2008; Mugisha-Kamatenesi *et al*., 2008).

The plant is also used to control fungal infections, dandruff, ringworm, abortion, bacterial infections, anthrax, rabies, gonorrhoea and as spermicidal for birth control. Furthermore, its juice from leaves is used to treat malaria, helmints, wounds, headache,

rheumatism, skin irritation, stomach pain, syphilis, cancer, constipation and diarrhoea (Misganaw *et al*., 2012). In Mbulu, *P. dodecandra* leaves are used by small farmers to control grain pests in stores with minimal loss quantity and quality.

2.3.3 Cynoglossum geometrium Bak and Wright

2.3.3.1 Plant Description and Distribution

C. geometrium bak and wright (synonyms: forget me -not) is an indigenous perennial herb of *Boraginaceae* family and occur widely at altitudes ranging from 1200 to 2100m. The species has an upright, branched, roughly hairy annual, growing to about 60cm with alternative simple leaves in which the lower are not distinctly stalked. The leaves are up to 6cm long x 1.25 cm wide, gradually narrowed to the pointed apex and the sessile base. The flowers are pale blue, less than 0.7cm across, and form a looser inflorescence with up to 15 flowers per branch. The mature fruits are 0.7cm across and covered with short and hooked bristles. The plant is distributed in the East African countries namely Tanzania, Uganda and Kenya (Russell, 1975).

Figure 2.12: *Cynoglossum geometrium*

Source: Author, (2014)

2.3.3.2 Traditional Uses of C. geometrium

C. geometrium species are used for treatment of ulcer, burns, inflammation, and allergy, antitumor and antimicrobial infections (Watt and Breyer-Brandwijk, 1962**).** The root tubers of the plant are used in Mbulu for treatment of measles in cattle.

2.3.4 Leonotis Nepetifolia

2.3.4.1 Plant Description and Distribution

L. nepetifolia is annual herb of *Labiatae* family. It is an erect, loosely branched annual plants that reaches 3 meters in height with a stout, 4-angled stem, paired and simple leaves and dense whorls of orange flowers in its single growing season. The leaves are smooth with serrate margins, triangular in shape which reaches 5-12 centimetres long. The upper leaves or bracts in the axils of which the flowers arise are long and narrow with more or less entire margin.

Figure 2.13: ***Leonotis nepetifolia***
Source: Author, (2014)

The stems are strongly angled and leaves are pairs opposite each other. The dense and circular masses of flowers are 5 - 6cm across and widely separated toward the top of the stem. Individual flowers are sessile about 1.8 - 2.5cm long. There is a curved, tubular,

hairy corolla which is divided above into tips; the upper longer than the lower and hooded, somewhat resembles a lion's ear. The calyx bears 5 long, pointed teeth of very unequal length. There are 4 stamens attached to the corolla tube, the lower pair longer than the upper and the 4 - lobed ovary developed into fruit of 4 nutlets (Russell, 1975; Wiart, 2007 and Udaya et al., 2013). This plant is distributed in African countries (Kenya, Uganda, Tanzania and Madagascar) and others like India, Brazil and Canada (Dhawan et al., 2013).

2.3.4.2 Traditional Uses of *L. neptifolia*

L. nepetifolia species is used for treatment of fever, analgesic reaction, diarrhoea, bronchial asthma, malaria, influenza, kidney diseases, rheumatism, dysmenorrheal, alleviate cough, skin irritations, intestinal worms, wounds, snake bite and indigestion. Also it is used as antifungal, antioxidant, antibacterial, larvicidal and pesticidal agent (Sobolewska *et al*., 2012; Udaya *et al*., 2013; Ngoci *et al*., 2014). In Mbulu district the leaves of the plants are used for treatment skin fungal disease in human

2.3.5 Ocimum Filamentosum

2.3.5.1 Plant Description and Distribution

This is an annual herb with a short root; erect stem, 15-30 cm high, obtusely 4 angular, pubescent with retrorse hairs, diffusely branched. Leaves opposite, 0.7-4.2 x 0.35-1.6 cm, oblong to ovate-oblong, cuneate to tapering to base, obtuse, faintly serrulate, glabrous and copiously dotted with oil globules on both surfaces; petioles 1-2.2 cm long. Racemes 3-5 cm long; verticils distant.

Floral leaves are linear-oblong, with a concave, glandular receptacle at base. Calyx tube is 2.5 mm long, subglabrous to sparsely pubescent without, glabrous within, upper lip

broadly ovate-oblong, to 8 mm long in fruit, slightly longer than the lower, lateral lobes of lower lip bimucronate or truncately serrated, median ones spinous-acuminate. Corolla is 6-7 mm long, without puberulous, pinkish-white; upper lip of 3 mm long, lower equally long. Stamens are much exserted, twice length of corolla; filaments glabrous. Nutlets globose, 1.5 mm long, smooth, mucilaginous when wetted. (Pullaiah and Silar Mohammed, 2013)

Figure 2.14: *Ocimum filamentosum*
Source: Author, (2014)

2.3.5.2 Traditional Uses of O. filamentosum

O. filamentosum are used for essential oil roduction, pot herbs, traditional medicine and insecticide control (Paton *et al* 1999)

2.4 Biopesticidal Properties

2.4.1 Antifeedants Properties

The idea of using insect antifeedants (feeding deterrents) gained potency in the 1970s and 1980s with the demonstration of the potent feeding deterrent effect of azadirachtin

and neem seed extracts to a large number of pest species. The discovery and demonstration of plant natural products as insect antifeedants has been performed on neem triterpenoids, clerodane diterpenes from the *Lamiaceae* family (Klein Gebbinck *et al*., 2002) and sesquiterpene lactones from the *Asteraceae* family (Gonzalez- Coloma *et al*., 2002).

2.4.2 Pests Repellence Activity

Introduction of several plant oils as natural alternatives to synthetic pesticide repellent involved oils of *citronella*, *eucalyptus*, or cedar wood. These materials can provide some protection, but the duration of their effect is limited (often < 1 h) (Fradin and Day, 2002). In tropical areas where mosquito-borne diseases are a threat, oil from plants probably remains the only reliable repellent. Oil of citronella and monoterpenes from *Rosmarinus officinate* and *Thymus vulgaris* are used in mosquito coils to repel mosquitoes from outdoor areas. Several veterinary products for flea and tick control on domestic pets contain *d*-limonene from citrus and peel (Maistrello *et al*., 2004).

2.4.3 Toxicity Activity

The botanical pesticides are biodegradable, broad spectrum with short period of activity, suitability for small scale farmers, less likelihood of resistance due to variable mixture of different plant chemical with reduced pest resistance and harmless to the environment. Still, unlike conventional pesticides that are based on a single active ingredient, plant derived pesticides consist of a range of chemical compounds which act concertedly on both physiological and behavioural processes. One plant species may possess substances with a wide range of activities; for example, extracts from the neem tree *Azadirachta indica* are antifeedant, antioviposition, repellent and growth-regulating. In contrast, the toxicity of conventional synthetic insecticides is mainly restricted to neuro-

muscular function (Ware 1983; EL-Wakeil, 2013)). Conventional synthetic insecticides require special safety procedures and equipment during production and application because of the exposure risks for humans, and environment (Schmutterer 1990; Childs *et al*., 2001).

2.5 Pharmacological Activities

2.5.1 Antioxidant Activities

Antioxidant are vital substance which posses the ability to protect the body from damage caused by free radical oxidative stress. They can be effective in preventing free radical formation of scavenging them by promoting their decomposition and suppressing such disorder (Altiok, 2010).

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen- derived free radicals is involved in the onset of many diseases such as rheumatoid, cancer, cirrhosis, arthritis and arteriosclerosis. Degenerative processes associated with ageing, endogenous metabolic processes and exogenous chemical in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals capable of oxidizing biomolecules, resulting in tissue damage and cell death (Halliwell and Gutteridge, 2003; Elmastas *et al*., 2007).

Occupational exposure to chemically and structurally diverse environmental pollutants including pesticides, toxic chemical wastes, direct and secondary hand cigarette smoke, gasoline exhaust, urban air pollutants ozone, radiation and physical stress produces free radical. These free radicals have generative effect on human health resulting in oxida-

tive deterioration of lipid, DNA, activation of procarcinogens, inhibition of cellular and defence system, changes in gene expression and induction of abnormal proteins that contribute significantly to human disease (Altiok, 2010).

Almost all organisms are well protected against free radical damage by oxidative enzymes from our body such as catalase, superoxide dismutase, glutathione peroxidise (Khan, 2010) or chemical compounds such as ascorbic acid, tocopherol, polyphenol compounds, glutathione, and carotenoids, (Niki *et al*., 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing.

Natural antioxidants from plants, animals and microbial sources have been extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress. This is because; currently available synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary butylatedhydroquinone (TBH) and garlic acid ester have adverse effects. On the other hand, natural antioxidants are generally harmless. Hence, there is an increasing trend to substitute them with naturally occurring antioxidants (Doss *et al*., 2012).

2.5.2 Antimicrobial Activity

The increasing antimicrobial resistance has lead to the study of plants products for searching new antimicrobials due to chemical diversity and structural phytochemistries (Gibbons, 2004; Clardy *et al*., 2006). Antimicrobial activities of plants and active components isolated from them are investigated against both gram-negative and gram-positive bacteria. For many antimicrobial agents, antimicrobial actions are initiated by interactions of the biocides with the bacterial cell wall membrane of the microorgan-

isms. These agents then penetrate into the cell and finally act at the target sites (Altiok, 2010).

Bacterial growth can be inhibited by phytochemicals through membrane disruption (Griffin *et al*., 1999; Monte, 2013), interruption of energy production due to enzyme inhibition (Monte, 2013) and inhibit microbial growth by change of microbial cell permeability (Mandal *et al.*,2005; Altiok, 2010) as well as inhibiting the synthesis of nucleic acids (Cushnine and Lamb, 2005).

Antimicrobials from plants usually interfere with microorganism activities of cell wall synthesis, cell membrane integrity, proteins synthesis, DNA replication and repair, transcription and intermediate metabolism (Tabez, 2005).

2.6 Phytochemical Profiling

Phytochemicals are biologically active compounds that provide health benefits for humans, animal and plants (Hasler *et al*., 1999; Saxena, 2013). Phytochemical screening of bioactive plants extracts has revealed the presence of phenols; alkaloids, flavanoids, terpenes, saponins, tannins and glycosides, of these, flavonoids and tannins have been linked to different biological activities (Ahmad *et al.,* 2006). A brief description of each of these chemical groups is given below.

2.6.1 Phenols

Phenolic compounds are secondary metabolites that have medical, biological, agricultural and chemical applications (Saxena, 2013). This include increased bile secretion, reduced blood cholesterol and lipid levels, antimicrobial activity (Silva, 2007), antiul-

cer, anti-inflammatory, antioxidant, cytotoxic, antitumor, antipasmodic, and antidepressant activities (Ghasemzadeh, 2010).

2.6.2 Alkaloids

Alkaloids are important for the survival and protection against for micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deferens) and as well as against other plants (Molyneux *et al*., 1996). Alkaloids have a lot of pharmacological activities including antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), antihypertensive effects (many indole alkaloids), and anticancer actions (dimeric indoles, vincristine, vinblastine). (Wink *et al.,* 1998; Saxena 2013). Some alkaloids have stimulant property like caffeine and nicotine which some are used as antimalarial e.g. morphine (Saxena, 2013).

2.6.3 Flavanoids

Flavonoids have broad biological and pharmacological activities which have reported to exert multiple biological property including antimicrobial, antioxidants, anti-inflammatory, cytotoxicity, as well as antitumor activities (Saxena, 2013). Also, flavonoids have useful properties containing enzyme inhibition, anti-allergic activity, oestrogenic activity, and vascular activity (Tapas *et al*., 2008). The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Saxena, 2013).

2.6.4 Terpenoids (Isoprenoids)

Terpenoids, which include hemiterpenoids, monoterpenoids, sesquiterpenes, diterpenes, sesterterpenoids, triterpenes, tetraterpenoids and polyterpenoids (Khan, 2010; Sexana, 2013), are responsible for plant direct defence, or as signals in indirect defence respons-

es that involves herbivores and their natural enemies (McCaskill *et al*., 1998). Volatile terpenes are produced by many plants to attract specific insects for pollination or expel certain animals using the plants as food. Less volatile but strongly bitter-tasting or toxic terpenes act antifeedants to protect some plants from being eaten by animals (Degenhardt *et al*., 2003; Sexana, 2013). Furthermore, terpenes play a role as plant growth regulators (phytohormones) and can have antimalarial (e.g. artemisin), anticarcinogenic (e.g. perilla alcohol), antimicrobial or diuretic (e.g. glycyrrhizin) activity, anti-ulcer and hepaticidal activities (Langenheim *et al*., 1994; Dudereva *et al*, 2004).

2.6.5 Saponins

Saponins are a group of secondary metabolites that form stable foam in aqueous solutions such as soap (Bohlmann *et al.,* 1998). They are known to have antimicrobial, antifungal (Lacaille-Dubois and Wagner, 2000), antiprotozoal, molluscicidal and antiviral activities (Takechi *et al*., 1999; Traore *et al*., 2000).

2.6.6 Tannins

Tannin-containing plant extracts have antioxidant, anti-inflammatory, antiseptic, and haemostatic properties (Dolara *et al.,* 2005). They are also used as astringents, diuretics as well as duodenal tumours (De Bruyne *et al*., 1999), In the dyestuff industry Tannins are used as caustics for cationic dyes (tannin dyes), and in the production of inks (iron gallate ink). In addition, they are used as coagulants in rubber production (Gyamfi and Aniya, 2002) as well as in beverage industry to clarify wine, beer, and fruit juices.

2.6.7 Glycosides

Glycosides are free radical scavengers, capable of preventing oxidative cell damage, and have strong anticancer (Okwu, 2004) and antimicrobial activities (Batista, *et al*.,

1994; Vijaya *et al*., 1995; Borris, 1996), *Streptococcus mutans* and *Shigella*. They are also used as counter irritants (Heinrich *et al.*, 2004), antifeedant (Kaufman *et al*., 1999) and incorporated into creams and ointments to treat abrasions, burns, skin irritation and constipation (Heinrich *et al*., 2004).

CHAPTER THREE

MATERIALS AND METHODS

The chapter covers sample materials and material collection, study area, research approach, botanical identification, preparation and extraction. Bioactivities against microbes including antifungal and antibacterial activities, antioxidant activity, and brine shrimps test for toxicity are also covered. Furthermore, bioactivity tests against selected pests and phytochemical screening are outlined.

3.1 Study Area

The study was carried out in Mbulu district, south western of Tanzania (Figure 3.1). Mbulu Distru area is approximately 7,695 square km (including Lake Eyasi) of which land area is approximately 6,700 square km. The altitude of the district range from 1,110m to 2,250m. This difference in altitude contributes the wide range of climatic conditions with mean annual temperature ranging between 17.3^0C and 23.4^0C. In addition, the mean annual rainful range from 400mm to 1,100mm. Mbulu district is dominated by mainly hunting and agro-pastoral communities. In this ditrict various socio-economic activities are practised ranging from smallholder rainfed cultivation, extensive grazing and afforestation to mechanized rainfed cultivation with medium to high inputs. The main crops grown in the study area includesmaize, beans, pigen pea, sorghum, wheat, vegetable, fruits and coffee (Magoggo *et al*., 1994).

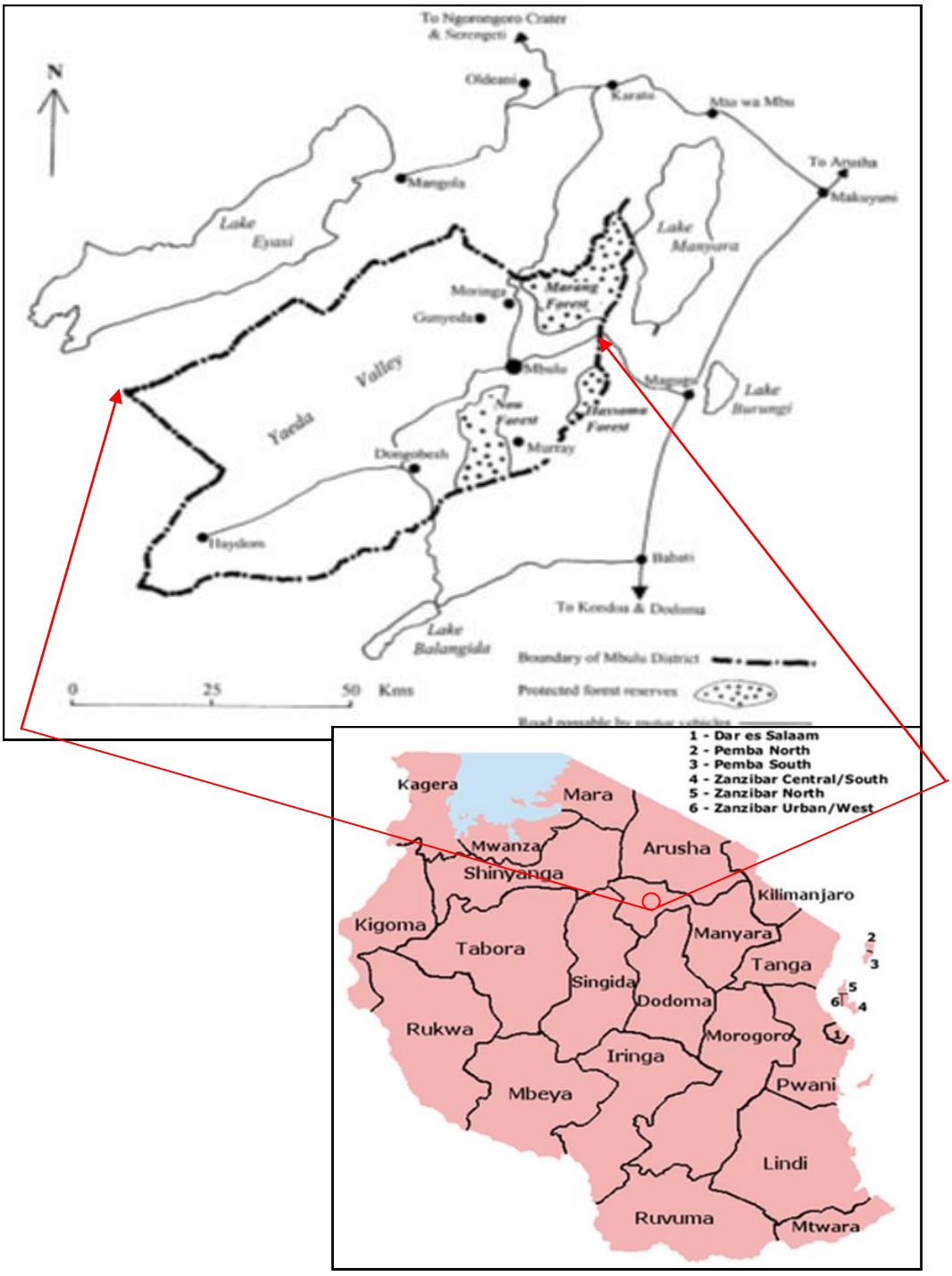

Figure 3.1: Map of Tanzania showing Mbulu District

Source: Google Earth

3.2 Plant Materials

The five selected plant species used in this study were selected from 66 identified species in Mbulu district according to their ethno-botanical uses. Plant species with little bioactivity information in literature were selected. The five selected plant species were

Gymnema, *sylverstre* roots, *Phytolacca dodecandra*, *Cynoglossum geometrium* roots, *Leonotis nepetifolia* leaves *and Ocimum filamentosum*. The fresh samples of these plant species were collected in March 2014 for further bioactivity tests. All selected plant species were tested agaist bacteria, fungi, BST and antioxidant. *Phytolacca dodecandra* species (leaves and roots) was testes against storage pests.

3.3 Research Approach

This study had two components; field works and laboratory works; Field work involved survey, collection and identification of various plant species used by agro-pastoral in Mbulu district. Laboratory work involved carrying out bioactivity tests, phytochemical screening of the selected plant species using standard laboratory procedures. These were complemented by literature search of the identified plant species. The schematic representation of the logical framework approach (LFA) is given in Figure 3.2. The field work justified that basic knowledge from substitence farmers and traditional healers provided baseline for scientific rationalization of selected five plant species. This knowledge were tested by standard laboratory method and complemented by literature review.

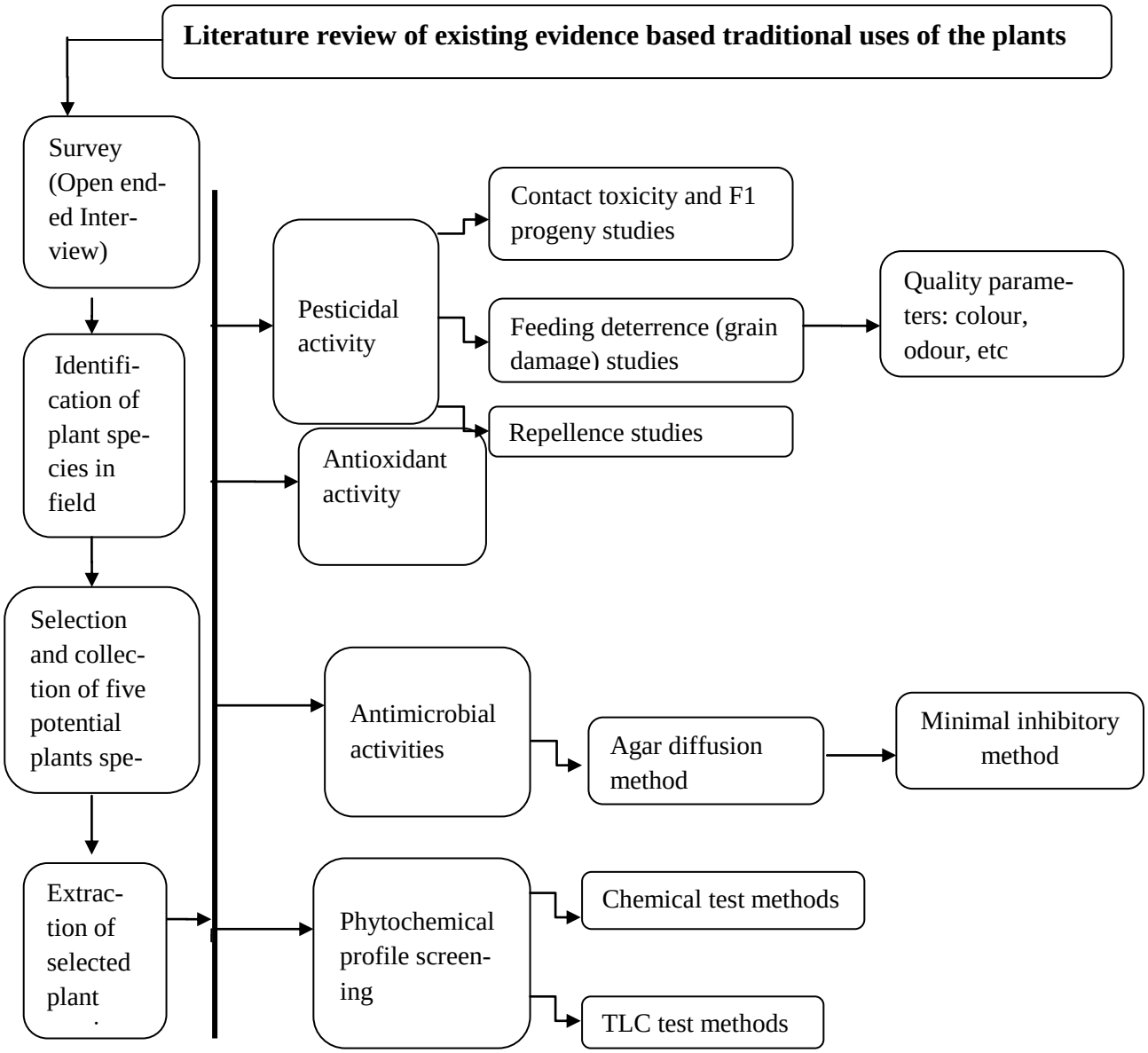

Figure 3.2: Logical Framework Approach

Source: author (2015)

3.4 Sample Collection and Botanical Identification

Medicinal and pesticidal plants were collected in Mbulu district according to ethnobotanical uses by open ended interview techniques. The collected plant species were identified at the Department of Botany, University of Dar es Salaam where voucher specimen were deposited. Five potential plant species were selected among the inventoried plants (Appendix one) for bioactivity studies according to literature review, where the plant species which are not documented were selected for bioactivites studies. The four (4) of the selected plant species tested for medicinal activity were *Gymnema sylverstre* root, *Cynoglossum geometrium* roots, *Ocimum filamentosum* roots and *Leonotis nepetifolia* leaves and one (1) tested for pesticidal activity was *Phytolacca dodecandra* root and leaves. However, *P. dodecandra* was tested for both medicinal and pesticidal activity. The activity of plant extracts against selected storage pests were conducted at the Medicinal Chemistry Laboratory, School of Pharmacy and Institute of Traditional Medicine (ITM), MUHAS. Also the phytochemical screenings of plant extracts were performed using TLC and chemical tests method at ITM, MUHAS as described in the subsection 3.10.1 and 3.10.2

3.5 Sample Preparation

The selected plant species were immediately separated into their components parts (leaves, twigs and roots) according to ethno-medical use and placed in the open container in a dark room. Then, all selected samples were allowed to air dry at room temperature in a dark room which have low humidity environment for about two weeks.

The components of each plant species were cut into small pieces sharp blade. Roots and barks were sliced into pieces of 1 - 2cm long, while leaves were cut into pieces approximately 1cm × 1cm. All samples were then dried at room temperature for another two

weeks and further oven dried at 35°C for 48 hours. Dry samples were ground into fine powders using a laboratory electric hammer mill. Finally powdered plant materials were then stored in air-tight glass jars in a cool place ready for extraction after two weeks.

3.6 Sample Extraction

Sample extraction was done using cold method (maceration) where plant powders were extracted using ethanol as solvent. Briefly, the grounded powder was weighed and soaked in ethanol (95% v/v) for 24 hours at room temperature. Later, the mixture was filtered using filter paper (Whatman No. 1, England) in Buchner funnel. The resulting crude extract was concentrated in vacuum using rotary evaporator, freeze-dried and then stored in a refrigerator at -4 $^{\circ}$C for various bioactivity tests.

3.7 Antimicrobial Activities

3.7.1 Test Organisms

Five gram-negative and one gram-positive bacteria were used for antibacterial tests. The gram-negative bacteria used were *Salmonella typhimurium* (ATCC 259239), *Escherichia coli* (ATCC 25922), *Shigella dysentariae, Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 10031) and gram-positive bacteria *Stapylococcus aureus* (ATCC 25923), Microorganisms used for antifungal tests included *Aspergillus niger* (mould) (ATCC 16404), *Candida albicans* (yeast) (ATCC 90028) and *Cryptoccoccus neofomans* (yeast) (ATCC 66037). All microorganisms were obtained at the Microbiology laboratory, Muhimbili University of Health and Allied Sciences (MUHAS) where standard cultures whose susceptibility on commonly used antibiotics have been well established. Antimicrobial (antibacterial and antifungal) of selected plant species were performed at the Microbiology laboratory, MUHAS.

3.7.2 Preparation of Culture Media

Growth media for culturing the microorganisms were prepared according to the manufacturer instructions provided on the label. Antibacterial and antifungal activities of selected medicinal plants ethanol extracts were tested using Agar well diffusion method as described by Barros *et al*., (2007c).as described below.

3.7.3 Antibacterial Activity

Bacterial Media (Muller Hinton Agar Media) for antibacterial activity were prepared using standard procedures. Thirty six grams (36g) of Muller Hinton Agar Media (Hi-Media) were mixed with distilled water in $1dm^3$ and then sterilised in autoclave at 15lb pressure at 121^oC for 15 minutes. The sterilized media were poured into Petri dishes and left to solidify. Then, wells of 6 mm diameter were made on the agar surface using corks borer. The bored plates were used for the antibacterial screening.

The prepared culture plates were inoculated with different bacteria by using plate method. The extracts were poured into the wells using sterile syringe. The plates were incubated at 37 ± 2 °C for 24 hours for bacterial activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were determined.

3.7.4 Antifungal Activities

Two hundred grams (200 g) of potato slices were boiled with distilled water in $1dm^3$ to prepare the potato infusions. Dextrose (20 g) were mixed with potato infusion agar (20 g)

was added as a solidifying agent. The contents were mixed and autoclaved. Wells of 6 mm diameter were made on the agar surface using corks borer and the bored plates were used for antifungal screening.

The prepared culture plates were inoculated with different fungi by using plate method. The extracts were poured into the wells using sterile syringe. The plates were incubated at 37 ± 2 $^{\circ}$C for 48 hours for fungal activity. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated as described in section 3.6.3.

3.7.5 Minimum Inhibitory Concentration, (MIC)

Broth dilution method was used to determine the MIC of extracts against the tested organisms that showed positive activities in the Agar Well Diffusion Method. Ethanol extracts from the five plant species showing antibacterial and antifungal activity were tested further on the same bacterial and fungal species to determine the Minimum Inhibitory Concentrations (MIC). The lowest concentration of an extract at which a tested organism that showed no any visible growth was taken as its MIC. This was determined by serial dilution method on ethanolic extracts. Bacterial (Muller Hinton broth) and fungal (Sabourand Dextrose Agar) growth media were prepared at concentration of 38 g/dm^3 and 65 g/dm^3 in distilled water respectively, autoclave at 15 Ib pressure for about 2 hours and sterilized at 120 $^{\circ}$C. The sterilised media were poured into Petri dishes and stored in a refrigerator at - 4^{0}C for MIC analysis. The Petri dishes poured with medium were divided into six parts by labelling at bottom with 1, 3, 5, 7, 11, and 12 numbers; the organisms were growth and incubating at 37 $^{\circ}$C for 24 hours for antibacterial activities and 48 for antifungal activities (National Committee for Clinical Laboratory Standards, 1990, 1997).

Stock solutions of plant extracts were prepared by dissolving 500 mg in DMSO (1mL). Serial dilution were performed in the 96 well plates by adding 50µl of media in 12 holes in replicates, Then, the extract (50 µL) was added serially up to the 8^{th} holes. At the 12^{th} hole standard commercial antibiotic gentamycin and antifungal fluconazole were added as positive control for bacterial and fungal tests, respectively. DMSO (50µL) was added in the 9^{th} hole as a negative control and 10^{th} and 11^{th} holes were left untreated. Then, microorganism culture (50µL) either bacteria or fungi was added to each hole. All 96 well plates were incubated at 37 $^{\circ}$C for 24 hours for antibacterial tests and 48 for antifungal tests respectively. The lowest concentration at which the tested organism which showed no visible growth were taken as its MIC. After every experiment, all experimental materials were sterilized in the autoclave and disposed as required for environmental and health safety regulations.

3.8 DPPH Free Radical Scavenging (Antioxidant) Activity

3.8.1 DPPH Qualitative Assay

Plant extracts were applied on a Thin Layer Chromatography (TLC) plate and sprayed with 2, 2-diphenyl-1-picryhydrazyl (DPPH) solution using an atomizer. Then, the plates were allowed to settle for 30min. The presence of antioxidant activity was indicated by white spots against a pink background.

3.8.2 DPPH Quantitative Assay

The DPPH reagent (0.1mM) was prepared by weighing of DPPH (0.04g) in 95% ethanol (1000 mL) and solution was stored in brown bottle. Different solutions of 1.25, 2.50, 3.75, 5.00, 6.25 and 7.5 mg/mL of DPPH standard were prepared as a reagent. Then, the prepared extract (4 mL) added to 16mL of 0.1mM DPPH solution. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The scavenging

activity on DPPH radical was determined by measuring the absorbance of the extract at 517 nm using UV-Visible Spectrophotometer. Synthetic antioxidant, Butylated hydroxyltoluene (BHT), was used as standard positive control. All determination of antioxidant was performed in triplicate and lower absorbance values of reaction mixture indicate higher free radical scavenging activity. (Barros *et al.*, 2007b, & 2008). The DPPH free radical scavenging activity (RSA) or percentage inhibition was calculated using the following formula:

$$\text{Percentage (\%) RSA} = \left(\frac{\text{ADPPH} - \text{AS}}{\text{ADPPH}}\right) \text{X } 100$$

Where: A_S is the absorbance of the solution when the sample extract was added and ADPPH is the absorbance of the DPPH solution (containing all reagents except extract solution).

The extract concentration providing 50% of radical scavenging activity (IC50) was calculated from the plotted graph of RSA percentage against extract concentration (Elmastas *et al*., 2011; Blois, 2002).

3.9 Brine Shrimps Test

The experiment was set according to Meyer *et al.*, (1982) and Zhao *et al.*, (1992) with some little modifications as described by Kidukuli *et al*., (2010). Briefly, brine shrimp (*Artemia salina* Leach) larvae were used as indicator animals for preliminary cytotoxicity assay of the selected plant species extracts (Meyer *et al*., 1982). In brief, artificial seawater was prepared by dissolving sea salt (38 g) in distilled water to make a concentration of 38 g/L and then filtered. The salt solution was filled into a tank that has been divided into two unequal compartments by perforated polythene wall. Shrimp eggs were later sprinkled into the covered part of the tank and a lamp was illuminated on the uncov-

ered part in order to attract the hatched shrimps. The mature *nauplii* were collected in between 36 and 48 hours of hatching (Baraza *et al*., 2007). The selected plant species extracts were dissolved in Dimethyl sulfoxide (DMSO) in vials in triplicate at an initial concentration 240 µg/ mL and decreasing up to 4 µg/ mL. In every vial containing the extract in solution, 10 brine shrimp larvae were added. An additional fourth set of vials containing only a solvent (DMSO) in 5 mL of artificial seawater and 10 shrimp larvae added as control (Meyer, *et al*., 1982). The number of survived larvae was established after 24 hours and the LC50 values concentrations required killing 50% of the shrimp larvae, the concentrations to give 100% mortality rates were obtained using probit analysis (Finney, *et al*., 1971). Percentage death was given by the formula:

Percentage death (%) = $\frac{Total\ nauplii - Alive\ nauplii}{Total\ nauplii}$ X 100%

3.10 Bioactivity Tests against Storage Pests

The activity of plant extracts against selected storage pests were conducted in terms of contact toxicity, repellence, feeding deterrence, first fillial generation (F1) progeny and grain quality studies as described in the following subsections.

3.10.1 Mass Rearing of the Pests

Selected unsexed adult *Sitophilus zeamais* and *Tribolium Castaneum* species (approximately 250) were introduced into a 1-Litre glass jars containing maize grains (500 g) and kept at 25–30°C, 65–70% Relative Humidity (RH) and 12:12 hours (light: darkness). The insects were allowed to lay eggs for 14 days and the whole procedure was done as described by Ogemah, (2003) and Ogendo *et al.,* (2004 & 2005). The jars were covered using plastic stoppers reinforced on the inside with 0.5 mm wire gauze to prevent the insects from chewing through them.

The beetles and flour frass were separated from the treated grains by repeated gentle sieving through layering of 3 mm and 1 mm mesh sieves, respectively. The grains were retained by the 3 mm sieve, the beetles were retained by 1 mm sieve and flour frass were retained in the holding pan. Those that did not come out during the sieving were forced out by probing with a plastic fibre. The grains and flour frass were then be returned into the jars and kept at 65–70% R.H until the adults emerge. The adults were also obtained by sieving as described above.

3.10.2 Contact Toxicity and First fillial Generation (F1) Progeny Studies

Maize grains each (40 grains) were weighed into glass jars (250 mL) and admixed with different plants species extract at six different dosages (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate. One batch of grains was treated with synthetic insecticide, actellic gold ^{TM} 2% dust (0.05% w/w) and this was used as positive control. Procedure of assessing contact toxicity was as described in Taponjou *et al*., (2002) and Ogendo *et al*., (2005).

Twenty (20) unsexed adult beetles, *S. zeamais* and *T. castaneum* (5–10 days old) were placed into each experimental jar. A controlled randomized design (CRD) with 3 replicates per treatment was used. The top of each jar was covered using plastic stoppers reinforced on the inside with 0.5 mm wire gauze to prevent the insects from chewing through them. The experimental units were kept at 25–30°C and 65–70% R.H.

The number of dead (ND) insects in each jar was recorded at 1, 3, 5, 7, 14 and 21 days after treatment (DAT). The adult *S. zeamais* and *T. castaneum* were removed from the grains in experimental jars at 21 DAT and the grains were returned into the jar and kept for F1 progeny counts. The numbers of newly emerged adult F1 progeny insects were

recorded at 28, 35 and 42 DAT. The percent reduction in adult emergence or reproduction inhibition rate (IR %) was computed as shown below:

Reproduction Inhibition Rate (%) = $(C_N - T_N) \times 100 / C_N$

Where C_N = number of newly emerged adult insects in the un-treated control and T_N = number of newly emerged adult insects in the treated grains.

3.10.3 Feeding Deterrence (Grain Damage) Studies

Maize grains each (40 grains) were weighed into glass jars (250 mL) and admixed with different plants species extract at six different dosages (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate. Each set of experiment was kept at 25–30°C and 65–70% R.H as described by Haines (1991), Taponjou *et al*., (2002) and Ogendo *et al*., (2004).

Later, twenty (20) unsexed adult insects *S. zeamais* and *T. castaneum* (5–10 days old) were introduced into the treated grains and allowed to feed. After 7 and 21 days, insects were removed and the amount of frass (flour) produced were determined by sieving the samples and weighing the resultant frass (flour). Percent grain damage was computed using the formula:

Weight loss (%) = (UNd – DNu) × 100/ (U (Nd + Nu))

where U – Weight of undamaged grains, D – Weight of insect-damaged grains, Nu – number of undamaged grains and Nd – number of insect damaged grains.

3.10.4 Repellence Studies

The repellent activity of the plant extracts against *S. zeamais* and *T. castaneum* were assessed using a locally made Y-shape olfactometer. The test sample extract dissolved in DMSO were applied onto a filter paper disc (Whatman No. 1, 1.8 cm in diameter), while

DMSO alone was applied on similar filter paper disc. The solvent was allowed to evaporate and the resulting treated and control discs were placed in the either arms of the olfactometer. Randomly selected adult pests (30 for each assay) of mixed sex and age were introduced into the olfactometer. Prior to the introduction of the test material, air suction were applied at the Y junction of the olfactometer by means of an aspirator pump to ensure that it did not become saturated with the test materials which are confined on the treated arm.

All bioassays were conducted and kept at 25–30°C, 65–70% R.H at six rates (0, 50, 100, 150, 200, 250 and 300 mg/mL) in triplicate. actellic gold^{TM}dust (2%) was used as a positive control and the same procedure for extracts was applied for the control. All experiments were performed in dark room (Karemu *et al*., 2013). The assays were left to run for 30 min and after that the number of insects in the control arm (NC) and that in the treated arm (NT) were counted. After each test, the olfactometers were thoroughly cleaned and dried. Percentage repellency (PR) values were computed using the following formula given by Ndung'u, *et al*., (1993):

$$PR= [(N_C - N_T)/ (N_{C +} N_{T)}] \times 100$$

A positive PR value indicated repellence by insects against pesticidal plants, while a negative PR value indicated attractance

3.10.5 Effects of the Extracts on Grain Quality

3.10.5.1 Disinfesting of Maize grains

Maize to be used in the experiment were disinfested under a gas tight sheet using phostoxin tablets at 5g PH_3 per tonne for 10 days to kill any latent insect infestation according to Haines (1991). The disinfested maize was kept in the laboratory under ambient condi-

tions. Sub-samples to be used for grain quality evaluations were further disinfested at 40 ºC in an oven for 4 hours (Bekele *et al.,* 1997; Ogendo *et al*., 2004) and allowed to cool for 2 hours before use.

3.10.5.2 Grain Quality Evaluations

The extract of selected plant species was admixed with disinfested maize grains (5 kg). An untreated sample and a synthetic insecticide treatment, (actellic gold™ 16g/kg pirimiphos-methyl+ 3g/kg Permethrin) 2% dust at the recommended rate of 0.05% (w/w) were used as comparative negative and positive controls for the extract treatments, respectively. A total of 4 treatments, each replicated 3 times, were arranged in a CRD on one metre high wooden benches in the laboratory. All treatments were kept at temperature of 25-30ºC, 38-69% RH and at L12:D12 hour's regime (light: darkness).

A cylindrical grain sampler (25mm in diameter) were used to take sub-samples (250 g) from each replicate according to the methods described by Haines (1991) after 0, 30, 60, 90,120 and 150 days of grains storage. The sub-samples were used to determine the grain quality parameters: moisture content (%), insect damage (%), seed viability index (%), grain colour and odour as described below.

3.10.5.3 Grain Moisture Content

A weighed subsample of about 100 grams of untreated maize grains was put in a capacitance grain moisture meter (LDS-1G, China). Direct temperature-corrected moisture content (%) readings were recorded for each grain sub-sample in triplicate.

3.10.5.4 Grains Damage

The grain sub-samples were assessed for damage arising from natural insect infestations after 0, 30, 60, 90,120 and 150 days of storage. Each sub-sample was separated into un-

damaged and insect-damaged grains. The numbers of grains in each category were counted, weighed and the percentage weight loss (percent grains damage) of maize grains in storage were computed according to the methods described in Haines (1991) and Ogendo *et al*., (2004) as follows:

$$\%\text{Weight loss} = UNd - DNu \times 100 / (U(Nd + Nu))$$

Where: U = weight of undamaged grains, D = weight of insect damaged grain, Nu = number of undamaged grains and Nd = number of insect damaged grains.

3.10.5.5 Seed Viability Index

The effects of extract treatments, storage duration and their interactions on seed viability were expressed as the percent germination as investigated over a 150-day grains storage period. Unbiased sub-samples of 100 undamaged grains were obtained according to the methods described in Haines (1991). The sub-samples (100-grains) were germinated on moistened cotton wool in Petri dishes arranged in a CRD with 3 replicates. The experiment was maintained under laboratory conditions as described above. The number of emerged seedlings from each Petri dish were counted and recorded after 7 days. The percent germination was computed according to the methods described by Zibokere (1994) as follows:

$$\text{Viability index (\%)} = (NG \times 100)/TG$$

Where: NG = number of seeds that germinated and TG = total number (=100) of test seeds placed in each Petri dish.

3.10.5.6 Grain Colour and Odour

The change in colour and odour of untreated and treated maize grains were assessed on a monthly basis for five months consecutively. Grain sub–samples were assessed for

change in colour and odour by modification of Ogendo *et al*., (2004) quick assessment method into a scoring scale of 1-5 defined separately for each of the two parameters. The scores at day zero (0) represent the values just before grain treatment.

Scoring for change in grain colour was done according to the following scale;

1 = No detectable change i.e. natural colour (white/gray) with a few yellow grains

2 = Slight change (≤5%) from natural colour to light brown

3 = Moderate change (>5 to 30%) from natural colour to brown

4 = Great change (>30 to 50%) from natural to dark brown

5 = Highly significant change (>50%) making grain unacceptable for human consumption.

Scoring for change in odour was done as follows:

1 = Grain is odourless

2 =Grain has little offensive odour

3 =Grain has moderately offensive odour

4 =Grain has very offensive odour making grain unacceptable for human consumption.

To obtain unbiased scores, each grain sample were coded and presented in a well lit and ventilated room for assessment. A panel consisting of six independent assessors scored for change in grain colour and odour (Ogendo *et al*., 2004). The assessors were allowed into the assessment room once at a time in rotation to ensure their scores were independent from each other.

These procedures were repeated on a monthly basis for five months and the same six panellists were retained over the entire assessment period (Kramer, 1956; Ogendo *et al*., 2004). Blank scoring sheets were used for the different assessment dates to ensure that the previous data do not bias subsequent scores.

3.11. Phytochemical Screening

3.11.1 Thin Layer Chromatography

3.11.1.1 Preparation of the Development Tanks and Mobile Phases

Four development tanks (6x12x15) cm^3 each lined with well trimmed filter papers to facilitate the saturation of the tank with the developing solvent. Solution of each developing solvents were then poured into the plates in tanks and covered to obtained equilibrium for a period of 15minutes. The developing solvents used in this study included chloroform: hexane (75:25; 50:50; 20:80), chloroform: methanol: hexane (1: 1:2), MeOH: $CHCl_2$: EtOAC (1:1:2), MeOH: EtOAC: H_2O: Acetic acid (10:30:1 drop: 5 Drops). The best mobile phase giving best results was presented (Harborne, 1984 & 1973). Development was allowed to proceed until the solvent front. The plate was then removed from the chamber and the solvent fronts immediately were marked with a pointed pencil (Harborne, 1984). The plate was then allowed to dry in a fume cupboard.

3.11.1.2 Sample Spotting on the Chromatoplates

The chromatoplates were allowed to develop in a development tank till the solvent front was about 5/6 of the plate. The plates were then removed and allowed to dry in open air after marking the solvent front. The position of the separated solutes was located by pointed pencil. Later the chromatoplates were then observed under UV light at a wavelength of 365 nm then sprayed with reagents. Coloured substances were seen directly when viewed against the stationery phase while colourless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the spotted regions, which they occupy (Harborne,1984 & 1973).

3.11.1.3 Tests for Alkaloids, Phenolics and Terpenoids

The chromatoplates were activated in an oven at 100^oC for about 15 minutes. After complete cooling, they were spotted using ethanol extracts. Sample was spotted 2cm apart

using a very thin capillary tube. An air blower was used to dry the samples spots so as to control the spot size. After the plates were developed, they were left to dry for about 10 minutes, then viewed under UV fluorescence light at wavelength 254 nm and 365 nm, and finally sprayed with the required detection reagent in the following subsection (Harborne, 1973. & 1984).

3.11.1.4 Dragendoff Reagent for Alkaloids:

This reagent was made by dissolving, bismuth subnitrate reagent (0.97 g) in acetic acid solution (10 mL) and water (40 mL). Then potassium iodide reagent (6.5 g) was dissolved in water (20 mL) making a 1:1 mixture of these two solutions (Harborne, 1973 & 1984). Lastly, the spray reagent was prepared by mixing the stock solution (1mL) with fresh acetic acid (2 mL) and water (10 mL). Orange-brown spots on yellow background on sprayed TLC indicated the presence of alkaloids and other nitrogen compounds.

3.11.1. 5 Ferric Ferrocynide Reagent for Phenols

Iron chloride (10%) $FeCl_3$ (aq)) was mixed with 0.01 g/mL Iron cyanide to get one solution. Then, freshly prepared solution of a mixture of Ferric chloride (0.1g) and Potassium ferricyanide (0.1g) in of distilled water (10 mL) formed the second solution. A spraying reagent was prepared by mixing the two solutions at 1:1 ratio. The formed solution was then sprayed to the plates and the plates heated at 110^oC in the oven. Instant change of colour to blue indicated the presence of phenols (Harborne, 1973& 1984).

3.11.1. 6 Vanillin Reagent Test for Terpenoids

Vanillin (10%) was dissolved in ethanoic acid – concentrated sulphuric acid (2:1) and sprayed onto the plates. After that the plates were put in the oven for 15 minutes. Pres-

ence of terpenoids was indicated by the separated colours of brown, dark green and purple colour (Harborne, 1973 & 1984).

3.11.1.7 Tests for Flavonoids

TLC plate with the extract was exposed to ammonia. The presence of flavonoids was indicated by yellow, pink, grey and brown spots on the plate (Harborne, 1984 & 1998).

3.11.1. 8 Tests for Anthraquinones

TLC plate was sprayed with a solution of CH_3OH (10 mL) and Potassium hydroxide (10 g). Change of the original yellow brown colour to purple indicated presence of anthraquinone (Harborne, 1984).

3.11.2 Qualitative Tests of Phytochemicals

Chemical tests were carried out using the ethanol extracts to identify the constituents as described by Sofowara (1993),Trease and Evans (1996, & 1997) and Harborne (1973, 1984 & 1998) as follows.

3.11.2.1 Test for Saponins

The presence of saponins in the plant extracts was determined as described by Kokate, (1996). Briefly, the extract was mixed with distilled water to make 20 mL and the suspension was shaken for 15 min. Formation of a 2 cm layer of foam indicated the presence of saponins.

3.11.2.2 Test for Tannins

In a test tube, water (20 mL) and dried plant powder (0.5 mg) were added, boiled and then filtered. Later, drops of Ferric chloride (0.1 %) were added in a filtrate (Buvaneswari, 2011). A brownish green or blue black coloration indicated the presence of tannins.

3.11.2. 3 Test for Flavonoids

To a portion of the aqueous extract (6 mL), dilute ammonia solution (5 mL) was added, followed by few drops of concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids (Buvaneswari, 2011).

3.11.2.4 Test for Glycosides

Glycosides in the extracts were tested using Liebermann's test. Crude plant extract (6 mL), was mixed with chloroform (2 mL) and acetic acid (2 mL). The mixture was cooled in ice and then few drops of concentrated H_2SO_4 were added. A colour change from violet to blue and then to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside (Yadav and Agarwala, 2011).

3.11.2.5 Test for Sterols and Triterpenoids

To sample extract (6mL), 2mL of chloroform and few drops of concentrated H_2SO_4 were added. Later the mixture was shaken well and then allowed to stand for some time. Whereas the appearance of a red colour in the lower layer indicated the presence of sterols, formation of yellow coloured layer indicated the presence of the triterpenoids (Yadav and Agarwala, 2011).

3.11.2. 6 Test for Alkaloids

To a portion of plant dry extract (0.5 g), chloroform (10 mL) was added. Then, chloroform was evaporated to dryness and the residue was dissolved in dilute hydrochloric acid (2 mL). After that, the solution was tested with dragendorff's reagent. An orange precipitate indicated the presence of alkaloids (Kumar *et al.*, 2011).

3.11.2.7 Test for Coumarins

A few drops of Sodium sulphate were added dropwise to a crude extract (5 mL) in a test tube. Appearance of a yellow colour indicated the presence of coumarins (Buvaneswari, 2011).

3.11.2.8 Test for Anthraquinones

A few drops of benzene were added dropwise to a crude extract (5 ml) in a test tube followed by ammonia drop. Appearance of a pink colour indicated the presence of anthraquininones (Buvaneswari, 2011).

3.11.2.9 Test for Phlobatannins

Aqueous extract of plant sample (10 mL) was boiled with aqueous hydrochloric acid (1%). Formation of red precipitate indicated the presence of phlobatannins (Khanna and Kannabiran, 2007).

3.11.2.10 Test for Phenols

Distilled water (5 ml) and few drops of neutral Ferric chloride solution (5%) were added to the plant extract (10 mL). A dark green color indicated the presence of phenol compounds (Yadav and Agarwala, 2011).

3.11.2.11 Test for Terpenoids

Presence of terpenoids in the extracts was tested using Salkowski test. Briefly, 10 mL of the extract were mixed with 2 mL of chloroform and few drops concentrated sulphuric acid and the layers heated for about 2 minutes. A reddish brown coloration of the interface showed the presence of terpenoids or gyayish colour (Yadav and Agarwala 2011)

3.11 Data Analysis

Data analyses were done using Microsoft office excel 2007 where all the experimental results for brine shrimps test, antioxidant and antimicrobial tests were expressed as mean ±S.D (standard deviation) of triplicate measurements. LC_{50} for brine shrimps test and antioxidant activities were determined using probit analysis method (Buss and Park-Brown, 2006). Pesticidal activity data were evaluated by using one-way analysis of variance (ANOVA). Non-parametric exact tests (Ogendo *et al.*2004) (Wilcoxon rank sum test or Mann U test) were used to test for any significant changes in grain colour and odour during storage. The presentations of all results were either in form of tables or graphs as shown in the next chapter.

CHAPTER FOUR

RESULTS AND DISCUSION

The chapter covers the ethno-botanical survey of biomedicinal and biopesticidal plant species available in Mbulu district. Bioactivities of selected plant species against selected microbes including antibacterials, antifungals and antioxidants are outlined. Furthermore, bioactivities of plant extracts against selected storage pests such as totoxicity, repellence, feeding deterrence and grains quality evaluation are analysed and discussed. Moreover, brine shrimp of selected plant species and phytochemical profiles for compounds like saponins, favonoids, alkaloids, sterols and terpenoids are assessed and discussed.

4.1 Ethnobotanical Survey of Biomedicinal and Biopesticidal Plants from Mbulu

4.1.1 Traditional use of plants in Agro-pastoral communities in Mbulu district

This study identified sixty six (66) plant species from thirty nine (39) different families which are used by subsistence farmers and traditional healers as indicated in Appendix 1. Out of these, fifty five percent (55 %) of the plant species are used for medicinal purposes, twenty nine percent (29 %) are used for pesticidal purposes and the rest are used for both medicinal and pesticidal purposes. In total, fifty two percent (52%) of the plant species are used to treat human diseases, sixteen percent (16 %) are used for veterinary uses and thirty one percent (31 %) are used to treat crop in the field, storage and domestic pests (Figure 4.1).

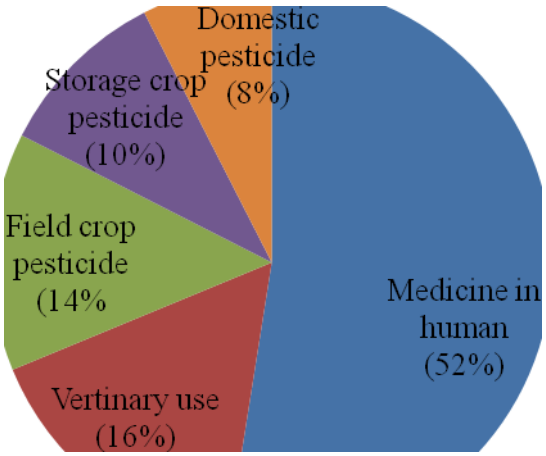

Figure 4.1: Categorazation of Use of Plant Species by Agro - pastoral of Mbulu

Most of the identified medicinal plants in the study area are belonged to *Minosoidaceae, Solanaceae,* and *Euphobiaceae* families. This is because these families are widespread and easily accessed. However, scientific studies support their widespread use in the area. Previous studies have shown these families have different phytochemicals which exhibit medicinal and pesticidal properties (Lui, 2003; Nalubega, 2010). For example, *Ephobiaaceae* family have phytochemicals that have antimicrobial and acaricidal properties (Adedapo *et al.,* 2005; Falodum *et al.,* 2006; Palombo and Adebola, 2008). Typical alkaloids of *Solanaceae* family are known to have pharmacologically important compounds (Osborm, 2003).

In terms of plant species, *Capsicum annum, Nicotiana tobacam* and *Solanum incanum* are the most widely used plant species. Bukenya, (2007) reported that these plant species are also used by farmers in Uganda to treat microbial disinfection in poultry. Research

indicates that these plant species exhibit good antimicrobial and other medicinal properties (Elujoba *et al.*, 2005; Bukenya, 2007; Olukayode and Adebola, 2008). This probably confirms their use by agro-pastoral communities in the study area. On the other hand, *Meliaceae* and *Euphorbiaceae* are the most used with pesticidal features. Like medicinal plants, these families are preferred for control of field and storage pests due to their availability. This means that these families are good candidates for pesticidal research (Mwine *et al.*, 2011).

4.1.2 Categorazation of Plant's Use by Route of Administration

In terms of administration, six administration routes were identified for treatment of diseases and controlling pests. These included oral, spotting, spraying, chewing, mix and inhalation of smoke from burnt plant parts (Figure 4.2). Out of these, forty six percent (46%) of the plants species were administered orally.

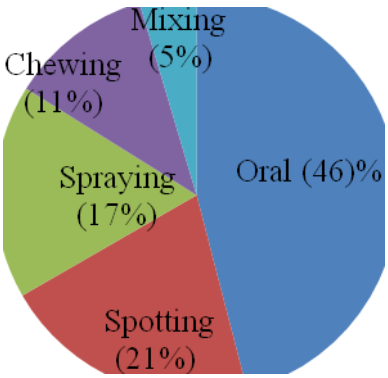

Figure 4.2: Categorization of Plant Species by Routes of Administration

Subsistence farmers and traditional healers used the oral route most frequently probably since it is easy to administer in this way and the route requires less skills (Nalubega, 2010). This finding are in agreement with Bukenya, (2007) study who reported that the

most common way of preparing and administering of the biomedicine to human and animals is oral administration.

4.1.3 Categorization of the Plants by Plant Parts

Twenty seven percent (27%) of the plants used in Mbulu district involved leaves in making concoctions, while others parts used include roots, fruits, seeds, barks, stems or the whole plant (Figure 4.3)

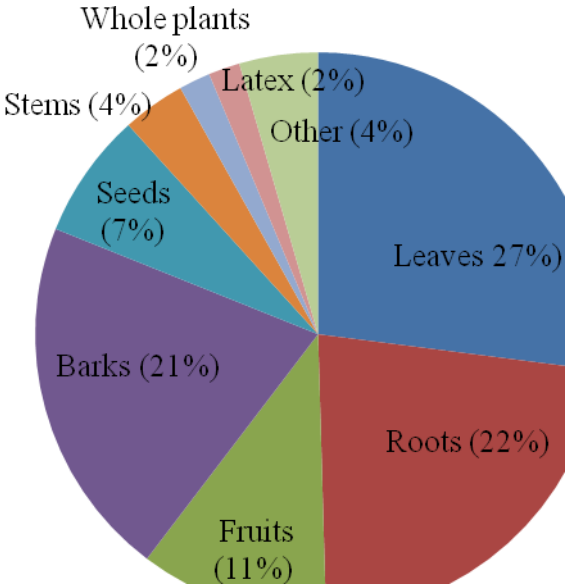

Figure 4.3: Categorization of the Plants by Plant Parts

This finding is in line with the results of some ethno-medicinal studies (Oliva *et al.*, 2007; Yineger and Yewhalaw, 2007). Pradhan and Badola, (2008) and Nalubega, (2010) reported that leaves are the most used plant parts in remedy preparations. Research also indicate that despite the considerable variations of plant parts used, most farmers consistently use the similar plant parts from particular species such as leaves, roots, fruits, husks, etc. Though the main difference was in the mixture applied by each individual farmer, hence leaves constituted a large portion (Mugisha-Kamatesi *et al.*, 2008).

4.1.4 Categorization of Plants by Method of Preparation

Thirty three percent (33%) of the plant species used were prepared by soaking in cold or hot water. The rest of the methods used were making juice, powders, fresh plants, and smoking the plant species as indicated in Figure 4.4.

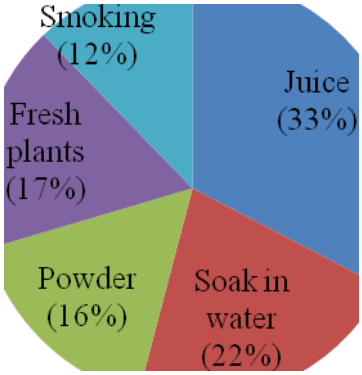

Figure 4.4: Categorization of the Plant species by the Method of Preparation

Most plants are prepared as juice by soaking in cold or hot water and probably this requires less time. This research also agreed with Bukenya, (2007) study who reported that making juice and administer the concoction orally is the most preferred way. The study showed evidence that farmers and traditional healers in Mbulu district use various indigenous practices and use a number of medicinal and pesticidal plant species to prevent and treat human, animal's diseases and controlling pests in field and storage crops.

4.2 Bioactivities of Selected Plant Species against Selected Microbes

Five plant species were tested for antibacterial, antifungal and antioxidant activities in order to determine their microbiological activites.

4.2.1 Antimicrobial Activities against selected plant species

4.2.1.1 Antibacterial Activities

P. dodecandra was bioactive to *E.coli, S. typhimurium, P. aeruginosa, K. pneumonia* and *S. aureus.* The highest activity as indicated by zone of inhibition was in *S. typhimurium,* followed by *P. aeruginosa* (Table 4.1). The bioactivity of *P. dodecantra* was 25% to 75% of the activity of gentamycin. This species was not active to *S. dysentriae.* Similarly, *L. nepetifolia* was active to all tested bacterial except *S. typhimurium*. However, the activity of L. *nepetifolia* was lower than that of *P. dodecantra* in all species.The bioactivity of L. *nepetifolia* was 25% to 38% of the gentamycin activity. *C. geometrium* showed bioactivity to *E.coli, S. typhimurium, K. pneumonia* and *S. aureus* only and its bioactivity was 25% to 53% of the gentamycin activity. *O. filamentosum* and *G. sylverstre* showed actitivies to four and three species only, respectively. Bioactivity of *O. filamentosum* was 27% to 48% of the gentamycin activity. Similarly, bioactivity of *G. sylverstre* was 31% to 46% of the gentamycin activity.

Table 4.1: MIC and Mean Inhibitory Zone of Selected Plant Species against Six bacterial Species

	E. coli		***S. typhimurium***		***P. aeruginosa***		***K. pneumonia***		***S. Aureus***		***S. Dysentariae***	
PLANT SPECIES	**Mean IZ**	**MIC**	**Mean IZ**	**MIC**	**Mean IZ**	**MIC**	**Mean IZ**	**MIC**	**Mean IZ**	**MIC**	**Mean IZ**	**MIC**
PDL	10±1	125	29.7±1.5	125	19±1	75	13.7±1.5	125	x	x	x	x
PDR	x	x	11.7±1.5	125	x	X	x	x	15±1	125	x	x
LN	15±2	125	x	x	10±1	125	10.7±1.5	125	15.3±1.5	75	10±1	125
CG	21±2	37.5	20±2.6	75	x	X	11.7±1.5	75	10±1	125	x	x
OF	15±1	125	x	x	x	X	11±2	125	19.7±1.5	125	11.3±1.5	125
GS	x	x	19±2	125	13±1	125	x	x	15.3±1.5	75	x	x
Gentamy-cin (Standard)	39.3±3.2	9.4	40.7±3.2	9.4	41±2.6	9.4	40.3±1.5	9.4	40.3±2.1	9.4	39.7±1.5	9.4

KEY: PDL = *P. dodecandra (*leaves*)*, PDR = *P. dodecandra (*roots), LN = *L. Nepetifolia*, CG = *C. geometrium*, OF = *O. filamentosum*, GS = *G. sylverstre*, IZ = Inhibition Zone in mm, MIC = Minimum Inhibition Concentration (mg/mL) and X = No activity

P. dodecandra leaf extracts was active against S. *typhimurium, P. aeruginosa* and *K. pneumonia* while root extract was active against *S. typhimurium* and *S. aureus.* These findings could be attributed to the positioning of plant parts on a plant. Plant defence theories suggest that chemical or structural defences should be maximized when and where browsing is most likely to occur (Mathai *et al*., 2000). Leaves are exposed and conspicuous, which makes the best targets for herbivore attack. It is not surprising, therefore, that plants tend to deposit and localise majority of secondary substances in exposed parts such as leaves, barks and fruit/flowers to act as deterrents to herbivores. Plants without conspicuous leaves like *Euphorbia* spp. utilize their green stems/latex for such a purpose (Manu *et al*., 2008). The result obtained from this study show that ethanolic extracts of selected plant species exhibit antibacterial properties. This justifies their traditional use as medicinal plants.

4.2.1.2 Antifungal Activities

P. dodecantra was bioactive against *A. niger, C. albicans and C. neofomans.* Leaf extract was active to all fungi except *A. niger,* while root extract was active to all fungi. Bioactivity of *P. dodecantra* leaf extract was 23% to 50% of the fluconazole standard activity. Furthermore, bioactivity of *P. dodecantra* root extract was 23% to 37% of fluconazole activity. The MIC of *P. dodecantra* was as low as that of fluconazole standard for *C. neofomans. L. nepetifolia* and *C. geometrium* extracts were active against all the tested fungi. Bioactivity of *L. nepetifolia* was 37% to 53% of fluconazole activity. Similarly, the bioactivity of *C. geometrium* was 23% to 32% of the fluconazole activity. *O. filamentosum* and *G. sylverstre* were both bioactive to *C. albicans* and *C. neofomans* only. Bioactivity of *O. filamentosum* was 26% to 31% of the fluconazole activity. Similarly, bioactivity of *G. sylverstre* was 23% to 25% of the fluconazole activity.

Table 4.2: MIC and Mean IZ of Selected Plant Species against Three Fungal Species

	A. niger		***C. albicans***		***C. neofomans***	
Plants Species	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC
PDL	x	x	9.3± 2.2	125	19.7± 2.1	9.4
PDR	15.0± 1.7	37.5	11.0±1.7	75	9.0± 1.6	125
LN	15.3±2.1	18.8	21.0±1.7	75	16.7±0.8	18.8
CG	9.3±0.8	125	10.0±1.4	75	12.7±0.8	37.5
OF	x	x	12.3±0.8	75	10.3±1.4	125
GS	x	x	9.3±2.2	125	10.0±1.4	125
(Fluconazole)						
Standard	41.0±1.4	9.4	39.7±2.1	9.4	39.3±0.8	9.4

KEY: PDL = *P. dodecandra (*leaves*)*, PDR = *P. dodecandra (*roots), LN = *L. Nepetifolia*, CG = *C. geometrium*, OF = *O. filamentosum*, GS = *G. sylverstre* = Inhibition Zone in mm, MIC = Minimum Inhibition Concentration (mg/mL) and X= No activity

The secondary metabolites in *P. dodecandra* may be responsible for this bioactivity (Mohamed *et al*., 1996). Other studies have shown that chloroform and ethyl acetate extracts of the aerial parts of *G. sylvestre* exhibited activity against *P. vulgaris* (Paul and Jayapriya, 2009; Fabio *et al*., 2013). The widely use of *G. sylvestre* in traditional ways of disease and pest control has a strong scientific basis. Gnaneswari and Venkata Raju, (2012) and Maobe *et al*., (2013) reported that ethanol extract of *L. nepetifolia* leaves showed strong activities against *Candida albicans.*

The antibacterial and antifungal activities could be due to the presence of secondary metabolites which are known to possess antimicrobial activities properties (Ngulde *et al*., 2013; Jaiganesh and Arunachalam, 2013). *L. nepetifolia* (*Lamiaceae*) is an important me-

dicinal plant which exhibited various biological activities such as antifungal and antibacterial activities due to presence of different bioactive compounds (Dhawan *et al.,* 2013). This is in agreement with Udaya Prakash et *al*., (2012) who observed that methanolic extract of *L. nepetifolia* leaves showed wide spectrum of antimicrobial potency. From this investigation, the results obtained provide evidence of therapeutic potency of L. *nepetifolia* as in traditional medicine.

The roots *of Cynoglossum geometrium* ethanolic extract have activity against bacteria (*E.coli, S. aureus, K. Pneumonia* and *S. typhimurium)* and fungi (*C. albicans*, and *C. neofomans*). Omwega and Paul (2012) reported that presence of flavonoids, tannins and cardiac phytochemicals inhibit growth of bacterial and fungal (Chakraborty and Chakraborti, 2010).

4.2.2 DPPH Free Radical Scavenging Activity

Antioxidant activity was present in *P. dodecandra* roots only. The percent absorbance of *P. dodecandra* was 90% (R^2 = 0.924) as compared to 98% (R^2 = 0. 8226) of the BHT standard (Figure 4.5). The LC_{50} for *P. dodecandra* root extract was 0.399 mg/m L compared to 0.034mg/ml of BHT.

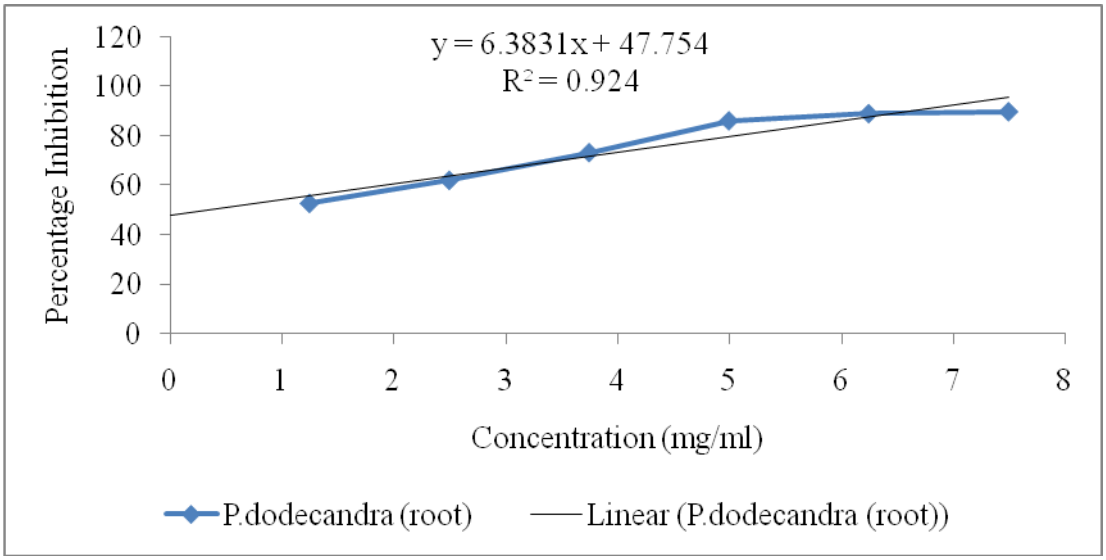

Figure 4.5: Free Radical Scavenging Activity of Different Concentration of Ethanolic Extract of *P. dodecandra* Roots by 2, 2-diphenyl-1-Picrylhydrazyl Radical.

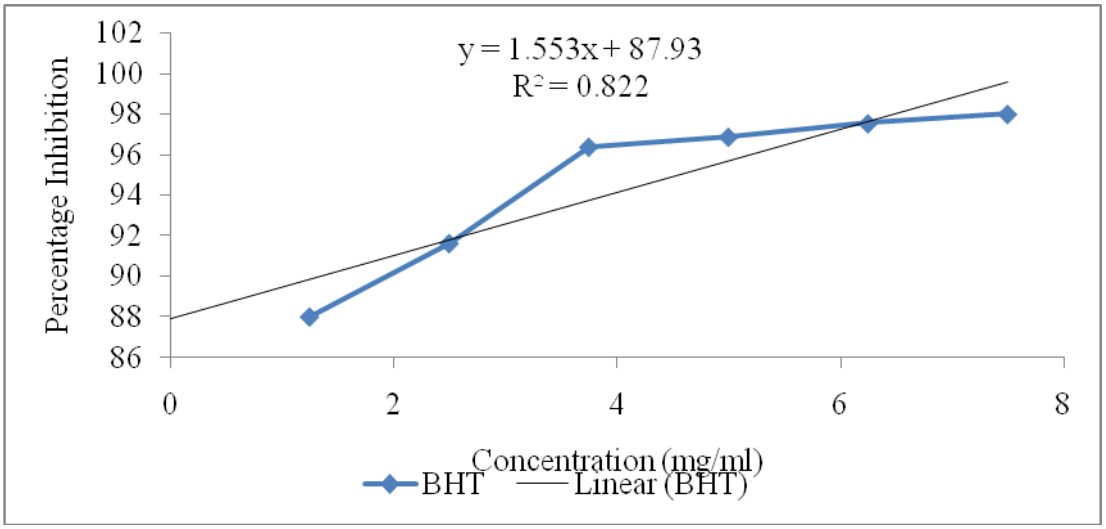

Figure 4.6: Free radical scavenging activity of different concentration of standard BHT by 2, 2-diphenyl-1-picrylhydrazyl radical

Scavenging effect of ethanolic extract of *P. dodecandra* roots increased with increased concentration and the change was significant ($p < 0.01$). Natural antioxidants present in the herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Maisuthisakul *et al.*, 2007 reported that total phenolic compounds are major components for antioxidant activity in plant species.

Antioxidant activity in plant extracts is due to the redox effect, which make it a reducing agent, hydrogen donor, hydrogen peroxide or lipid peroxide scavenger as well as singlet oxygen quencher (Chaangwei, 2009). This offsets the imbalance between reactive oxygen species and antioxidant defence system that may increase the oxidative burden and lead to damage of macromolecules that play a role in pathological processes of various diseases. The presence of antioxidants in plant is shown by the exhibited strong anticancer, heap-protective, antiviral and several other activities (Khalaf *et al*., 2008; Jain *et al*., 2014).

4.3 Brine Shrimp Cytotoxicity of Selected Plant Species

The results of the brine shrimp lethality bioassay test for the extracts of selected plant species are presented in Table 4.3 Crude ethanolic extracts of *P. dodecandra* leaf exhibited the highest LC_{50} compared to root extract. *O. filamentosum* crude extracts indicated to have LC_{50} of 28.08 µg/mL. In general, the LC_{50} extracts of all selected plant species were higher than the LC_{50} of the Potassium permanganate ($KMnO_4$) standard (LC_{50} =3.39 µg/mL).

Table 4.3: Cytotoxicity of Ethanol Crude Extracts from Selected Plants Species

Plant Species	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Regression Equation	R^2
PDL	4.57	88.25	y = 3.0963x + 2.9755	2.9522
PDR	34.74	726.09	y = 3.03x + 0.3312	0.8562
GSR	133.4	6166.22	y = 2.4026x - 0.1059	0.8898
CGR	71.09	1581.28	y = 2.9692x - 0.4985	0.9033
OFR	28.08	3904.41	y = 1.867x + 2.2957	0.9475
LNL	119.42	5286.40	y = 1.1140x + 4.1476	0.8459
PP	3.39	100.61	y = 2.7171x + 3.5586	0.9089

KEY: PDL = *P. dodecandra* leaf; PDR = *P. dodecandra* (root); CGR = *C.geometrium* (root); GSR = G. *sylverstre* (root); OFR = *O. filamentosum* (root); LNL = *L. nepetifolia* (leaf) and PP = Potassium Permanganate

Plant toxicity is important in assessing the safety of plant products by the societies. The plant's intrinsic toxicity and problem of overdose can be assessed by brine shrimp test, which is considered as convenient method for preliminary assessment of toxicity due to high sensitivity of the *nauplii* to different chemical substances (Jamil, 2010; Mungenge *et al*., 2014). The numbers of novel antitumor and pesticidal products have been identified and later isolated using this bioassay (Sam, 1993).

Jooste (2010) and Moshi *et al*., (2010) classified the brine shrimp cytotoxicities such that LC_{50} < 100 µg/mL are very toxic, 100 µg/mL <LC_{50} < 500 µg/mL are toxic, 500 µg/mL < LC_{50} < 750 µg/mL are moderate toxic and LC_{50} > 1000 µg/mL are not toxic. The LC_{50} values depicted by *P. dodecantra*, *C. geometrium* and *O. filamentosum* extracts indicate the presence of potential of cytotoxic compounds in the plant species. This is supported by Peteros and Mylene, (2010) and Rahman *et al*., (2014).

The results also have shown that the degree of lethality varies with exposure to different dose levels of the test samples. The degree of lethality was directly proportional to the concentration applied and mortality increased gradually with the concentration of the tested samples, which is supported by Peteros and Mylene, (2010) and Rahman *et al*., (2014).

The brine shrimp cytotoxicity assay is a convenient method for preliminary assessment of toxicity and has been used for the detection of fungal toxins, food additives, plant extract toxins, heavy metals, cyanobacteria toxins, pesticides antimicrobial and cytotoxicity testing of dental materials (Rajen *et al*., 2012; Aziz *et al*., 2013; Zimudzi, 2014; Screeshmeal and Nair, 2014). There are particularly positive correlations reported between brine shrimp lethality and cytotoxiciy toward the 9KB cell line (human nasopharyngeal carcinoma) and

other solid tumors as well as for the P388 cell line (*in vivo* murine leukemia) (Sam, 1993). Hence, low LC_{50} is indicative of the antitumor activity of the plant extract. For example, LC_{50} of *croton bonplandianum* at concentration of 0.06mg/mL indicated antitumor properties (Ajoy and Padma, 2013).

4.4 Bioactivities of Plant Extracts Against Selected Storage Pests

Bioactivity against storage pests was only determined to *P. dodecandra.* This is due to the fact that ethnobotanical evidence gathered during the survey (Appendix 1). So, *P. dodecandra* extracts were tested for contact toxicity, feeding deterrence, repellence as well as quality parameters as described in detail in the following subsections.

4.4.1 Contact Toxicity of P. dodecandra

P. dodecandra leaf extracts at concentration of 150 mg/mL killed 98% of *S. zeamais* and 99% of *T. castaenum* at day 3, respectively (Figures 4.7 and 4.8). The same concentration of P. *dodecandra* root extracts killed only 5% of S. *zeamais* and 67% of T. *Castaneum* at day 3 (Figures 4.9 and 4.10). No mortality was recorded from untreated control maize, whereas maize treated with actellic gold^{TM} dust achieved 100% mortality within one day. Generally, the mortality percent increased as concentration increased and when duration of contact with treated maize increased and ($P < 0.05$).

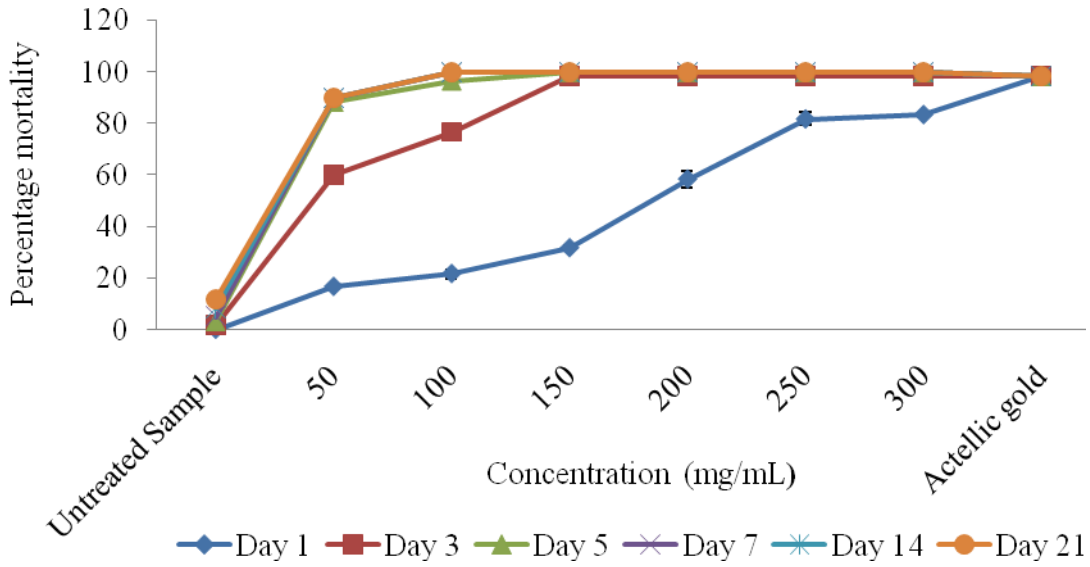

Figure 4.7: Percent Mortality of S. *zeamais* Using *P. dodecandra* Leaf Extracts

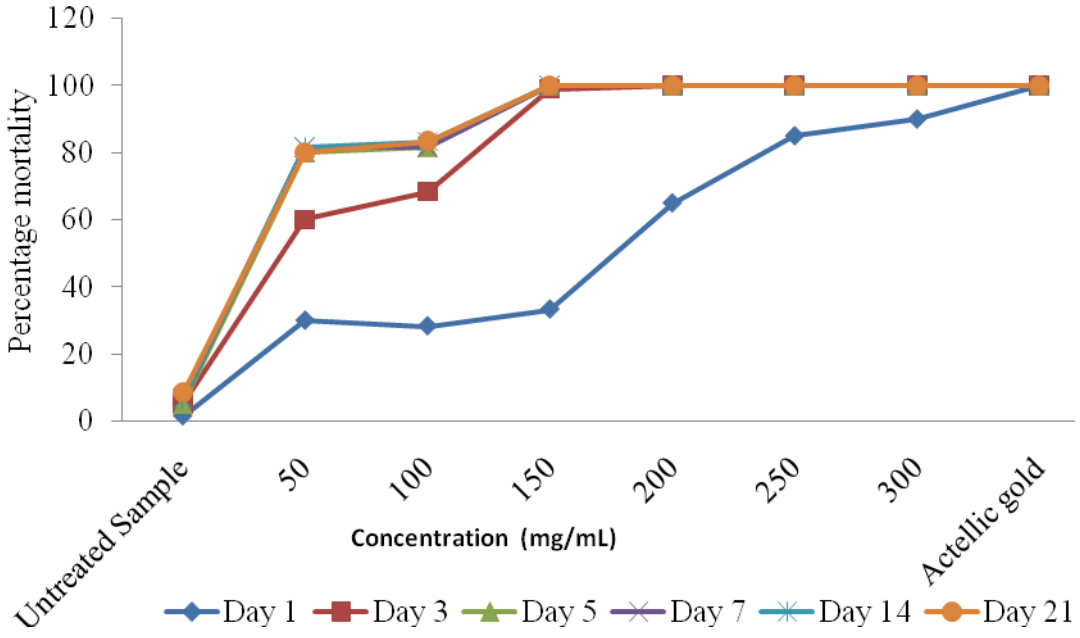

Figure 4.8 Percent Mortality of T. *castaneum* Using *P. dodecandra* Leaf Extracts

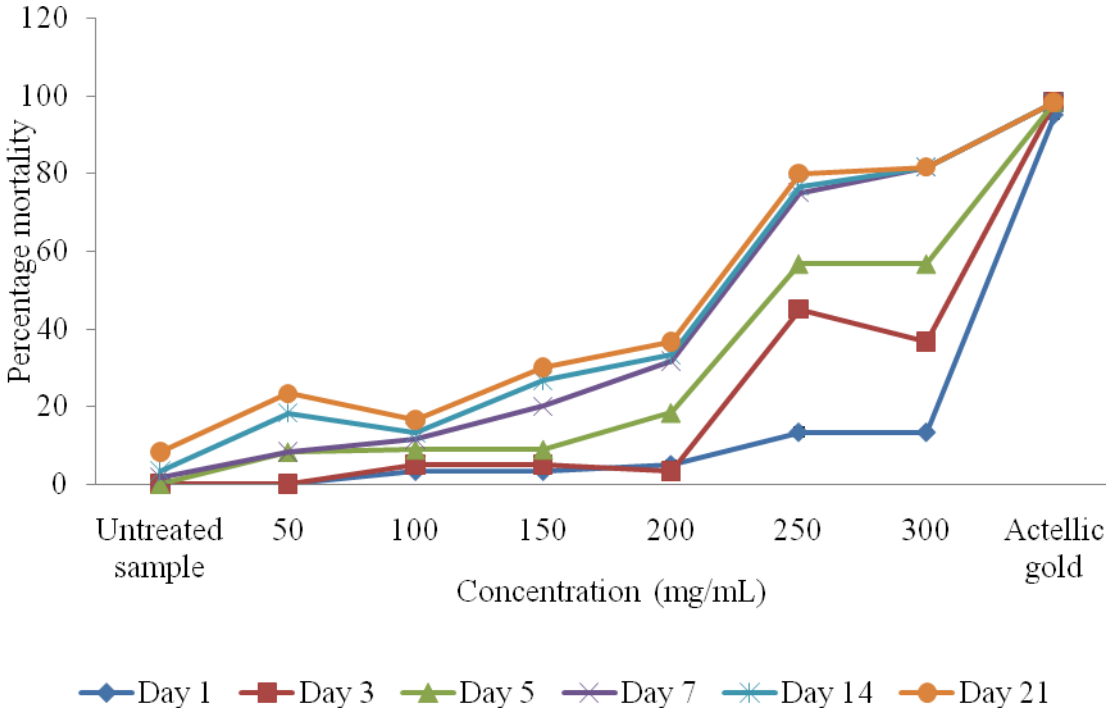

Figure 4.9: Percent Mortality of S. *zeamais* Using *P. dodecandra* Root Extract

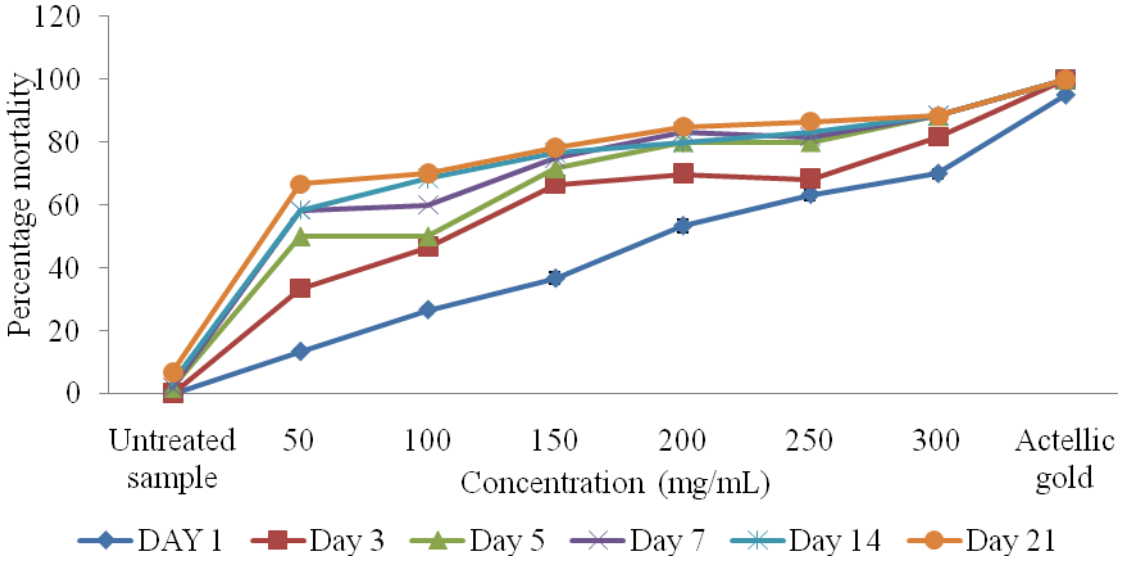

Figure 4.10: Percent Mortality of *T. castaneum* Using *P.dodecandra* Root Extracts

The results showed that crude extracts of *P. dodecandra* leaves had strong toxicity compared to *P. dodecandra* roots against adult *S. zeamais* and *T. castaneum*. Toxicity was significantly dependent upon the concentration of extract applied and duration of contact

with treated maize grains compared to untreated control. Percent mortality increased with increasing dosage (concentration) and duration of exposure to extract, where low mortality was observed in first day of treatment. As time of exposure proceeds, there was progressive increase in the toxicity of the botanical compounds in roots. The toxicity of crude extracts was influenced by plant parts (leaves and roots), contact duration expose (days), concentration applied and corresponding factors of interactions. The fact that *P.dodecandra* leaf extract at 150 to 300 mg/mL caused 100% mortality of adult *S. zeamais* and *T. castaneum* on 3 DAT (Figure 4.7 and 4.8) while *P. dodecandra* root extracts at 250 – 300 mg/mL caused between 80% to 81% mortality of S. *zeamais* and between 80% to 86% mortalty of T. *castaneum* on 21 DAT. (Figure 4.9 and 4.10). This findings are similar to previous study done by Mugisha-Kamatesi *et al*., (2008) who reported that *P. dodecandra* powder from dry leaves were used to treat grain storage insects by subsistence farmers around lake Victoria basin. Toxicity induced by plant species in the current study are similar to the findings of Kasa and Tadese (1996) in which crude powders from eight plant species caused between 58 and 88% mortality of *S. zeamais*.

It is evident from the above results that *P.dodecandra* leaf extract is a potential protectant against *S. zeamais* and *T. castaneum*. The broad spectrum bioactivity of this botanical extract of *P.dodecandra* with the local availability and processing make them acceptable and cost effective alternative to synthetic pesticides in small holder agriculture. Hence, this plant - based products hold good promise for inclusion in the Integrated Pest Management (IPM) strategies.

4.4.2 Feeding deterrence of *P. dodecandra*

P. dodecandra leaf extracts at concentration of 150 mg/mL and above deterred *S. zeamais* and *T. castaneum* for damaging maize grains from day 1 to day 21 similar to actellic

gold™ (Figures 4.11 and 4.12). That is there was 0% insect damage in both maize treated with *P. dodecantra* leaf extract and those treated with actellic gold™. In the untreated samples, h owever, there was up to 10% insect damage by day 21 in which ($P < 0.05$).

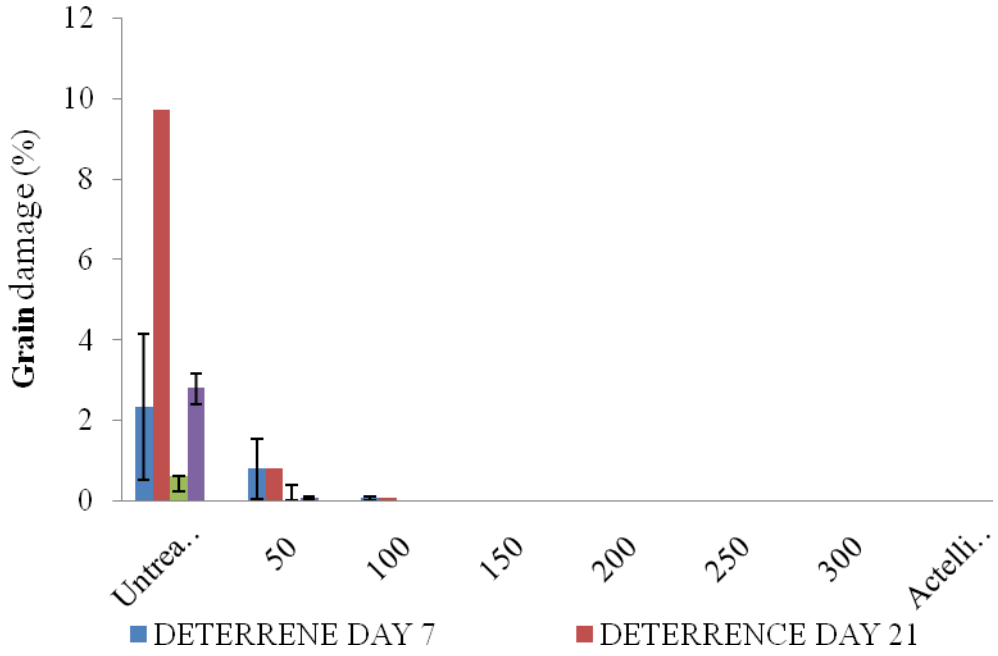

Figure 4.11 Percent Damage of *S. zeamais* Pests in Maize Applied with *P. dodecandra* Leaf Extracts

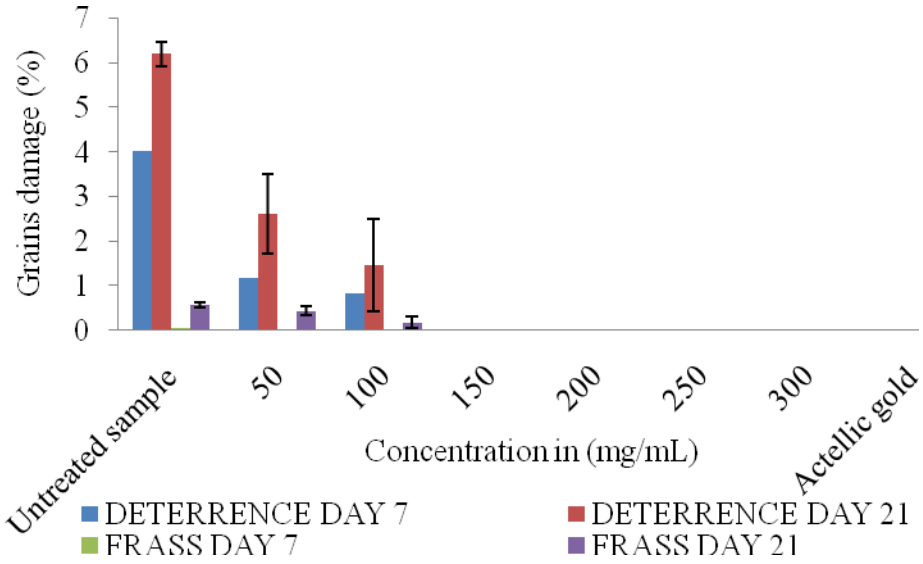

Figure 4.12: Percent Damage of *T. castaneum* Pests in Maize Applied with *P. dodecandra* leaf Extracts

Root extracts of P. *dodecntra* had little feeding deterrence compared to leaf extracts at the concentration of 150 mg/mL (Figure 4.13 and 4.14). Insect damage was also observed in the treated samples. However, the percentage insect damage was decreasing with increasing concentration of the root extract. In general, as the concentration of test sample increased the percent of damage grains decreased.

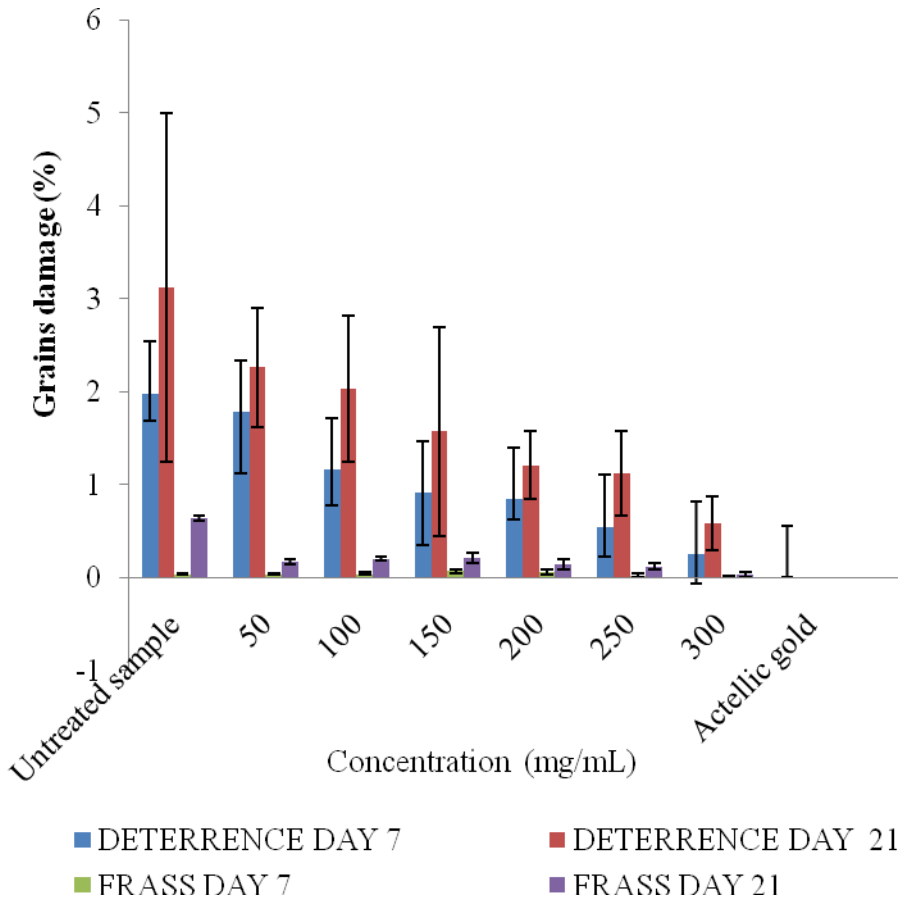

Figure 4.13: Percent Damage of *S. Zeamais* Pests in Maize Applied with *P. dodecandra* Roots Extract

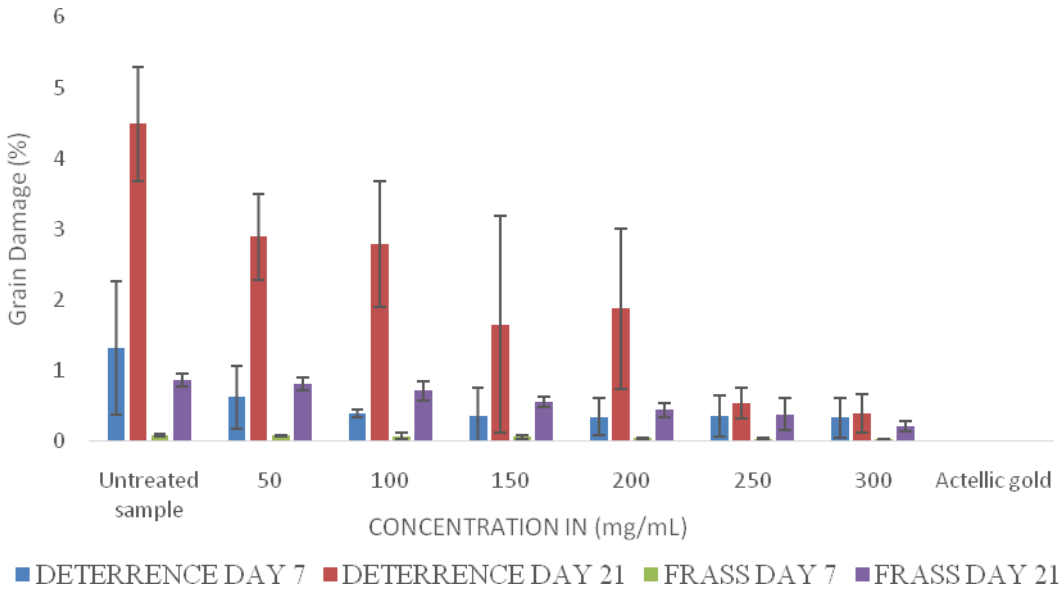

Figure 4.14: Percent Damage of *T. castaneum* Pests in Maize Applied with *P. dodecandra* Root Extract.

The crude extracts of *P.dodecandra* leaves exhibited strong dose and contact duration dependent deterrence as expressed in terms of reduced grain loss (percent damage) and amount of frass produced during the insects feeding activities. As observed in the choice bioassay, grains admixed with ethanol extracts of *P. dodecandra* leaf and roots were deterrent reducing adults *S. zeamais and T. castaneum* compared to the untreated control. Untreated samples had the highest damage and weight frass percent in all cases.

The antifeedant property of any plant material depends on active constituents of plant part such as triterpenoids, iridoids, gycocides which may be responsible for the observed insecticidal properties (Sharma *et al*., 1995). Previous studies reported presence of antifeedant compounds in plant species; *A. indica, T. vogelli, and L. camara* (Butterworth and Morgan 1968; Rembold 1995; Ogomah 2003; Chebet *et al*., 2013). The bioactive isoflavonoids, decalin, hydroxyfuran, nimbin and salinnin are reported to have sterilizing effects on eggs of insects (Ibrahim *et al*., 2000; Ogendo *et al*., 2005). Decalin is reported to have regulato-

ry effects on growth and development while hydroxy furan has antifeedant effects (Ascher 1993; Sclar 1994). Studies have indicated that *T. vogelli* leaves and seeds contain rotenoids tephrosin and daguelin) which have antifeeding deterrents (Isman 2006; Adabayo *et al*., 2007; Koona *et al*., 2007; Ogendo *et al*., 2008). Chebet *et al*., (2013) reported that *L. camara*, seeds provide 80% protection of stored grains from bruchid beetles over a 6-month period. The fact that *P. dodecantra* have a considerable reduction in damage by the adult *S. zeamais* and *T. castaneum*, the finding holds good promise for their adaption and rationalized use for grains protection in smallhold agriculture (Alonso-Amelot and Aviva-Nunez, 2011).

The observed grains protection properties of crude extracts treatments could partly be attributed to a modification of the physical properties of stored grains that reduced inter-granular air spaces thereby discouraging insect's penetration, feeding and amount of oxygen available. In that sense, the crude extracts succeeded in inhibiting insect feeding and oviposition. In addition, the adult insects mortalities recorded in the study may largely be due to starvation. Hence, the antifeeding of test botanicals may be attributed to their bioactive constituents (Chebet et al*., 2013).*

4.4.3 Repellence activities of *P. dodecandra*

P. dodecandra leaf extract demonstrated a moderate repellence ranging from 15.85% at 50 mg/mL to 57% at 300 mg/mL for *S. zeamais* and from 20.99% at 50 mg/ mL to 66.67% at 300 mg/ mL for *T. castaneum* (Figure 4.15). The repellence of *P. dodecantra* leaf extract at 150 mg/ mL was 75% of the repellence activity of actellic gold^{TM} standard against *S. zeamais* and 88% of repellence of actellic gold^{TM} standard against *T. castaneum.*

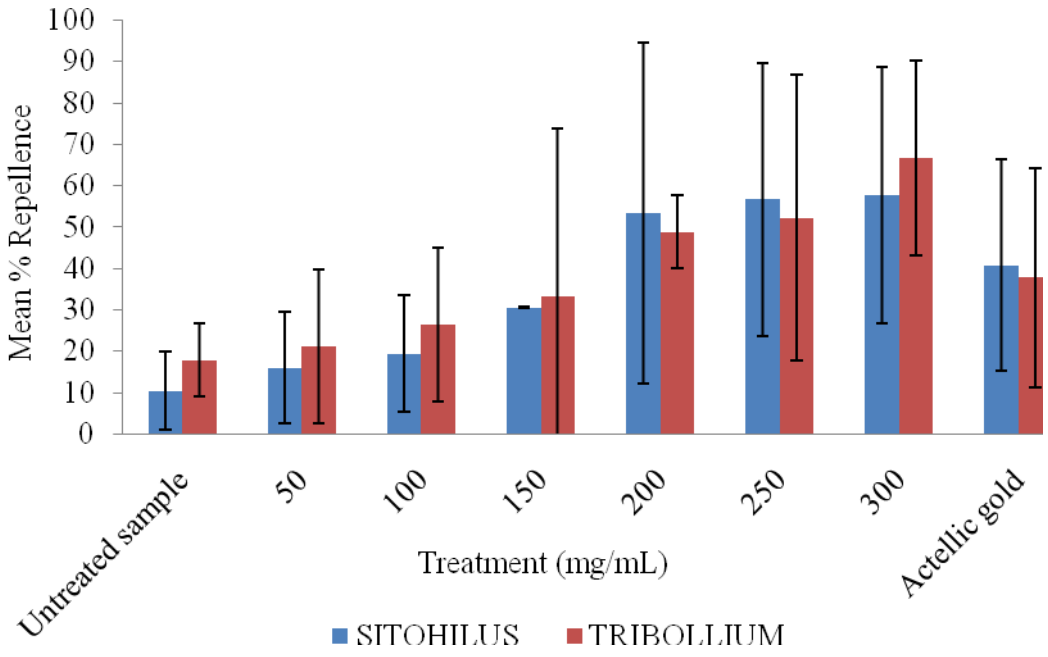

Figure 4.15: Mean Percent Repellence of Tested Pests against *P. dodecandra* leaf extract

Unlike leaves, *P. dodecandra* root extracts indicated a weak attractance percent ranging from 5.11% at 50 mg/ mL to 44.43% at 300 mg/ mL for *S. zeamais* and from 20.99% at 50 mg/ mL to 56.18% at 300 mg/ mL for *T. castaneum* (Figure 4.16). In contrast, actellic gold^{TM} had repellence percent of 40.67% and 37.68% for *S. zeamais* and *T. castaneum*, respectively. Results from the repellence and attractance bioassay showed sigficance ($P < 0.05$) difference between the untreated control and treated samples. There were also significance differences between actellic gold^{TM} and two insect at different concentration ($P < 0.05$). The percent repellence (PR) values for all insects' treatment significantly increased with increasing concentration.

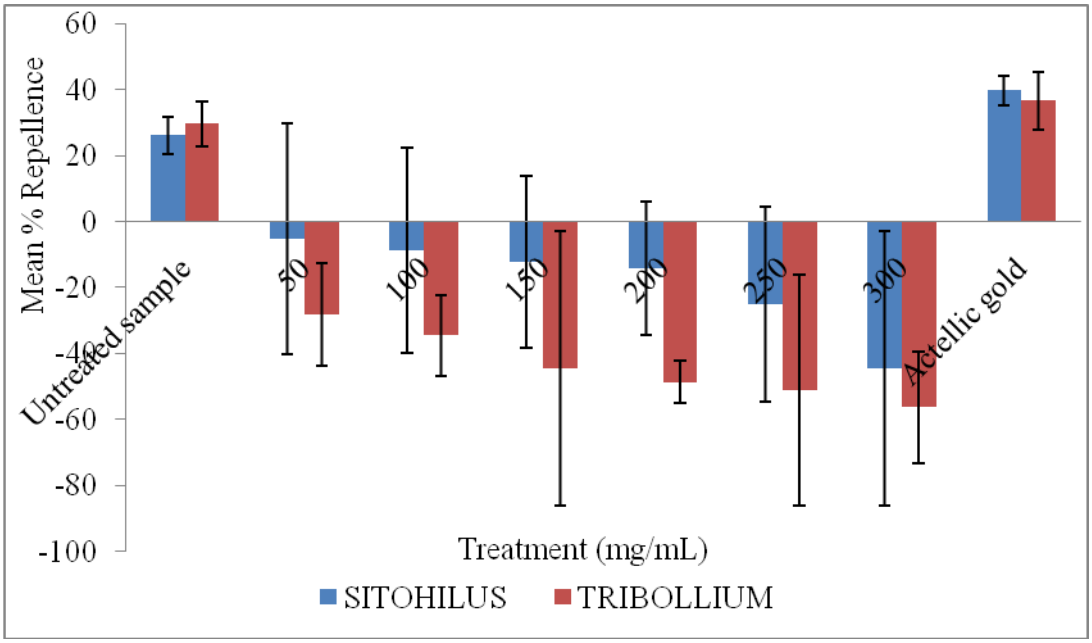

Figure 4.16: Mean Percent Attractance of *P. dodecandra* Root Extracts *Against* Tested Pests

Maize grains treated with *P. dodecandra* leaf was significantly moderately repellent against *S. zemais* and *T. castaneum* while maize grains treated with *P. dodecandra* roots was significantly weakly attractance against *S. zemains* and *T.castaneum* (Figure 4.15 and 4.16). The degree of repellence and attractance was greatly influenced by plant parts, dosage extract applied and phytochemical properties (compound). The essential oils from plants contain sulphur and saponin compounds which potentially acts as repellent (Ascher, 1993). For example, *Ephestia cautella, L. camara* and *T. vogelli* are good repellents to major storage pests (Bekele *et al*., 1997; Ogendo *et al*., 2005; Shehu *et al*., 2010). Also, Blum and Roitberg (1999) reported neem oil repel various insects including moths.

The observed repellent activity could partly be attributed to the presence of volatile constituents such as monoterpenes and sesquiterpenes which were well known repellents of phytophagous (biting) insects by acting in vapour form. (Debboum *et al*., 2007; Wang *et*

al., 2008). In addition, aromatic compounds from plants have long been used in daily life as insects repellent because are generally regarded as safe and economically and socially friendly compared to synthetic pesticides (Chebet, 2013).

The repellent effect of *P. dodecandra* leaves may have important implications on post harvest protection system, since they facilitate maize weevils to depart from the treated grains (Karemu *et al*., 2013). The repellent action of *P. dodecandra* leaves suggest that there exist good potential for the use of this plant species as grain protectants in the traditional resource-poor farming communities in sub-sahara Africa. Sustainable use of botanical pesticides will boost the food security in that environment in which synthetic pest control is uneconomical (Ogendo *et al*., 2005).

4.4.4 First fillial generation

The results of adult *S. zeamais* and *T. castaneum* of F_1 progeny counts were presented in Figure 4.17 and 4.18. The tested botanical extract of *P.dodecandra* leaf and root has shown reduction of F_1 progeny emergence. The results showed that crude extract of *P.dodecandra* leaf has strong reduction of F_1 progeny in *S. zeamais* and *T.castaneum* by 100% at 150mg/mL and 4.47% at 300mg/mL compared to root. All botanical treatments induced significant reduction in *P.dodecandra* F1 progeny emergence compared to the untreated control. Positive control of actellic gold^{TM} has 100% reduction of F_1 progeny emergence. as indicated in Figure 4.17 and 4.18

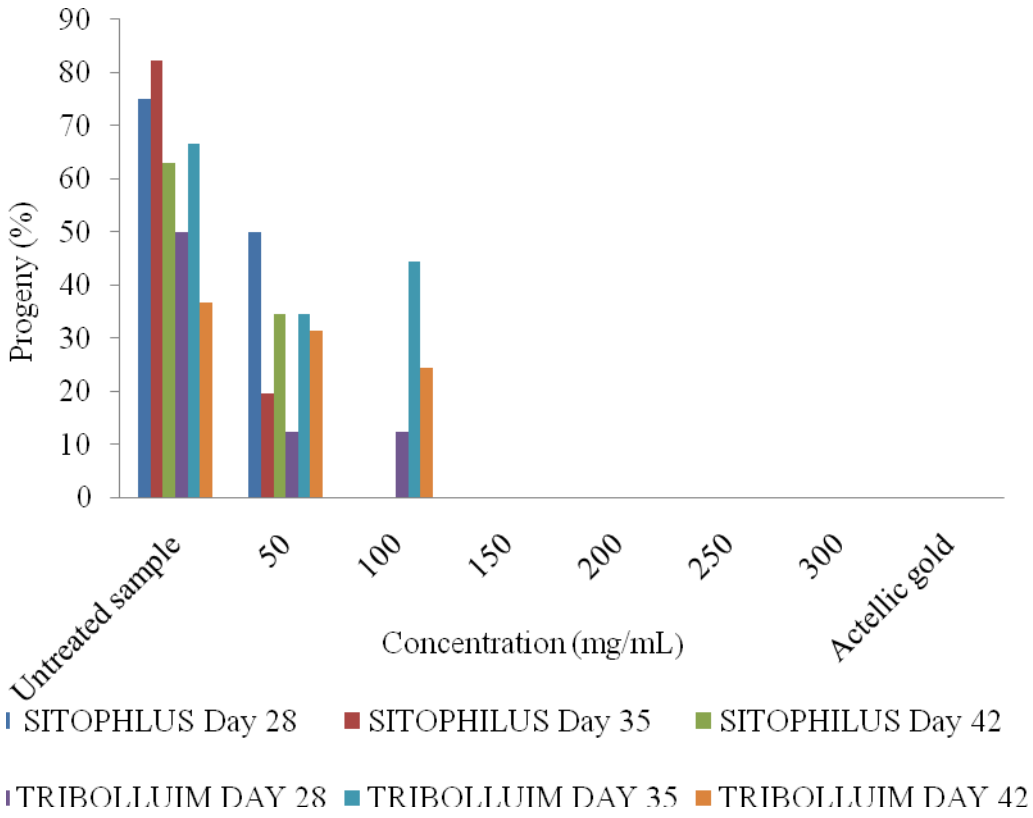

Figure 4.17: First Fillial Generations of *S. zeamais and T. castaneum* Pests in Maize applied with *P. dodecandra* leaf Extracts

The filial generation progeny decreased as concentration of *P. dodecantra* increased. The adult *S. zeamais* and *T. castaneum* F_1 progeny counts in maize grains treated with crude potential extract were insignificantly ($P > 0.05$.) affected by the plant part used and concentration applied. Results have shown a clear dose-dependent reduction in the adults *S. zeamais* and *T. castaneum* F_1 progeny count at concentration between 150 to 300 mg/mL of *P. dodecandra* leaf extract and concentration of 300mg/mL of *P. dodecandra* root extract. Leaf extracts at high concentration reduced F_1 progeny count to 0% (100% reduction) similar to synthetic insecticide, actellic gold^{TM} dust at 0.02% (w/w). Similarly, root extracts reduced F_1 progeny count to 6.67% (93.3% reduction) compared to the untreated control.

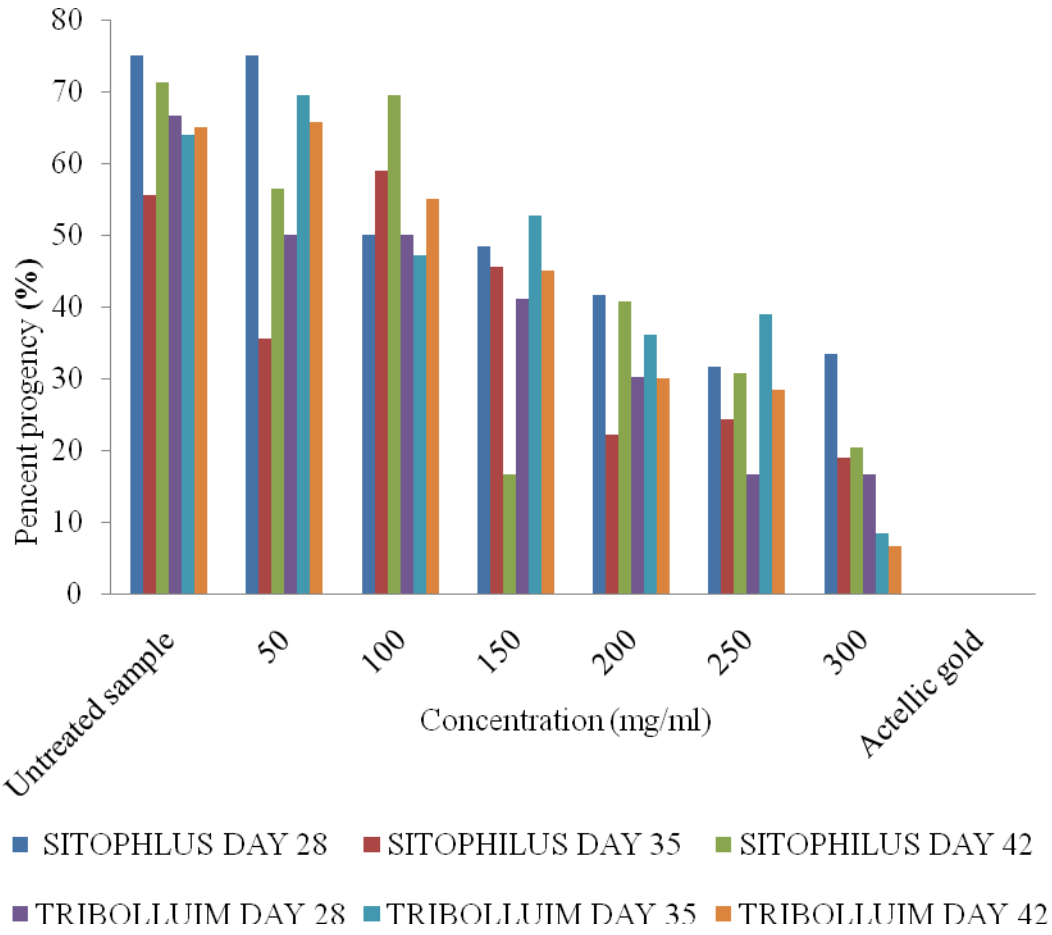

Figure 4.18: First Fillial Generations of *S. zeamais and T. castaneum* Pests in Maize Applied with *P. dodecandra* Root Extracts

The reduction of F_1 progeny emergence in the treated grains might be due to factors such as increased adult mortality, in addition to ovicidal and larvicidal properties of the tested *P. dodecandra* leaf and root extracts. This confirms the findings observed by Tapondjou *et al*., (2002) noted that dry ground leaves of plants can inhibit oviposition and subsequent progeny production of pests. The act of weakening of adults of maize pests by botanical extract may make them lay fewer eggs than normal leading to less hatchability to larvae and final metamorphosis to adults. Chebet, (2013) reported that the maize grain treated with *L. camara T. vegolii and A. indica* powder recorded 71.6%, 69.7% and 85.6% F_1 progeny reduction respectively. Different botanicals effectiveness at higher dosage to various insect pests have been reported by several authors (Avyaz *et al*., 2010; Sivakumar *et*

al., 2010; Adedire *et al*., 2011; Ileke and Oni, 2011; Mahmoud, et *al*., 2011; Fekadui, 2012).

4.4.5 Grains Quality

The result of treatment used and baseline of grains quality evaluation at start of the investigation (zero days) are presented in Table 4.4 and 4.5 respectively.

Table 4.4: Treatments Used for the Grains Quality Evaluation

Treatments	Description	Dosage
T1	Untreated grain sample (control)	0 mg/ mL
T2	Actellic gold^{TM} 2% dust	0.05 w/v
T3	Ethanol extract of *P. dodecandra* leaf	150 mg/mL
T4	Ethanol extract of *P. dodecandra* root	300 mg/mL

Table 4.5: Baseline Parameters for Grains Quality

Quality parameter	Value (± S.E, where applicable)
Grains moisture contents (%)	14.7 ±0.14
Insect damage (%)	00 ± 00
Seed viability (%)	86 ± 0.3
Grains odour	odourless
Grains colour	natural white with few yellow gains

4.4.5.1 Change in Moisture Content of Grains

The effects of botanical pesticide on the percent grain moisture content over a given duration of storage are presented in Figure 4.19.Generally; there was a slight increase in moisture content of maize treated with botanicals as compared to controls. This increase was however, significant ($P < 0.01$). This indicated that the extracts have little impact on the moisture content of the stored maize.

Result showed that the moisture content of treated and control maize grains were significantly influenced by duration of storage as indicated in Figure 4.19 and Table 4.4. Ogendo *et al,*. (2004) reported that percentage moisture content of maize grains treated with followed a more or less similar trend. Storage products are usually hydroscopic and as such they absorb or desorb moisture from the surroundings. As there was change in temperature of the environment, the moisture holding capacity of the air increases three times (Haines, 1991). When stored products release moisture to the surroundings during low humidity, moisture content of grains is depressed.

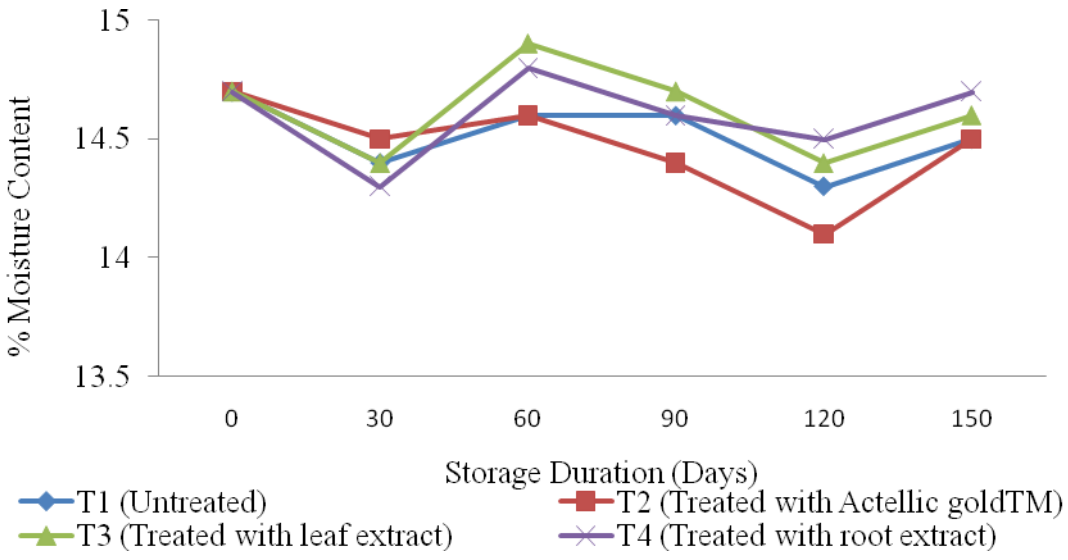

Figure 4.19: Effect of Biopesticide on Moisture Content in Stored Maize

When the relative humidity and air temperature vary, the maize grains moisture content is likely to vary (Ogendo, *at el*., 2004). This is justifiable particularly in the coastal tropics where ambient humidity and temperature tends to change throughout the year.

4.4.5.2 Change in Seed Viability of Grains

Percent germination was not significantly (P > 0.05) decreasing with storage duration for untreated maize grains, grains treated with actellic gold^{TM} and grains treated with botanical pesticides(Fig. 4.20). Germination decreased rapidly from 30 to 90 days for botanical treatments and stabilised at around viability index of 29% after 120 days. The decrease of percent germination of seeds treated with *P. dodecantra* extract dropped from 86% on day zero to around 30% on day 90 as compared to 73% of the actellic gold^{TM} standard. However, this decrease was not significant (P >0.05). In addition, there are no significant effects of treatment and storage duration by treatment interaction on the percent germination. Correlation analysis results showed that seed viability was weakly correlated with the grain moisture content, botanical treatments and storage duration. Grains treated with leaf extracts had the lowest germination viability after 90 days.

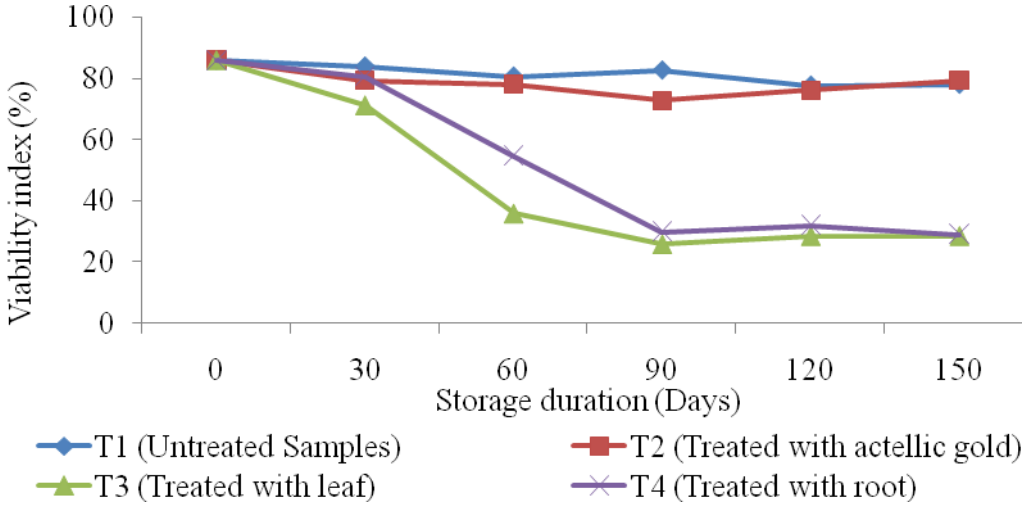

Figure 4.20: Change in Viability of Maize Grains Treated with *P. dodecandra* Extract During Storage

The plant extracts have ability to affect the germination or growth of other plants due to phytotoxins that can alter the physiological and biochemical processes changes occurring inside the seeds (Maryam, 2010). For example, the percentage germination decreased from 90% of control to 50% when the crop seeds were treated with neem oil extract for 4 hours at 0.08mg/mL due to botanical compounds (Rath *et al*., 2013). Studies indicate that decrease in the number of germinated seeds of various crops after treatment with botanical extracts is attributed to phytotoxins which is common in many plant species (Araar, 2009; Babu *et al.,* 2014; Mishra, 2014; Mendez and Mannuel, 2014). However, some extracts have no effects on seed germination (Pandely *et al.,* 1986; Kasa and Tadese, 1995; Ogendo *et al.,* 2004).

4.4.5.3 Grains Damage during Storage

The level of grains damage was higher in untreated (control) maize grains than those treated with actallic gold^{TM} and *P. dodecandra* leaf and root (Fig. 4.21). Results showed that there was no significant effect ($P > 0.05$) of treatment storage duration, and treatment by storage duration interaction on the level of grains damage on the stored grains. The percent of grains damage increased as duration of storage increased. This can be explained probably by the volatility or biodegradability of bioactive constituent over time. However, there was still a considerable reduction in grains damage (5%) for the 5 months of storage. Unlike the treated grains, the untreated maize grains suffered high percent grains damage compared to grains treated with actellic gold^{TM} 2% dust and the botanical treatments (Figure 4.21).

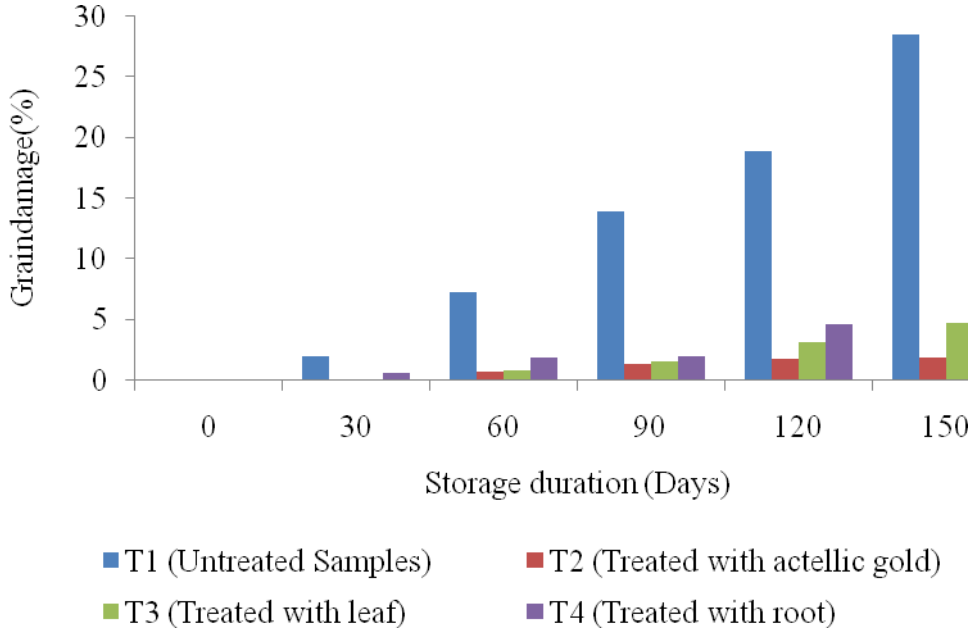

Figure 4.21: Effect of *P. dodecandra* Extract on Percent Grains Damage on Stored Maize Grains

The result of grains damage percent during storage of untreated maize grains, grains treated with *P. dodecandra* and those treated with actellic gold^{TM} were presented in Figure 4.21. The present study is in agreement with a study done by Ahmed *et al*., (2014) who observed that *Callosobruchus maculates* seeds treated with extracts of neem seeds for six month reduced insect damage of the grains from about 95% to around 23%. A similar study by Ogendo *et al*., (2004) reported that maize grains treated with *L. camara* and *Tephrosia vogelii* Hook for five month had small insect damage percent of up to 0.7%. The relatively high insect damage observed in this study could be due to high humidity that always prevails in coastal areas, which agrees well with the observation by Schulten, (1996). Also, Musundire *et al*., (2015) reported maize grains in storages treated with plant powders on *E. grandis* and *T. minuta* can reduce grains damage and live insect infestation with no adverse effects on seed germination, colour and odour.

4.4.5.4 Effect of Botanical Treatment in Colour and Odour Change in Grains during Storage

Figure 4.22 shows the mean evaluation scores of colour for treated samples with *P. dodecandra* extracts as compared to those treated with actellic gold^{TM} 2% dust and untreated maize grains. Result showed that there was no significant ($P > 0.05$) effect of storage duration, quality assessors, botanical treatments and storage interaction on the colour of the stored maize grains. The Wilcoxon scores (rank sum) increased with increased storage duration. The effect is higher in *P. dodecandra* leaf treatment compared to root treatment. This could be due to chlorophyll materials available in the leaves. Eliminating the chlorophyll may reduce this effect.

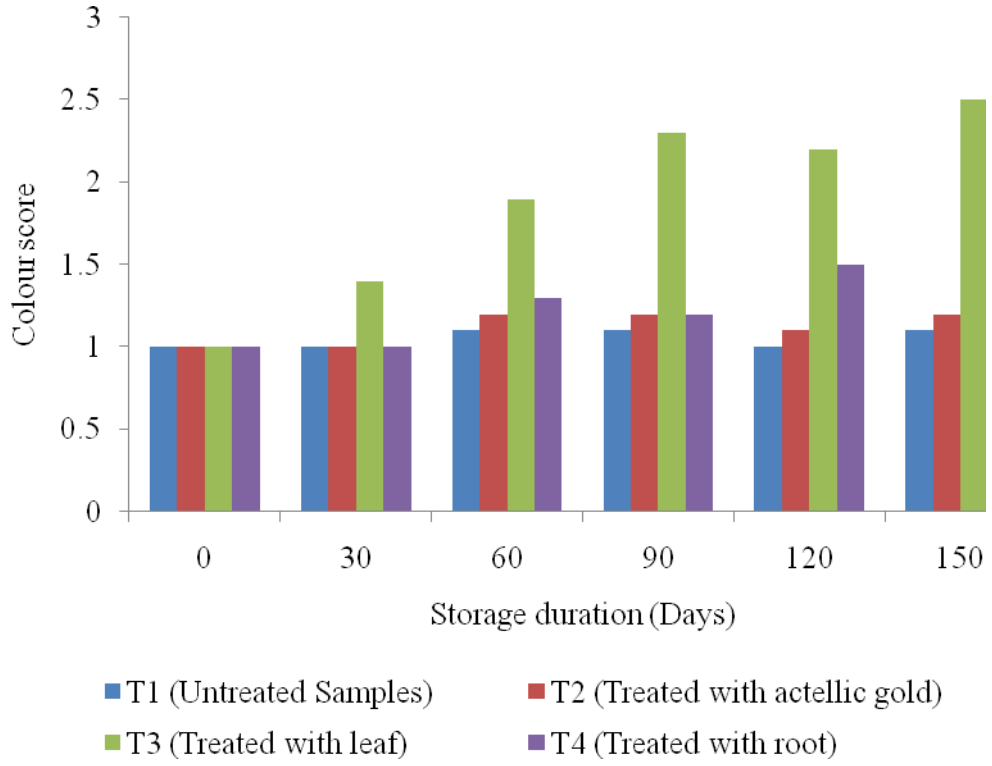

Figure 4.22: Effect of *P. dodecandra* Leaf and Root Extracts in the Mean Scores for Grains Colour over 150 days of Storage

The results of change in odour during storage of untreated maize grains, those treated with actellic gold^{TM} 2% dust and those treated with ethanol extract of P. *dodecandra* leaf and

root are presented in Figure 4.23. Result showed that there was significant ($P < 0.05$) storage duration, quality assessors, botanical treatments and storage interaction effects on the odour of the stored maize grains. The Wilcoxon scores (rank sums) increased at 30 days of storage for botanical treatment and decreases to 120 days of storage. The effect is higher in *P. dodecandra* leaf compared to root treatment.

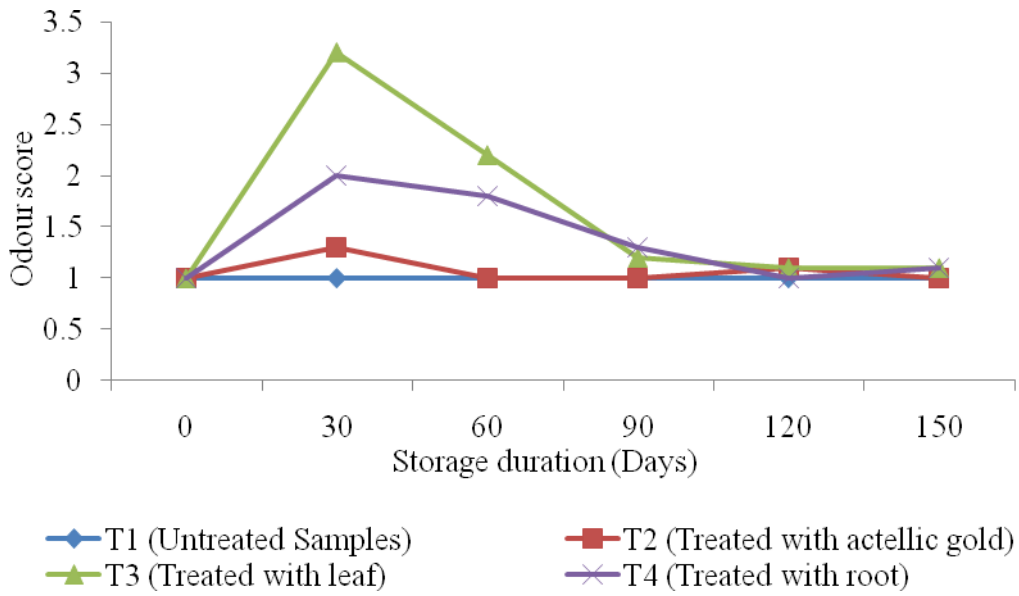

Figure 4.23: Mean Evaluation Scores for Maize Grains odouras Affected by *P. dodecandra*

The Wilcoxon scores for colour quality increased as duration of storage increases while in odour parameter scores increases from zero days to 30 days, then decreases to 90 days and became constant until 150 days of storage for the treated maize grains with *P. dodecandra* extracts. The untreated and actellic gold^{TM} treated maize grains had slight changed of score during storage period. This implies that the concentration decreased with time, possibly due to the volatility of the bioactive compounds (Ogendo *et al.*, 2004). The evaluation results indicated that admixing maize with botanicals slightly changed grain colour and odour over the five months of storage similar to study by Ogendo, (2000) and Musundire *et al.*, (2015). This could be an indication that constituents from the botanical plant powders were not absorbed by the grains (Jayasekara *et al*., 2005; Ogungbite *et al*.,

2014). The deterioration of colour in maize grains are due to high relatively moisture, high relative humidity, storage period and respiration process in grains which develop growth of fungal such as *Aspergillus favus, Penicilliun* spp and *Fusarium* spp which produce mycotoxins especially aflatoxinis (Suleiman et al., 2013) . The high value for colour change was below 2.5 which indicate slight change, therefore is tolerable to local market.

4.5 Phytochemical Screening of Selected Plant Species

The summary of the phytochemical constituents of five selected plant species is presented in Table 4.6. The analysis has indicated the presence of widespread compounds like saponins, favonoids, alkaloids, sterols and tripenoids which determime the bioactivities of selected plant species. Anthraquinone and phabtanins were present only in *L. nepetifolia* (leaf) and *G. sylvestre* (root).

Table 4.6: Qualitative Analysis of Phytochemical Constituents of Selected Plant Species

	Phytochemical Constituents									
Plant species	Saponin	Alkaloid	Sterol	Tritepenoid and terpene	Phenol	Tannins	Flavonoid	Proteins and amino acid	Glycocide	Coumarins
P.dodecandra (leaf)	+++	++	+++	+++	+	_	++	_	++	_
P.dodecandra (root)	+++	_	+++	+++	++	_	+++	++	+	-
L.nepetifolia (leaf)	+	+++	++	+	++	++	++	++	++	+++
O. filamentosum (root)	+	+++	+++	+	_	+++	+++	+	+	++
C. geomentrum (root)	++	_	_	_	_	++	+++	++	+	_
G. sylvestre (root)	++	++	+++	+++	++	_	-	++	+	_

Legend:

(-): Absence of phytochemical compounds; the extract remains clears with no clears change. (+): The trace of phytochemical; faint changes against dark background. (++): Presence of phytochemical; definite colour change noticed. (+++): Intense presence of phytochemical; strong change noticed.

The phytochemical screening of the bioactive plant extracts has revealed the presence of alkaloids, proteins and amino acids, tannins, flavanoids, sterols, terpenes glycosides, phlobatanis, phenol, saponins and anthraquinone (Table 4.6). Tannins have been reported to have antibacterial, antifungal and antidiarrheal activities (Ahmad *et al.*, 2006; Nathathe and Ndip, 2011; Ogbonna *et al*., 2013; Ngulde *et al.,* 2013; Ahmad *et al.,* 2014). Secondary metabolites such as saponins, tannins, alkaloids and cardiac glycosides have been reported to treat diseases such as typhoid, haemorrhoids, impetigo and malaria (Okigbo *et al*., 2009; Ogbonna *et al.,* 2013; Adeogum *et al.,* 2014;). Anti-inflammatory effects have also been reported for these phytochemicals including flavonoids (Lui, 2003). Thus the observed activities could be associated with the presence of these phytochemicals in the bioactive extracts.

4.5.1 Tannins

Tannins were present only in *Ocimum filamentosum and Leonotis nepetifolia.* These may be responsible for antibacterial activity demonstrated by these plant extracts (Table 4.6). Previous studies have shown that tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis, they bind proteins and adhesion inhibit enzyme and complex with cell wall (Iqbal *et al.,* 2006). Tannic acid which is a mixture of gallic acid esters of glucose can be used as a topical preparation for cold sores (Heinrich *et al*., 2004). Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been associated with the presence of tannins (Haslam, 1996). One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam, 1996; Yadav and Agarwala, 2011). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, and cell envelope transport pro-

teins, they also complex with polysaccharide, that it's good antibacterial, antioxidant and antifungal activity (Brownlee *et al.,* 1990; Somkuwar and Kamble, 2013;).

4.5.2 Alkaloids

Alkaloids were present in most selected plant species tested such as *Gymnema sylverstre*, *Phytolacca dodecandra* leaf, *Ocimum filamentosum and Leonotis nepetifolia.* The presence of these compounds in the plant species may be responsible for the observed antibacterial and antifungal activity (Table 4.6). Studies have demonstrated that alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein, and membrane phosipholipids biosynthesis (Shelton, 1991). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine is an important representative of the alkaloid group and harmane, which is attributed to their ability to intercalate with DNA **(**Phillipson and O'Neill. 1987). Yadav and Agarwala (2011) reported that alkaloid have common biological properties such as cytotoxicity, analgesic, antispasmodic, and antibacterial properties. Saxena *et al*., (2013) reported in their review that alkaloids have many pharmacological activities including antihypertensive, antiarrhythmic effects, antimalarial activity, anticancer action, stimulant property, analgesis, and antimicrobial (antifungal and antibacterial) activity. This probably explains the reason why the plants containing this group of natural products displayed antibacterial, antioxidant and antifungal activity.

4.5.3 Sterol Glycocides

Sterols and sterol glycocides were present in all selected plants tested except *Cynoglossum geometrium* (Table 4.6). These may also be responsible for the observed antibacterial, and antifungal activity demonstrated by these selected plant species extracts. Earlier studies have shown that sterols posses antibacterial, antifungal, antimicotic activity and act as inhibitors

of tumor promotion *in vivo* (Yasukawa *et al*., 1991). Sterols were found to inhibit tumor promotion in two-stage carcinogenesis in mice and anti-inflammatory activity after topical application (Kasahara *et al*., 1994). Sterols exhibit inhibitory effect on HIV reverse transcriptase (Akihisa *et al*., 2001). Sheikh *et al.*, (2013) reported that cardiac glycosides are stimulant in body in case of cardiac failure. Cardiac glycosides have strong activity on the heart and have been used in the treatment of heart failure and have pesticidal properties (Okwute, 1992).

4.5.4 Flavonoids

Almost all selected bioactive plant extracts contained flavonoids in this study except *Gymnema sylverstre.* Their presence in the plant species may also be partly responsible for the observed activities (Table 4.6). Flavonoids being phenollic compounds are water soluble antioxidants and free radical scavengers which are capable of preventing oxidative cell damage and have strong anticancer activity (Okwu, 2004). Alan and Miller, (1996) reported the ability of flavonoids to scavenge hydroxyl radicals, superoxide anions and lipid peroxide radicals. Catechins (strong antioxidants), the most reduced form of the C_3 unit in flavonoid compounds occur in oolong green teas ,that is why they have important dietary additive significance in food (Kaufman *et al.*, 1999). Sheikh (2013) reported that flavonoids are good against oxidants, allergies, inflammation, platelet aggregation, microbial ulcers, hepatoxins, viruses and tumors. Harborne and William (2000) reported that flavonoids possess useful properties such as anti-inflammatory, estrogenic enzyme inhibition, antimicrobial, hypoglycemic, anti-allergic, antioxidant vascular and cytotoxic and antitumour activities. This forms the basis for use of these plant species by farmers in the management of human diseases.

4.5.5 Phenols

Phenols were present in *Gymnema sylverstre*, *Phytolacca dodecandra*, *Cynoglossum geometrium, and Leonotis nepetifolia* (Table 4.6). These compounds are reported to be toxic to microorganism (Savoia, 2012; Adeogum *et al*., 2014). Phenolic compounds are most ubiquitous groups of plants metabolites which posses biological properties including anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, natural anti-oxidant, cardiovascular protection, cell proliferation activities, improvement of endothelial function as well as inhibition of angiogenesis (Ali *et al.*, 2008; Yadav and Agarwala 2011). Sexana *et al.*, (2013) reported that phenol is a strong antioxidant. Phenol also increases bile secretion, anti-plasmodic, anti-depressant activities, anti-ulcer, purgative, stomachache, constipation, decrease blood pressure, cholesterol reduction; reduce lipid level and antimicrobial activities (Gibson *et al*; 1998; Mathai 2000; Sexana *et al.*, 2013).

4.5.6 Saponins

Saponins were present in all selected plants extracts as indicated in Table 4.6. These compounds are reported to be good candidates for treating fungal and yeast infections, so they serve as natural antibiotics, fight against infections and microbial invasion in the body (Sheikh *et al.*, 2013). Somkuwar and Kamble (2013) reported that saponins are traditionally used as detergents, pesticides, molluscicides and a foaming agent. Saponins are known to produce anti-inflammatory activity and erythrocyte haemolysis (Ngulde *et al.,* 2013). Triterpene saponins have been shown to be effective in diseases conditions such as asthma, diabetes, atherosclerosis and cancer (Khan *et al*., 2011; Ngulde *et al.,* 2013). Sodipo *et al.*, (2000) reported that saponins produce inhibitory effects on inflammation, precipitation and coagulation of red blood cell, formation of foams in aqueous solution, hemolytic activity, and cholesterol binding and bitterness properties. Many saponins are known to be antimicrobial (antifungal and antiviral), protect plants from insects attack, anti-protozoan, hypochlolesterolaemic and anti-carcinogenic (Takechi *et al.*, 1999; Saxena *et al.,* 2013). Thus,

these phytochemicals might be responsible for the healing properties of the Plants as claimed by the farmers and traditional healers in this study.

4.5.7 Terpenes

Terpenes or terpenoids were present in *Gymnema sylverstre*, *Phytolacca dodecandra*, *Ocimum filamentosum and Leonotis nepetifolia* (Table 4.6). This class of compounds is reported to be active against many bacterial infections (Barre *et al* 1997; Sheikh *et al* 2013). Somkuwar and Kamble (2013) reported that terpenoids are responsible for analgesis and inflammatory activities. Anti-feedants, anticarcinogenic, antimalarial, anti-ulcer and antimicrobial activities have also been reported (Dudareva *et al.*, 2004). The presence of these compounds in the plant species may contribute to the observed antibacterial, antifungal and anti-pest activity.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Medicinal and pesticidal plants are cheap, available, less side effect than synthetic medicinal and pesticidal to the rural subsitance farmers. Validation of herbs used in various localities in order to incorporate them into healthcare in humans, animals and also pests control in field and storage crops is essential. This move may not only preserve the scarce foreign exchange but also promote the spirit of plants protection and environmental conservation. Studies of natural product still represent a very promising way to discover pharmaceutical and pesticidal compounds important for the potential treatment of wide variety of diseases and pests in homes, field and storage crops, respectively.

5.1 Conclusion

This documented indigenous knowledge of the identified plant species provides a baseline foundation for further research for drugs and pesticides and therefore improving health and food production for poverty reduction. The documentation particularly among agro-pastoral communities will ensure that the knowledge is passed on from one generation to the next.

The study has established and documented the important medicinal, pesticidal, acaricidal and poisons plants used in Mbulu district and their use. The studied plant extracts demonstrated varying biological activities in different aspects. Further properties were indicated in their pesticidal activity against stored grains and other anti microbial activity partly based on their chemical constituency. Apparently, the associated effects on grain colour and odour, variation in the moisture content as well as seed viability index suggested a need of some knowledge to the users prior to the use of the botanicals. The botanical pesticide

(*P.dodecandra*) showed relatively high toxicity to the stored pests (*S. zeamais* and *T. castaneum*) than others. Botanical pesticides and storage duration influenced the moisture content of stored maize grains and reduced seeds viability. Therefore P. dodecandra is not suitable for storage of grains that will be used as seeds for growing. The *P. dodecandra* treatments caused only marginal changes in colour and odour which should have virtually no impact on the local market value of treated grains. In addition, *P. dodecandra* reduced grains damage to an appreciable extent.

All crude extracts of the five selected plant species under investigation exhibited concentration- dependent activity against both gram-positive, gram-negative bacteria, and fungal (yeasts and moulds). Highly pronounced antimicrobial activity was displayed against fungal (yeast and mould), which serves as a clear indication of the potentials of these extracts from selected plant species. Most selected plant species extracts were poor antioxidant activity in DPPH assay with exception of *P. dodecandra* roots.

Selected plant species screened for cytotoxicity against the brine shrimp, most species showed LC_{50} values less than 100µg/mL promising to be antitumor compounds. This study has further confirmed that these selected plant species contain phytochemical bioactive compounds.

5. 2 Recommendations

The current study gives a baseline information further research. The knowledge brought forward by this study includes the biologically active plant species of medicinal and pesti-

cidal importance available in Mbulu District. It also highlighted on the major groups of chemical compounds constituted in the species which may partly contribute to their potency efficacy. Furthermore, the common local uses of the species are well documented.

Apparently, knowledge gaps exist between the findings and the applications of the findings. The following are recommended for this baseline research;

i) Identify more factors that may affect the bioactivity of the plant species; extract solvents, part of plant, chemical constituents, co existence in equillibrium etc.
ii) Further studies of the bioactive plants from Mbulu District. Isolation purification and characterization of pure bioactive compounds for synthetic industrial uses.
iii) More tests on the pure compounds to establish the source of activity and hence structural modifiction of the compound for best activity.
iv) Promote the collaboration of the medical sector with the traditional practitioners for experience and knowledge sharing for promotion of the phytochemical compounds for use. It should note that although some plants are used locally their active doses for different treatments are uncertain.

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APPENDIX: List of Medicinal and Pesticidal Plant Species Screened and their Reported Local Uses in Mbulu District

Scientific name	Family name	local name	Part used	Pest/disease treated	Method of preparation
Acacia hockii	*Mimosoideae*	Narmo awak[1,a, y]	Bark	To treat bacterial diseases such as measles,gonorrhea, and vomiting food poison in stomach	Soak cold or hot water
Accacia milotica	*Mimosoideae*	Kantzi[1,4,a,y]	Roots, barks,	Roots are used to treat genitor urinary; barks are used as stimu-lants and tooth brush.	Powder, Soak in cold or hot water
Accacia seyal	*Mimosoideae*	Qarbu[1,4,a,y]	Barks, gum	Human medicine, tannin and dye	Powder, Soak in cold or hot water
Adansonia digitata	*Bombaceae*	Gendaryandi[1,a,y]	Root, barks	To treat malaria, goiter cancer and diabetes	Powder, Soak in cold or hot water
Albizia amara	*Mimosoideae*	Tsori[5,a,y]	Roots and bark	To wash clothes (pesticidal soap), Crop protection in fields (leaves)	Soak in cold or hot water or fresh
Annona cherimola	Annonaceae	Matopetope[1,2,a,x]	Green fruit, seeds leaves	Insecticidal in field crop and veg-etable, fruit and seeds to treat worms in animal and human, also are used in cancer treatment.	Powder, Soak in cold or hot water
Argemone mexicana	*Papaveraceceae*	Xaano dishimo[3,d,y]	Latex	To treat skin disease	Fresh stem
Azadirachta indica	*Meliaceae*	Mwarobaini[**1,2,a,z**]	Leaves and seeds	Crop storage, veterinary, medici-nal soap and medicinal over 40	Dry powder, soak in cold or

				diseases	hot water
Scientific name	**Family name**	**local name**	**Part used**	**Pest/disease treated**	**Method of preparation**
Azanza garckena	*Malvaceae*	Thaqay[1,4,a,y]	Fruit and bark	To treat diabetes	Powder and fresh fruit
Balanites aegyp-tiaca	*Balanitaceae*	Hhowi[1,2,c,y]	Fruit, bark	To kill snails host of bilharzia and roots are used to treat dirty urine	Soak in cold
Bridelia micrantha	*Euphobiaceae*	Fitselmo[1,3,a,y]	Roots and barks	Human medicine and to attracts caterpillars and birds	Juice and fresh plants
Capiscum genus e.g *Capsicum annuum*	*Solanaceae*	Pilipili[1,2, b,x]	Fruits	Army warm in field crop, culi-nary, and new castle treatment in chickens	Mix in water, soaking
Carica papaya	*Caricaceae*	Papai[1,2,a,z]	Leaves and seed	Field pesticide crops and to treat diarrhea in human , tenderizing of meat and Prophylaxis	Soak in cold or hot water
Carissa edulis	*Apocynaceae*	Titiwi[1,4,a,y]	Fruit , root	To treat gonorrhea, syphilis, uri-nary treatment, soap and typhoid in human.	Soak in cold and hot water
Cassia absus	*Fabaceae*	Mlutulutu[2,a,y]	leaves	potent against many pests in field crops	Soak in cold water and dry powder
Cassia sin neanda del	*Caesalpiniaceae*	Lalangidako [1,a,y]	Barks	Deworming, malaria skin disease and tapeworm	Soak in hot water
Catha edulis	*Celastraceae*	Warfi[4,a,y]	Leaves	Stimulant, and to treat asth-ma,coughs, stomach ache and	Fresh leaves

chest pains

Scientific name	Family name	local name	Part used	Pest/disease treated	Method of preparation
Chenopodium procerum hocha	*Chenopodiaceae*	Hhishhinsi[3,d,y]	Leaves	Insect repellent	Fresh leaves
Cmniphrora mekari Egl	*Burcraceae*	Hangatle[3,a,y]	Barks	To treat skin disease and UTI	Juice
Combretum molle	*Combretaceae*	Gendaamo[1,3,a,y]	Root and steam	To treat hookworm, snake bite, stomach pains, fever dysentery, leprosy and remove bad smell from milk container	Smoking, powder and soak in hot water
Commiphora Africana	Burseraceae	Naamo[2,b,y]	Roots, bark, fruits, resin, or gum Latex	To control ticks in animals	Soak in cold water
Croton dichogamus	*Euphorbiaceae*	Girgirmo[5,b,y]	Leaves and steams	Pesticidal plant for storage crop protection, brush teeth, UTI treatment in women	Dry powder or soak in cold water and hot water
Croton megalocarpus	*Euphorbiaceae*	Ayloi[1,a,z]	Barks	Purgative and smoke from firewood can irritate eyes	Soak in hot or cold water
*Cynaglossum geometriu*m Bak and Wright	*Boraginaceae*	Xaslslaamo[1,4,b,y]	Roots	To treat measles in cattle	Fresh roots or juice
Erthrina abyscinica	*Papilionoideae*	Qanqari[2,3,a,y]	Leaves,	To treat skin diseases and wound	Soak in hot wa-

			root, seeds and back	in human and cattle, also seeds are poison.	ter and dry powder
Scientific name	**Family name**	**local name**	**Part used**	**Pest/disease treated**	**Method of preparation**
Euclea divinorum	*Ebenaceae*	Minighiti[4,b,y]	Roots , stem and barks	Tooth brushes, worms in humans and gonorrhea	Fresh plant parts
Euphorbia tirucali	*Euphorbiaceae*	Manyari[1,2,a,z]	Leaves	Field pesticide crops, ticks control and veterinary to treat new castle in poultry and remove placenta in animals	Soak in cold water
Galinsoga parviflora	*Asteraceae*	Qalmirboo[2,b,y]	leaves	Pesticdal plants in field crops	Soak in cold water, juice
Grewia bicolor	*Tiliaceae*	Lagangir awak[1,a,y]	Bark and roots	To treat intestinal problems, syphilis chest pain and colds	Decoction, juice
Gwewia villosa	*Tiliaceae*	Amu[1,c,x]	Fruit and seeds	Fruit to treat tapeworm and fruit to increase body immunity in human and animals	Juice and prepared as food
Gymneme sylvestre	Asclepladaceae	Tsiti[1,c,y]	Root and leaves	To treat gonorrhea and control snail in water	Powder
Hoslunqlia opposita	*Labiatae*	Elwahhoki[5,d,y]	Latex	To treat skin disease	Juice
Julbernardia globiflora	*Caesalpionideae*	Hhewassi[1,c,y]	Barks	To treat cough and snake bite in human	powder
Kigelia Africana (K	*Bignoniaceae*	Mangaffi[1,c,y]	Fruits,	To treat asthma, decrease weight,	Juice

aethiopum)			leaves, and bark	new castle in poultry and unripe fruit is poisons	
Scientific name	**Family name**	**local name**	**Part used**	**Pest/disease treated**	**Method of preparation**
Lantana camara	*Verbenaceae*	----------[5,b,y]	Leave	To control weevil in maize and sorghum	Powder
Leonotis nepetifolia	*Labiatae*	Giro[3,d,y]	Leaves	To treat ring in skin	Juice
Nicotiana genus eg *Nicotiana tobaccam*	*Solanaceae*	Tumbaku/ , Tumati[2,b,x]	Leaves and flow-er	Field and storage crop pesticide, ticks control and veterinary to treat bloat in livestock, Prophy-laxis	Soak in cold water, juice
Ocimum filamento-sum Forssk	*Lamiaceae*	----------[1,b,y]	Leaves and roots	To treat amoebiasis and stomach ache	Soak in water
Osris lanceolata	*Santalaceae*	Kipaa't[1,3,y]	Bark and roots	Perfume, and to provide a tonnic blood	Powder and smoking
Phytollaca dodec-andra	*Phytolaccaceae*	Thoxi[2,a,y]	Berries, leave, root	Crop protection in stores and field, and to treat ring in human	Soak in water, fresh
Plectranthus cf ele-gans Britton	*Labiatae*	Ulwandi [5,d,y]	Leaves	To control bedbug and viroboto	Powder
Plumbago zeylanica	*Plumbagina-ceae*	Xaanoumang [I,b,y]	Roots	To treat measles in cattle	Soak in cold water or juice
Rhus natelensis	Anacardiaceae	Sioo[1,3,a,y]	Leaves and seeds	Neutralize poison, body lotion, purgative , stomach ache, abor-tion and pain killer in the external body	Soak in hot wa-ter, fresh

Scientific name	Family name	local name	Part used	Pest/disease treated	Method of preparation
Rhus natelensis	Anacardiaceae	Sirongi [4,b,y]	Barks and leaves	Tooth brushes	Fresh plant parts
Salvadora persica	*Salvadoraceae*	Mswaki/Fura[4,a,y]	Roots, bark, stem	Tooth brushes which make mouth clean and prevent tooth decay	Powder fresh root or steam
Securdaca longi-pendunculata	*Polygalaceae*	Furudangw[1,b.y]	All part	Poisons, snake bite, cough, abor-tifacient, relive toothache, anti-tapeworm, malaria, anti-pain,antibacterial and Brussels	Powder, soak in water , juice
Solanum anguivi	*Solanaceae*	Avitamo[3,b,y]	Fruits	To control Jigger or sand fleas in children	Juice
Solanum incanum	*Solanaceae*	Hhangali[1,4,b,y]	Roots , and fruits	Roots to treat stomach com-plaints and fruits to treat teeth	Fresh roots and soaking in hot water
Syzgium guineense	*Myrtaceae*	Matharmo[1,a,y]	Leaves, roots, barks	Anti-worm in animals, tannin, dye and fruit	Powder, soak in water, juice, and chewing
Tagetes minuta	*Asteraceae*	Bangi[1,2,b,y]	leaves	Insect repellent in crop storage	Fresh plants
Tanacetum cinerar-iae folium	*Aseraceae*	Pareto[2,b,x]	Flower	Accarides to treat ticks in live-stock, control of worm in young plants in the field crops	Soak in cold water, juice
Tephrosia pumila (lam) pers	*Papilionaceae*	----------[1,d,y]	Roots	To treat measles in cattle	Soak in cold water or juice
Tephrosia vogelii	*Fabaceae*	Tupatupa[2,3,a,z]	leaves	Pest repellent, fishing and crop	Dry powder,

				protection in store and ticks control	soak in cold water
Scientific name	**Family name**	**local name**	**Part used**	**Pest/disease treated**	**Method of preparation**
Terminalia sericea	*Combretaceae*	Bukuumo, Sarakwi[1,3,b,y]	Leaves, barks and roots	Barks are red dyes, leaves are used to treat stomach ache, diarrhea, snake bite and wounds	Juice
Terminalla stuhlmaniii Engl	*Combretaceae*	Katalaisha [1,a,y]	Barks	Amoebiasis	Soak in cold water
Trema orientalis (T.guineansis	*Ulmaceae*	Slaragahhi[1,a,y])	Leaves and barks	Antidote to poison, tannin , cough and deworming	Juice
Vangueria Madagascariensis	*Rubiaceae*	Baranqu[1,a,y]	Roots and back	To treat worms in animals	Soak in cold water
Vangueria infausta	*Rubiaceae*	Baranqu[1,a,y]	Leaves and roots	To treat Malaria, pneumonia and worms in animals	Juice, fresh
Warburgia ugandensis (W.salutaries)	*Canellaceae*	Sakwenay [1,a,y]	Leaves, roots, barks fruits, twigs	Leaves, bark, fruits and young shoot can be used in curries. Roots are used anti-pain killer.	Juice, fresh
Withania somnifera (L) dunal	*Solanaceae*	Tloqomo[3,b,y]	Roots	To treat fresh wound	Juice
Xanthoxylum chaly beum	*Rutaceae*	Morongi[1,4,a,y]	Leaves, roots barks and fruits	To treat carving malaria, typhoid,Bruises,and romantics’	Juice and fresh

Scientific name	Family name	local name	Part used	Pest/disease treated	Method of preparation
Ximenia afra (x-americeana var caffra)	*Olacaceae*	Maayangu[1,3,a,y]	Fruit , roots and leaves	To treat cough, malaria, psycho-logical illnesses, cancer, and smooth inflamed eyes	Juice, and fresh
Ximenia americana	*olacaceae*	Tahhamanto, tarantu[3,4,b,y]	Seeds, leaves, roots and barks	Oil for soap, body and hair oil and softening leather	Soak in cold or hot water
Ziziphus mucronata	*Rhamnaceae*	Ghalyandi[1,3,4,a,y]	Leaves and roots	To treat boils and skin infection, stomach and chest com-plaint,increase blood and poul-tices	Juice, soak in cold water and fresh

Key:

1. **Methodb of adminision**:1 = Oral, 2 = Spraying , 3 = Spotting, 4 = Chewing 5 = Mix
2. **Type of plant species**: a = Tree, b = Shrub, c = Climbing and d = Herb
3. **Sustainability**: x = Cultivated, y = Wild and z = Both cultivated and wild