ANTIFUNGAL ACTIVITY AND PHOTOSENSITIZATION EFFECT OF PLANT EXTRACTS ON AFLATOXIN PRODUCING *Aspergillus flavus* (RAPER AND FENNEL)

NJOKI LOISE MUMBI.

B. SC (BIOLOGY), UNIVERSITY OF NAIROBI

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY)

SCHOOL OF BIOLOGICAL SCIENCES

COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES

UNIVERSITY OF NAIROBI

2016

DECLARATION

I Njoki Loise Mumbi declare that this thesis is my original work and has not been presented for the award of a degree in any other University.

Signed: ……. Date: ……

Miss Njoki Loise Mumbi

Supervisors

This is to certify that this thesis has been submitted for examination with our approval as supervisors

Signature: …… Date: …….

Prof. Sheila Okoth
School of Biological Sciences
University of Nairobi
P. O Box, 30197-0100
Nairobi

Signature: …… Date: ……..

Dr. Peter Wachira
School of Biological Sciences
University of Nairobi
P. O Box, 30197-0100
Nairobi

DEDICATION

To my husband Kennedy Mwangi, daughter Sharlene Wambui, mother Mary Njoki and sibling Joseph Githua. Thank you for your encouragement, support and for believing in me. God bless you.

ACKNOWLEDGEMENTS

My sincere gratitude goes to the Almighty God for giving me the strength to undertake this study. This study was successful because of material and moral support from my mentor and supervisor Prof. Sheila Okoth. My sincere recognition and appreciation goes to her and my supervisor Dr. Peter Wachira for his continued guidance, advice and support in the course of this study. I am grateful to the University of Nairobi for offering me a chance to advance my studies through a scholarship. My gratitude goes to the technical staff especially the botanist Mr. Patrick Mutiso, technical staff from School of Biological Sciences, Centre for Biotechnology and Bioinformatics, Chemistry and Veterinary departments from the University of Nairobi for the various important roles they played in my research. I am very grateful to my fellow students in the Msc. Microbiology class 2014 for every kind of support that they gave me. More gratitude goes to my entire family and friends for their support, encouragement and patience.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

AF: Aflatoxin

AFB1: Aflatoxin B1

AFB2: Aflatoxin B2

AFG1: Aflatoxin G1

AFG2: Aflatoxin G2

AFSA: Alliance for Food Sovereignity in Africa

ANOVA: Analysis of variance

CLSI: Clinical and Laboratory Standards Institute

CFU: Colony forming units

DMSO: Dimethyl sulphoxide

FAO: Food and Agriculture Organization

GRAS: Generally Recognized as Safe

HPLC: High performance liquid chromatography

IARC: International Agency for Research on Cancer

JECFA: Joint FAO/WHO Expert Committee on Food Additives

NCCLS: National Committee for Clinical Laboratory Standards

PDA: Potato dextrose agar

PDB: Potato dextrose broth

MIC: Minimum inhibitory concentration

TLC: Thin Layer Chromatography

WHO: World health organization

ABSTRACT

Aspergillus flavus is a saprophytic fungus exhibiting weak parasitism. Some strains produce aflatoxins and are known to be aflatoxigenic while those that do not produce aflatoxins are atoxigenic. Aflatoxins are mycotoxins produced mostly by *A. flavus* in cereals, nuts and spices. In Kenya, aflatoxins have been a major problem in some semi arid areas of Eastern, Central and Coastal regions. They have caused death in both human and livestock. Aflatoxins are known to be mutagenic and carcinogenic.

Many strategies are being tried to fight aflatoxin contamination of food and feed. These methods include cultural practices, biological and chemical control. However, none of these methods have fully succeeded in controlling aflatoxin contamination. This study was carried out to test plant extracts as an alternative source for controlling aflatoxin contamination. Using ethno botanical knowledge, six plants namely; *Ocimum lamiifolium*, *Solanum aculeastrum*, *Lippia kituensis*, *Syzygium cordatum* and *Prunus africana* were collected from the Central region of Kenya. *Spinacia oleracea* was obtained from the local market. Efficacy for aqueous (distilled water) and organic (dichloromethane: methanol 1:1 v/v) extracts of the medicinal plants was tested against two identified strains (UONV017 and UONV003) of toxigenic *A. flavus*. The evaluation of antifungal activity of the medicinal plant extracts at different concentrations 600 mg/ml, 450 mg/ml and 300 mg/ml was done using disc diffusion method. The organic plant extracts displayed antifungal activity that depended on concentration and fungal strain variations. The aqueous plant extracts did not show significant ($P \leq 0.05$) antifungal activity. *Solanum aculeastrum* organic extracts had the highest zone of inhibition compared to other plants against both strains of *A. flavus*. The mean inhibition diameter was 18.50 ± 0.71 mm for strain UONV017 and 11.92 ± 0.94 mm for strain UONV003. The plant extract with second highest antifungal activity was *Syzygium cordatum* with a mean inhibition diameter of 17.00 ± 1.26 mm

against strain UONV017 and 10.27 ± 0.32 mm against strain UONV003. The highest concentrations (600 mg/ml) achieved the highest zones of inhibition. *Prunus africana* had the lowest activity at 300 mg/ml against strain UONV003 with a mean inhibition diameter of 3.00 ± 0.90 mm. For both strains, *S. aculeastrum* and *S. cordatum* at 600 mg/ml compared favorably with the standard antifungal control (Apron star 250mg/ml). Minimum inhibitory concentrations (MIC) were determined for organic extracts using broth (PDB) microdilution method. Organic extract of *S. aculeastrum* against strain UONV017 had the lowest MIC at 25 mg/ml. Photosensitization was carried out by reacting the photosensitizer (plant extract) in solution with toxigenic *A. flavus* spores with visible light (420 nm; 10, 20 and 40 minutes) and determining the viability of fungal spores after the reaction by counting the colony forming units. Samples reacted with light had significantly ($P \leq 0.05$) lower numbers of Colony Forming Units (CFU) compared to the controls which were not reacted with light. Both aqueous and organic extracts exhibited photosensitization activity. For aqueous extracts against *A. flavus* strain UONV017, *Solanum aculeastrum* was the most effective at a concentration of 600mg/ml and the longest time duration of 40 minutes with 2 CFU's; the no light control had 35 CFU's. For the aqueous extracts against *A. flavus* strain UONV003, *Syzygium cordatum* was most effective with 3 CFU's followed by *Solanum aculeastrum* with 4 CFU's at both 600 mg/ml and 450 mg/ml for the highest time duration (40minutes); the controls had 34 and 43 CFU's respectively. The most effective organic extract was *Syzygium cordatum* with 4 CFU's against *A. flavus* strain UONV017 and *Solanum aculeastrum* with 9 CFU's against *A. flavus* strain UONV003; controls had 51 CFU's and 69 CFU's respectively. Duration of exposure to the light was directly proportional to the inactivation of toxigenic spores; treatments exposed to light for longer durations had the least number of CFU's. Findings of preliminary phytochemical tests indicated

that both aqueous and organic plant extracts contained several bioactive compounds namely; saponins, tannins, flavonoids, terpenoids, glycosides and alkaloids. Organic extract of *Spinacia oleracea* contained all the bioactive compounds named above. Findings showed medicinal plant extracts in this study can control conidia viability hence with further development can control toxigenic fungal spread

CHAPTER ONE

1.1 Background of the study

Aspergillus flavus is a fungus which is ubiquitous in nature and constantly encountered in agricultural soils (Hua, 2013). They are saprophytic, exhibit weak parasitism and contaminate a range of cereals, nuts and spices which serve as food and feed (Klich, 2007). *Aspergillus flavus* have two morphotypes; S strain and L strain. Communities with the S strain of *A. flavus* have been associated with acute mycotoxin poisoning (Probst *et al*., 2011). Mycotoxins are secondary metabolites produced by fungi and they are toxigenic (Pitt, 2000). Aflatoxins are a type mycotoxins produced by some species of *Aspergillus*. Types of aflatoxin produced by *Aspergillus* are B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and M1 (AFM1) (Yin *et al*., 2008). Aflatoxins B1 and B2 are the main aflatoxins produced by *Aspergillus flavus* (Okoth *et al*., 2012). *Aspergillus flavus* are the main fungi producing aflatoxin (Hua, 2013).

Aflatoxins are known to have carcinogenic, mutagenic and lethal effects and have been classified as class 1 poisons by the International Agency for Research on Cancer (IARC) (Pitt, 2000). Aflatoxicoses which is an infection caused by ingesting aflatoxins was first reported in Kenya in 1982 (Probst and Cotty, 2010). More outbreaks were officially reported in 2001, 2004, 2006 2008 and 2010. The out breaks occurred in four districts which are adjacent to each other in Eastern and Central provinces. Three hundrend and seventeen cases were reported during the 2004 aflatoxin contamination outbreak with 125 fatalities (Shephered, 2008). In the 2006 outbreak, there were 53 fatalities (Schmale and Munkvold, 2009). In 2010 the government declared 2.3 million bags of maize unsafe for consumption due to toxicity related to aflatoxin

contamination and 3 cases of death were reported (Njuguna, 2010). Kenya is the only African country with recurrent outbreak of acute aflatoxicoses (CDC, 2004).

Aflatoxins occur in a variety of food products which are highly consumable worldwide and these include: maize, groundnuts, cottonseeds, spices (Okoth *et al*., 2012). Aflatoxins also contaminate mouldy feed hence dairy products like milk, meat products and eggs (Rustom, 1997). Estimates show that 25% of the world's food is contaminated by mycotoxins including aflatoxins each year as reported by FAO (Thippeswamy *et al*., 2014). In Africa, maize which was introduced 500 years ago is a staple food. Maize (*Zea mays*) therefore becomes the primary avenue through which aflatoxins get into the human system causing carcinogenic and other lethal effects (Probst, 2010).

Aflatoxin contamination occurs majorly in tropical and subtropical regions where climatic conditions and storage methods favour growth of fungi (Thippeswamy *et al*., 2014). High humidity, temperature and drought conditions which are natural occurrences favour aflatoxin production by *A. flavus* (Wagacha and Muthomi, 2008). Kenya being part of sub-saharan Africa which has high humidity, temperature and drought seasons hence becomes susceptible to numerous fungal growths. Agronomic and socio-economic factors also contribute to increased fungal proliferation. Socio-economic status of most sub-saharan Africans leave them few options of choosing high quality over low quality food products (Hell and Mutegi, 2011). Kenya being a developing country has great concern in regard to aflatoxin effects because 4.5 billion people in developing countries are exposed to uncontrollable amounts of aflatoxins (Shukla *et al.,* 2008*).*

Methods being tested in aflatoxin control include physical methods like aeration or rapid drying (Munkvold, 2003), chemical synthetics and pesticides (Bassapa, 2009) and biological control using other microorganisms like yeast, bacteria or atoxigenic strains *Aspergillus* (Reddy *et al*., 2010). However, these methods have their shortcomings; they are costly and others like chemical synthetics are hazardous to health due to chemical residues which also cause environmental pollution (Kohl, 2011). Regulation policies have been set to control aflatoxin contamination. United States Food and Drug Administration has set a thresh hold for aflatoxin levels in food stuff to 20 parts per billion (ppb). Japan and European Union (EU) however has set the thresh hold at below 4 parts per billion (Hua, 2013). Policies are not well enforced in developing countries including Kenya due to corruption and poverty levels which lead to malnutrition and lack of food security (Cotty *et al*., 1994).

These shortcomings leave a gap because the problem of aflatoxin contamination is unsolved among subsistence farmers. As an alternative to these methods scientists have gained interest in use of natural extracts from plants as a control for aflatoxin contamination. This is because natural extracts from higher plants are biodegradable, renewable in nature and safe to human health (Kaigongi *et al*., 2014). Plant extracts come in different forms which may be oils, crushed leaves, roots, stem, bark and flowers.

African communities in the early times used different plant parts prepared in different ways as cure for diseases and injuries for themselves and their livestock. Many drugs and pesticides used in the current society are derived from plants known to have ethno-botanical and ethno-medical

properties (Kaigongi *et al*., 2014). Aromatic substances in plants specifically secondary metabolites like alkaloids, flavonoids, saponins, glycerides and tannins, are able to protect plants from invaders such as fungi, bacteria and nematodes (Murugan *et al*., 2013). According to World Health Organisation (2001), 80% of African and Asian communities rely on traditional herbal medicines for primary healthcare. This is because herbal medicines are safer and cheaper compared to synthetic medicines (Paranagama *et al*., 2003, Kaigongi *et al.,* 2014). Estimates show that among all traditional medicine, only less than 10% have been evaluated for pharmacological uses. This brings in the significance of doing research on plants bioactive agents hence the medicinal value. This could boost the health and economic status of Kenya due to the safety, availability and affordability of herbal or traditional medicines.

Natural plant extracts from leaves and other parts have also been tested against toxigenic molecules by passing them through visible light a process called photosensitization. The bioactive compounds in plant extracts are excited and activated by light in presence of reactive oxygen molecule. The reactivated molecules react with toxigenic molecules destroying and killing them (Temba *et al*., 2016; Al-fawwaz and Al-khaza'leh, 2016). Visible part of blue light has been reported to be the most effective part of the light spectrum to stimulate phototrophism in plants (Lucca *et al*., 2012). The strategy has also been tested *in vitro* against *A. flavus* using extracts called curcumin (*Curcuma longa*). Temperature and pH were maintained constant and findings indicated there was significant reduction or destruction of the toxigenic fungal spores (Temba *et al*., 2016). In using photosensitization as a method to control toxigenic fungi, it is recommended that the photosensitizer in this case plant extracts should be safe for human

consumption, cost effective and easily activated using light of an appropriate wavelength (Ormond and Freeman, 2013).

The aim of this study was to evaluate locally known medicinal plant parts from Central region of Kenya for their antifungal activity, photosensitization effect on growth of toxigenic *A. flavus* and phytochemical composition.

1.2 Problem statement

Aspergillus flavus infest a wide range of cereals which are staple foods in Kenya like maize, sorghum, millet and groundnuts (Mburugu *et al*., 2015). Toxigenic *A. flavus* produce mycotoxins called aflatoxins which contaminate these cereals. Aflatoxins also contaminate feed hence products like meat, milk, cheese and eggs get contaminated when animals consume the aflatoxin contaminated feed (Lizzaraga- paulin *et al*., 2011, Herzallah, 2013). Aflatoxins have been detected in maternal and cord blood specimens in 14 out of 83 Kenyans tested. Breast milk samples tested in Ghana, Sudan and Kenya were detected to be contaminated with aflatoxins. (Maxwell *et al*., 1989). A cross sectional study carried out in Kenya revealed aflatoxin contamination in 78% of human blood serum samples (Yard *et al*., 2007). Outbreaks of aflatoxin contamination have been reported in Kenya since 1981 with more than 200 deaths and fatalities and a case of aflatoxicosis is reported each year (Okoth *et al*., 2012).

Aflatoxin production by *A. flavus* is influenced by aeration, moisture, temperature and substrate (Wagacha and Muthomi, 2008). Aflatoxin contamination occurs in warm humid climates, and drought affected regions (Hell and Mutegi, 2011). The moisture and climatic conditions in some Central and Eastern regions of Kenya which have subtropical climate favour aflatoxin production hence ingestion (Cotty and Jaime- Garcia, 2007). Adverse climatic conditions in Kenya are pre

disposing factors for aflatoxin contamination. Farmers are unable to harvest their crops at the right time and the rains may coincide with the harvesting season causing crops in the field to be mouldy hence easily colonized by fungi which lead to aflatoxin contamination (Waliyar *et al*., 2014). It is recommended that cereals should be dried quickly to 10- 13% of moisture levels before storage to avoid aflatoxin contamination (Hell *et al*., 2008). This is difficult for Kenyan farmers due to adverse changes in climate (Mburu *et al*., 2014).

A greater period of food storage increases the chances of aflatoxin contamination. In Kenya, small scale farmers use the traditional storage facilities made of wood, thatch or mud which are covered with iron roofing sheets or thatch and placed on raised surfaces. Farmers also store their grains in polypropylene bags which are not airtight. These facilities are prone to excessive heat, high humidity, poor aeration and insect damage leading to fungal infestation on the stored food products hence aflatoxin contamination. It is difficult to introduce new storage facilities like cement or metal bins that could lower aflatoxin contamination due to their high cost which the small scale farmers cannot afford (Hell and Fandohan, 2010).

Small scale farmers posses misinformation on how to identify aflatoxin contamination since it is odourless, tasteless and colourless hence not easily detectable (Ramesh *et al*., 2013). Currently there is no simple and cheap method for testing aflatoxins and the small scale farmers cannot afford the available testing methods due to complexity and high cost (Unnevehr and Grace, 2013). With these shortcomings, the problem of aflatoxin contamination mostly in Kenya and developing countries remains persistent.

1.3. Justification and significance of the study

Different strategies are being tested to control aflatoxin contamination which has been associated with liver damage and cancer in human and animals (Muluvi *et al*., 2015) and mainly contaminate the staple foods in Kenya (Okoth *et al*., 2012). Biological control involves use of microorganisms like yeast, atoxigenic fungi (aflasafe) and bacteria. However these methods has shortcomings in that some of the microorganism's survival is dependent on specific climatic conditions while others may alter the natural environment by negatively affecting non target organisms or accumulating to harmful levels (Ehrlich, 2015). Cultural methods like storage and drying practices have also been used but they are not efficient due to the drastic changes in climate that lead to increased moisture and temperature levels hence increased fungal proliferation (Munkvold, 2003). Chemical control is hazardous hence raising safety concerns, it lowers nutritional quality of the food products due to the build up and non biodegradable chemicals and it is also expensive (Kohl *et al*., 2011).

These strategies therefore, have not eliminated aflatoxin contamination. Some extracts from medicinal plants with antifungal activity have proved antifungal activity against *A. flavus* (Kiswii *et al.,* 2014). Reaction of plant extracts activated by light against toxigenic *A. flavus* and other toxigenic molecules has also proved to be a viable alternative in management of aflatoxin contamination (Lucca *et al*., 2012, Temba *et al*., 2016). Plant extracts are biodegradable and easily available (Kaigongi *et al*., 2013) and this makes them a safer and cheaper alternative method to control aflatoxin contamination. In this study, the antifungal, phytochemical and photosensitization activities of *Ocimum lamiifolium, Solanum aculeastrum, Prunus africana,*

Lippia kituensis, Spinacia oleracea and Syzygium cordatum extracts from Central Kenya were screened to provide an alternative control strategy for aflatoxin contamination.

1.4. Research objectives

1.4.1. General objective

To determine antifungal activity and photosensitization effect of novel plant extracts on growth of toxigenic *A. flavus.*

1.4.2. Specific objectives

i) To evaluate effect of selected medicinal plant extracts on growth of toxigenic *A. flavus.*

ii) To evaluate effect of selected plant extracts in combination with light (photosensitization) on growth of toxigenic *A. flavus.*

iii) To identify bioactive agents of the selected plant extracts.

1.5. Research hypotheses

i) The extracts of selected medicinal plants have an effect on growth of toxigenic *A. flavus*.

ii) The selected plant extracts in combination with light have an effect on growth of toxigenic *A. flavus.*

iii) The selected plant extracts have bioactive agents that may inhibit growth of toxigenic *A. flavus.*

CHAPTER TWO: LITERATURE REVIEW

2.1. *Aspergillus* species

Aspergillus is a saprophytic fungus. It belongs to the genus *Aspergillus* which has more than 200 diverse species (Carris *et al*., 2012). Some species of *Aspergillus* are economically important like *Aspergillus oryzae*, *Aspergillus niger, Aspergillus sojae* and *Aspergillus awamori*. The four species produce various useful enzymes which are useful in fermentation. These four *Aspergillus* species have been used in the food industry for long hence they have been included in the Generally Recognized As Safe (GRAS) list of the United Stated Food and Drug administration (Machida *et al.*, 2008). On the contrary, *Aspergilli* are known to produce some secondary metabolites which are toxigenic to human, plants and animals. Some of *Aspergillus* known to produce toxigenic secondary metabolites include: *A. flavus, A. parasiticus* and *A. niger* which produce mycotoxins (Dorner, 2004). The mycotoxins are known to be carcinogenic and mutagenic (Van *et al*., 2007). Aflatoxins are key mycotoxins produced by *Aspergillus* and they are widely spread in tropics and sub tropics (Hua, 2013). Pietrio Antonio Micheli a priest and botanist, in 1729 described asexual spore heads (conidiophores) of a number of common moulds. Heads of some moulds showed rows of spores radiating from a globular central surface which resembled aspergillum which was an instrument used to sprinkle holy water on his congregation hence the origin of the name *Aspergillus* (Moselio Schaechter) (Machida and Gomi, 2010).

Aspergillus flavus belongs to Kingdom Fungi, Phylum Ascomycota, Order Eurotiales, Class Eurotiomycetes, Family Trichocomaceae, Genus *Aspergillus* and Species *flavus*. It is an imperfect filamentous fungus which is an opportunistic pathogen. It causes invasive and non-invasive aspergillosis in humans, animals and insects (Yu *et al*., 2008). *Aspergillus* is also an

opportunistic pathogen of plants which is of key importance as it produces aflatoxins. Aflatoxin which is highly carcinogenic is associated with drought stressed cereals and oilseeds like maize, cottonseeds and peanuts (Diener *et al*., 1987). *Aspergillus flavus* is commonly a soil fungus which causes it to be a key consideration in agriculture because of its production in plants which are at the bottom of foodchain. This makes it harmful to livestock and human beings (Klich, 2007). The lifecycle of *A. flavus* is explained by its saprophytic growth in plant remains like maize kernels that are infected and remain in the soil contributing to the primary inoculum. That primary inoculum is then spread through water, wind, insects and bird damage (Kiswii *et al*., 2014).

2.2. Health and economic significance of *Aspergillus flavus*

Foliar and aerial environment are the main environments in which *A. flavus* is adapted (Martins *et al*., 2000). It is dominant in crops like corn, cottonseed and nuts (Diener *et al*., 1987). Occurrence of aflatoxins in foods and feeds is influenced by environmental conditions related to storage and these are controllable. External factors like climate and internal factors like strain specificity, strain variation and instability of toxigenic properties are hard to control (Hussein and Jeffrey, 2001). Types of aflatoxin produced by *Aspergillus* are B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and M1 (AFM1) (Yin *et al*., 2008). Aflatoxins B1 and B2 are the main aflatoxins produced by *Aspergillus flavus* in Africa (Okoth *et al*., 2012). In an experiment, corn samples were analysed for aflatoxin and over 90% of the samples contained AFB1 and AFB2 which are produced by *A. flavus* (Diener *et al*., 1987).

Production of secondary metabolites called mycotoxins (aflatoxins) by toxigenic *A. flavus* makes the strain to be of great importance. This is because worldwide contamination of foods and feed

with mycotoxins is a very significant problem (Dorner, 2004). Aflatoxins have significant effects on plants, animals and humans including illnesses and economic losses. In animals and human there are both acute and chronic effects which depend on the species type and susceptibility of the species involved. Monogastrics are highly susceptible compared to ruminants because rumen microbiota in ruminants is able to degrade mycotoxins to some extent (Hussein and Jeffrey, 2001). Before 1960, *A. flavus* was known to produce aflatoxins named "*Aspergillus flavus* poisons" which caused poisoning and deaths in thousands of poultry, pigs and trout. The main cause of death in trout was liver cancer and the effect of aflatoxins to human beings is through transfer of the poisons from feed to food. Aflatoxin B1 is a potent hepatocarcinogen in rats and trouts and is known to induce carcinoma at rates below 1µg/kg^-1 (Cotty *et al*., 1994). Aflatoxin B1 has recently been proven to be genotoxic (Zain, 2011). Aflatoxin B1 is the most common in mammals and it is very hepatoxic causing liver damage, carcinogenic and mutagenic effects. In dairy cattle AFB1 and AFB2 are transformed into hydroxylated aflatoxin M1 and aflatoxin M2 found in milk produced by animals which have fed on aflatoxin contaminated feeds (Van *et al*., 2007; Zain, 2011). High levels of aflatoxins in feed can cause animal death leading to economic loss. Lower concentratins of aflatoxins have some other effects on domestic animals like decreased immunity, low productivity, increased healthcare and veterinary costs and funds invested in research and application to reduce occurrence of aflatoxins (Cotty *et al*., 1990).

Well enforced policies, regulations and abundant supply of food protect human populations from aflatoxin effects in developed countries. Unfortunately, in developing countries poverty leads to low food supply and regulation policies are missing or not well enforced hence human exposure to aflatoxin ingestion and contamination is a common occurrence (Cotty *et al*., 1994). Liver

cancer in human beings has been associated with relatively high levels of aflatoxin exposure through consumption of affected food like maize or peanuts (Cotty *et al.,* 1994). People working in industries that involve production and processing of commodities with aflatoxin contamination may be infected through inhalation (NTP, 2011). Dermal routes are also a possible entry of aflatoxins into the body systems. Generally, diseases caused by aflatoxins are called aflatoxicosis (Van *et al*., 2007).

In plants which are the base of the food chain and the route through which aflatoxins get to food chain, economic losses are of key importance. The infected food crops are disposed and also the control methods like chemical and pesticides as well as regulation of environmental factors may be costly which leads to great economic loss (Reddy *et al*., 2010)

2.3. Methods of aflatoxin control and management

Useful advances have been made in aflatoxin detection methods and control strategies. Significant advances have also been made on the biochemistry, genetics and regulation of aflatoxin production. Biosynthetic pathways of aflatoxin, clustering of biosynthetic genes and functions of these genes have been elucidated in great details. Despite all these advances, the problem of aflatoxin contamination especially in developing countries inclusive of Kenya is not yet solved (Bhatnagar *et al*., 2006). Mouldy food substances are key to aflatoxin contamination but non-mouldy and raw materials are also contaminated by aflatoxins (Magan and Aldred, 2007; Lizzaraga *et al*., 2013). Occurrence of aflatoxins is facilitated by insects transfers especially wasps, flies, bees and birds to foods where spores germinate, mycelium is produced and aflatoxins are excreted (Lizzaraga *et al*., 2013). Aflatoxin contamination also occurs during food processing. Specific foods like maize kernels, peanuts and almonds have proved to be a

suitable media for growth of aflatoxin producing *Aspergillus*. However, fruits and vegetables if contaminated contain very small amounts of aflatoxins. Some naturally mouldy foods like jams, potato chips and crips and pickles show no occurring amounts of aflatoxins (Krogh, 2013).

Many management and control strategies are being tested on aflatoxins. Some management methods used include cultural practices like drying to reduce moisture contents, development of resistant varieties, selecting appropriate dates for planting and harvesting susceptible crops depending on the climate of the area, using recommended practices to control insects that feed on plants increasing aflatoxin occurrences, crop rotation and use of irrigation practices to reduce effects of inadequate rainfall which promotes aflatoxin occurrences (Paranagama *et al*., 2003). Aflatoxins are deeply embedded in the kernels so simple washing is not a possible means of removal. However, use of polar solvents like alcohols and ketones for extraction to technically remove aflatoxins is possible. Heating however has no effect on aflatoxins. Biological control, biotechnological control, chemical methods and regulatory policies have also been used (Basappa, 2009).

Synthetic pesticides are the chemical control that has been widely used in control of toxigenic *A. flavus*. Examples of these pesticides include mixture containing propionic acid as the active ingredient, fumigant phosphines or dust formulations like pirimiphos methyl. However, fungicidal treatment has not shown great success in large scale application. Oxidizing agents readily destroy aflatoxins; hydrogen peroxide and ammonia have also been effectively used in oil seeds (Goldblatt, 1971). However, worldwide use of some synthetic pesticides has several disadvantages like high cost of the pesticides, handling precautions which if not well followed

may cause some hazards, risk to human health, long degradation which leads to environmental pollution. Synthetic pesticides are also known to harm and kill non-target organisms like pollinators which also lead to reduced productivity. With awareness of all these negative effects, there is need to offer alternative safer methods to control aflatoxins (Paranagama *et al*., 2003).

Genetic approaches have been developed as they can lead to development of commercially resistant varieties. It has been reported that seed coat for cotton seed called hard seed is able to resist growth of aflatoxin producing *Aspergillus* unlike the cotton seed without the hard coat which is considered susceptible (Goldblatt, 1971). Regulation policies have also been used to control risk of aflatoxins. Basically, regulations are based on known toxic effects. Currently, aflatoxins considerd most significant are B1, B2, G1, G2 and M1. Regulations that relate to protection of consumer against ingestion of aflatoxins have been set in many countries. Several factors determine the decision making process of setting the limits or regulations. Some of these factors include toxicological data available, occurrence of data and detailed knowledge about data sampling and analysis. By the end of the year 2003, about one hundrend countries which covers about 85% of the world's population, had set regulations or limits for aflatoxins in food (Van *et al*., 2007).

Regulations set to control aflatoxin contamination are mainly based on scientific opinions of authoritative bodies like FAO, WHO, Joint Expert Committee on Food Additives of the United Nations (JECFA) and the European Food Safety Authority (EFSA). Adequate sampling and analysis has some requirements which puts high demand on some organizations as well for example the European Standardization Committee (Van *et al*., 2007). These regulations are only

operated successfully in developed countries hence the developing countries like Kenya are still far from eradicating the aflatoxin ingestion and exposure problem (Van *et al.*, 2007).

Biological control which involves the use of other microorganisms is also being tried. It is considered safer for environment and human health unlike the chemical methods (Hua, 2013). Biological control being very promising has led to use of several organisms in control of aflatoxin producing *Aspergillus* species mostly *A*. *flavus* and *A*. *parasiticus*. These include bacteria, yeast and non-toxigenic strains of the causal organism *A. flavus* (Reddy *et al*., 2010; Hua, 2013). Many field successes have been achieved by applying non-toxigenic strains of *A. flavus* to the soil with crops that are susceptible like maize, peanuts and cotton. The applied strains occupy the same ecological niche as the toxigenic strains so they compete and exclude them when crops are susceptible to infection (Dorner, 2004; Yin *et al*., 2008)). Bacteria like *Bacillus subtilis, Lactobacillus* species and *Pseudomonas solanecearum* isolated from the non-rhizosphere of maize soil have been proved to lower aflatoxin production, accumulation and contamination by toxigenic *A. flavus* (Nostro *et al.*, 2000). These achievements were in laboratory experiments. Yeast species like *Pichia anomala* and *Candida krusei* have also shown great promise as biocontrol in laboratory tests against toxigenic *A. flavus*. Bacteria and yeast having been tested under laboratory conditions, more tests in the field are required to determine their success and efficacy in the field (Hua *et al.,* 1999).

2.4. Natural plant extract as a control strategy for toxigenic *Aspergillus flavus*

Use of natural plant extracts to control toxigenic *A.flavus* is also a promising method. Plant extracts have been proved useful in development of eco friendly antifungal agents (Thippeswamy *et al*., 2014). This is because natural plant extracts have no harmful chemicals,

have low or selective toxicity to animals and human beings and less environmental hazards hence widely accepted. Plant extracts from medicinal plants have been used for long to control many diseases (Kaigongi *et al*., 2013). The plant kingdom has the most biologically active compounds that can offer resistance against pests and other infectious agents like toxigenic fungi (Razzaghi and Rai, 2013). In Kenya, many medicinal plants are still in use today where knowledge about the effectiveness of the extracts is passed from one generation to the next through word of mouth. The antimicrobial agents in these plants if extracted and tested are able to work against toxigenic strains including aflatoxins (Kiswii *et al*., 2014). Plants have a wide diversity; despite some natural plant extracts being tested against aflatoxin producing *A. flavus*, many more plants within our localities which have high antifungal activity have not yet been exploited in the aflatoxin control strategy (Kaigongi *et al*., 2013).

Plant extracts may include oils, leaves, stems, flowers and bark. Natural plant extracts with proved success against aflatoxins occur worldwide and in Kenya the natural extracts have also been used. Saponins from tropical trees and plants have useful antifungal activities which work effectively on human diseases. Plants from the tropics therefore, are greatly investigated to find out more useful saponins. Biological and chemical methods are applied in parallel to attain these saponins. Biological activities include use of simple bioassays to test activity of extract of specific plants. Chemical screening involves taking the extracts through high performance (HPLC) liquid chromatography coupled with mass spectral analysis (Hostettmann *et al*., 1996).

With the role of food protection more important and challenging than before, there is a great need to seek efficient measures to control aflatoxins. Both qualitative and quantitative measures

need to be taken. Several natural plant extracts have been tested against aflatoxin producing fungi or *Aspergillus*. For instance essential oils extracts from fresh leaves of *Ocimum gratissimum* have been tested against aflatoxin producing *A. flavus* in stored peanut (*Arachis hypogaea*). The minimal inhibitory concentration of essential oils was found to be 7.5 µl/ml for *A. flavus* and *A. parasiticus*. The essential oil was proved to have strong fungicidal and inhibitory effects to aflatoxin production (Adjou *et al*., 2013)

Some medicinal plants were selected for bioactivity analysis in Tanzania. Extracts of *Cineraria grandiflora, Pavonia urens, Marratia fraxinea* and *Clutia abyssinica var. usambarensis* were made using ethyl acetate, methanol, cold water and boiling water .The extracts exhibited significant levels of antimicrobial activities (de Boer *et al*., 2015). In India, hexane, ethyl acetate and methanol extracts of 45 medicinal plants were tested for antifungal activity. Two known antifungal agents were used as control experiments. Most extracts inhibited more than four fungal strains. Of all the 45 tested plants, a wide spectrum of bioactive compounds was detected in specific plants like; *Punica granatum, Azima tetracantha, Toddalia asiatica, Cassia fistula* and *Cinommomum verum* (Duraipandiyan and Ignacimuthu, 2011).

In Kenya several medicinal plants have been tested using disc diffusion method and proven to exhibit antifungal activities. Effects of baobab (*Adansonia digitata*) extracts were tested on vegetative growth and aflatoxin secretion of *A. flavus* and *A. parasiticus*. Different fruit extracts of baobab fruit extract (1.5, 3, 5 and 7%w/v) and essential oil (0.5, 1, 3 and 5%v/v) were used. The fruit extract of baobab inhibited total aflatoxin production with up to 20.4-68.5% for *A. flavus* and 11.9-69.1% for *A. parasiticus*. Inhibition for aflatoxin B1 production ranged from

29.9-79.2% and 13-68% for the two strains respectively. Highest inhibition of total aflatoxin and aflatoxin B1 secretion were shown by the essential oils extract. Fruit extracts and essential oils of *Adansonia digitata* are proved potential biocontrol and biopreservative of food products against toxigenic *Aspergillus* species (El Nagerabi *et al*., 2013).

Inhibition capacity of leaf extracts of *zimmu (Allium sativum L.*Allim cepa*) against *A. flavus* was tested *in vitro*. Seventy three percent (73%) inhibition of *Aspergillus flavus* growth was exhibited. Medicinal plants like *Solanum nigrum (solanaceae*), *Chenopodium album (*Chenopodeceae*), Curcuma longa (*zingiberaceae*), Acalypa indica (*euphorbeaceae*)* and *Eucalyptus globulus (*myrtaceae) have all been tested and their antifungal activities proven. Their aqueous leaf extracts were prepared by grinding 10 grams of the leaves with 10 ml of sterile distilled water and filtered through two layers of muslin cloth. The extract was centrifuged at 7500 grams for 20 minutes and the supernatant made upto 10ml with distilled water. The extract was then sterilized and tested against toxigenic *Aspergillus* species and showed some favourable degree of aflatoxin inhibition (Sandosskumar *et al*., 2007).

A study was also conducted on acetone extracts of seven common species and different plant parts found in South Africa. The extracts were tested for antifungal activity against some fungi species like *Aspergillus, Fusarium, Penicillium, Trichoderma, Phytophthora, Colletrichum* and *Pythium*. The plant species used were; *Cestrum laevigatum* (flowers and leaves), *Nicotiana glauca* (flowers, leaves and seeds), *Solanum mauritianum* (flowers and leaves), *Lantana camara* (flowers, fruits and leaves), *Datura stramonium* (seeds), *Ricinus communis* (leaves) and *Campuloclinium macrocephalum* (leaves and flowers). All extracts proved moderate to good

inhibition activities in all the fungi species tested. The minimum inhibitory concentration (MIC) was ranging from 0.08 mg/ml to 2.5 mg/ml. In all extracts, leaf extracts were more effective than seed and flower extracts (Mdee, 2009).

Extracts (aqueous and organic) of plants like *Lippia javanica, Amaranthus spinosus, Tagetes minuta* and *Vigna unguiculata* were tested for antifungal activity against four fungi species; *A. flavus, A. parasiticus* and two *Fusarium* species. Dried powder parts of the aerial parts of these plants were extracted with hexane, dichloromethane, water and ethanol. Their antifungal activity was tested using serial microdilution assay. Findings were recorded every day over 120 hours and apart from the water extracts all the others showed significant inhibitory activity especially against *Fusarium*. *Aspergillus* species showed no inhibition but conidium formation was stimulated on plates treated with extracts in visual comparison with those growth controls. Hence, chemical constituents of these plant species if developed can be suitable fungal controls hence aflatoxin controls (Thembo *et al.*, 2010).

2.5. Photosensitization mediated by plant extracts against toxigenic *Aspergillus flavus*

Photosensitization involves use of plant extracts to kill toxigenic cells; it is also a potential control. Photosensitization occurs when a photodynamic agent (photosensitizer) is activated by light energy of an appropriate wavelength causing cellular injury or cell inflammation. Some plant extracts have acted as photodynamic agents (Motimer and Ronaldson, 1983).

Photodynamic inactivation is a new light based technique which offers a promising alternative control method against mycotoxigenic fungi. The technique involves light hitting the photosensitizer molecule in this case the plant extract. The molecule becomes excited and is

reactivated from singlet to triplet state and it directly reacts with the toxigenic molecule or passes its energy to molecular oxygen which also becomes reactive and reacts with the toxigenic molecule and kills it (Temba *et al*., 2016).

Extracts from *Curcuma longa* were investigated in a study as a control on spores of toxigenic *A.flavus.* In the study, the plant proved to be an effective photosensitiser when combined with visible light at an appropriate wavelength (420 nm) for varying time durations. The study was conducted *in vitro* and *in vivo* where *A. flavus* spores were treated with different photosensitiser concentration and light dose. The treated samples showed reduced count of fungal spores as compared to the untreated samples (Temba *et al*., 2016).

Increasing tolerance to the available fungicides has developed the need of new strategies to control pathogenic fungi (de Menezes *et al*., 2014). Antimicrobial photodynamic is a promising alternative antifungal discovery; it can be used to control fungal contamination and its consequences in animal hosts or fungi in the environment (Al-fawwaz and Al-khaza' leh, 2016). Exposure of the photosensitiser (plant extract) to light of an appropriate wavelength initiates a photochemical reaction leading to production of reactive oxygen species such as peroxides and singlet oxygen leading to nonspecific oxidative damage; this causes the subsequent binding and death of the fungal cells without significant harm to the host (de Menezes *et al*., 2014). In comparison with available fungicides commonly used, the multiple and variable targets of reactive oxygen species reduce chances of selecting tolerant microorganisms. Photosensitization has a key advantage in that unlike many conventional fungicides that kill only metabolically

active cells, it is able to kill both metabolically active and dormant structures such as conidia (de Menezes *et al*., 2014).

Some studies have reported use of antimicrobial photodynamic activity with different photosensitisers to kill fungi of several genera (Tardivo *et al*., 2005). These include *Aspergillus*, *Candida*, *Cryptococcus*, *Metarhizium* and *Trichophyton*. Reports on the photodynamic inactivation of plant-pathogenic fungi are not common; most of these studies were performed by using natural compounds as photosensitisers (Towers and Hudson, 1997).

A study was carried out where viability *Penicillium digitatum* conidia was tested using blue light and a dye Erythrosine (ERY) acting as a photosensitiser. Blue light alone or the photosensitizer alone did not reduce the viability of the non germinated conidia as compared to the conidia viability control. The control was comprised of no light and no photosensitiser treatment. However, non-germinated conidia treated with light and photosensitiser significantly reduced colony forming units (CFU) by 40 and 70% with blue light of 80 and 100 J cm_2 respectively compared to control (Lucca *et al*., 2012). Natural plant extracts have these dyes and other compounds which may act as photosensitiser against toxigenic *A.flavus* hence providing an alternative safer control strategy.

2.6. Antimicrobial activity of selected medicinal plants

2.6.1. *Ocimum lamiifolium*

Ocimum lamiifolium belongs to the family Labiatae. It is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicines to treat different diseases like

upper respiratory infections, diarrhea, headache, skin diseases, coughs and conjuctivis. *Ocimum lamiifolium* is an erect, hairy and perennial; several feet in height. In Ethiopia leaves are squeezed and extract used to treat coughs, colds and skin infections. In Kenya the leaf extract is used as a disinfectant and insecticide (Runyoro *et al*., 2010).

Oils of Ocimum *basilicum* and *Ocimum gratissimum* from different leaves in Kenya have been tested for antifungal activity against *Fusarium* species and they proved to be effective. Oil from leaves and flowering tops of *Ocimum basilicum* from Sagan contained linalool (95%). Flowering parts and leaves from Yatta. Samples contained mainly camphor (32.6% and 31% respectively) and linalool (28.2% and 29.3% respectively). Eugenol was the main component in *O. gratissimum* leaves from both Yatta and Sagana. Flowering parts had less eugenol. Oils from both regions showed some antifungal activity against the *Fusarium* species. The activity was attributed to the presence of the compound eugenol (Dambolena *et al*., 2010).

2.6.2. *Solanum aculeastrum*

Solanum belongs to the family Solanaceae. It shows a variety of activity attributed to the presence of saponins and steroidal alkaloids. Analysis with thin layer chromatography (TLC) and fast atomic bombardment mass spectrometry, using crude aqueous and methanol extracts of berries and root bark of *Solanum arundo* and *Solanum aculeastrum* showed presence of glycosides and glucoalkaloids. They showed molluscidal activity (Kariba, 2000).

Crude extracts of dichloromethane, methanol and aqueous extracts of *Solanum incanum* (fruits) showed 40% antifungal activity to human pathogenic fungi (Hussein *et al*., 2007). *Solanum tomentosum* has also been tested for antimicrobial activity. Methanol, acetone and water extracts

of the leaves were used. Growth inhibition was determined using agar dilution assays against bacterial and fungal strains. Though the activity of extracts on the test fungi was generally low, methanol extracts showed suppression to the growth of fungi with inhibitory percentage ranging from 47.22% to 50.56% in *Aspergillus Niger* and *Fusarium oxysporum (*Aliero and Afolayan, 2006).

Solanum aculeastrum has been used to treat human and animal disorders especially cancers and stomach disorders in South Africa. Traditional Zulu community used the fruit washed and boiled in herbal medicine to treat cancer, toothache and ringworms. Fruit and leaf extracts were investigated for antimicrobial activity against five fungal strains. The methanol extracts inhibited the growth of fungi with inhibitory percentage ranging from 60.26%-100% and 56%-100% in *A. flavus* and *Penicillium notatum*. The water extracts were effective in inhibiting growth of *Penicillium notatum* (69.89%) and acetone extracts inhibited growth of *A. flavus(* non toxigenic) *(*100%) and *Penicillium notatum* (64.81%) (Koduru *et al.,* 2006). In Somali, crushed filtrate of *Solanum arundo* fruits is massaged to treat itch and scabies. Seeds are soaked in oil which after cooling is applied to the teeth as an analgesic. In the Kenyan society, roots are pounded and soaked in water or chewed and used as cure for fever. The Rendille people of Kenya apply fruit juice on wounds of camel and sheep (Gurib- Fakim, 2008).

2.6.3 *Lippia kituensis*

Lippia belongs to the family Verbenaceae. *Lippia* is a genus of flowering plants. They are aromatic due to their essential oils (Stashenko *et al*., 2008). Leaves of certain *Lippia* species like *Lippia graveolens* can be used as a culinary herb (Pilau *et al*., 2011). Other therapeutic functions of genus *Lippia* include remedy for gastrointestinal and respiratory disorders, antimalarial

(Kariuki *et al*., 2016), anti-inflammatory, sedative and hypertensive effects. Ethanol extracts of *Lippia* leaves or multiflora were used to treat sorghum (*Sorghum bicolor)* which was infected with *A. niger.* After treatment with Lippia extracts, sorghum seeds had high level of germination both in root and shoot hence proving the antifungal activities of *Lippia multiflora* (Anjorin and Iheneacho, 2008).

Findings of Camphor isolated from *Lippia kituensis* indicated that it is a stong repellant against maize weevil (Kosgei, 2014). In central Kenya, methanol extracts of fresh leaves of *Lippia kituensis* showed inhibition of 16.66 mm against *Candida albicans* (Omwenga *et al*., 2011). *Lippia kituensis* has also been used to cure joint pains in Eastern part of Kenya (Wambugu *et al*., 2011)

2.6.4 *Syzygium cordatum*

It belongs to the family Myrtaceae. It is a medim sized tree (6-20) m in height. Its biophysical limits are an annual rainfall of (750-1200) mm and altitude of (0-1800) m. The plant is native in Swaziland and many African counties like Gabon, Zambia, Tanzania, Uganda and Kenya. The hypoglycemic activity of leaf extracts of *Syzygium cordatum*, have been tested using non-diabetic and streptozotocin induced non diabetic rats. Oral administration was used as the method of administration. The study proved that leaf extracts of the *Syzygium cordatum* had compounds that were effective in mild diabetes mellitus and glucose tolerance impairment (Musabayane *et al.,* 2005).

In some places the roots and bark are also boiled and the decotion used as a remedy for indigestion and giddiness; the leaf extract has been used as treatment for diarhoea (World Agro

forestry Centre). Crude methanol and water extracts of 32 medicinal plants including *Syzygium cordatum* were screened *in vitro* for antifungal activity against *Candida albicans* standard strain. The results proved that the extracts contained therapeutic compounds with potential antifungal activity (Steenkamp, 2007). However, the individual antifungal activity of *Syzygium cordatum* has not been tested. Essential oils from *Syzygium cordatum* leaves and bark were screened and found to have various bioactive compounds which may have antifungal properties (Challanavar, 2011).

2.6.5 *Prunus africana*

It belongs to the family Rosaceae (Bii *et al*., 2010). Its local English name is red stinkwood. The local name in central Kenya is 'Muiri'. It is native to the tropical forest of sub-saharan Africa and Madagascar. Liquid extracts from *Prunus africana* bark are used to treat benign prostatic hyperplasia and prostate gland hypertrophy (Prostate cancer) (Ingram *et al*., 2015). Leaves extract is inhaled to treat fever or to boost appetite (Simon *et al*., 2015). Water is added to the pounded bark and the red liquid is used for stomach-ache. The bark of *prunus africana* has been used as remedy for liver problems and constipation (Adongo, 2013). In central Kenya among the kikuyu community, the leaf extracts have shown antibacterial and antifungal activity (Mwitari *et al*., 2013).

The stem bark and leaves of *P. africana* is used to treat chest pain, malaria and fever traditionally (Adongo, 2013). A study was carried out to test the antibacterial and antifungal activity of methane and hexane bark extracts of *P. africana*. Disc diffusion assay was used to evaluate the antimicrobial activity of the specific bacteria and fugal strains. Standard discs of 6 mm diameter impregnated with 10 *ul* of the organic extract were used. The discs were placed aseptically on

sterile media (Mwitari *et al*., 2013). The methanol extracts had significant activity on several bacterial strains and only one fungal strain; *Trichophyton mentangrophyte*. The organic extracts of *P. africana* were found to shut down the expression of IL 7 Mrna at a concentration of 50μg/ml. The extracts however had no antifungal activity against *Candida albicans* and *Cryptococcus neoformans* (Bii *et al.,* 2010).

2.6.6 *Spinach oleracea*

It belongs to the family Amaranthaceae. Spinach has dark green leaves indicating it has high levels of chlorophyll and health promoting carotenoids like beta carotene, lutein and zeaxanthin. Anti-inflammatory and anticancerous effects are evident in these phytochemicals. They are also important for healthy eyesight and prevention of cataracts (Bergquist, 2006). Antioxidant activity of *S. oleracea* leaf extracts has been proved (Ko *et al*., 2014). The chlorophyll contained in spinach has been found effective in blocking the carcinogenic effect of amines generated when grilling foods at high temparature. The beta carotene in spinach excellently lowers the risks of developing diabetes (Watson and Preedy, 2009). Aqueous, ethanol and N-hexane extracts of fresh spinach were tested against several bacterial strains which are mammalian pathogens like *Lactobacillus*, *Staphylococcus* and *Salmonella* among others. The study was carried out in Pakistan.The ethanol extracts inhibited all the pathogens while the aqueous extracts inhibited some. *Spinacia oleracea* is therefore considered a potential source of new antimicrobial agents. *Spinacia oleracea* is known to contain many antioxidants which can bind many toxigenic molecules (Iqbal *et al*., 2012). *Spinacia oleracea* has been proved to have antioxidants that can protect against harmful radiations like gamma rays in a test that was carried out using mice (Bhatia and Jain, 2004).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Collection of plant material

Choice of plants for antimicrobial screening was based on ethnobotanical knowledge recorded at the University of Nairobi Herbarium. The various medicinal plant parts in this study: *Ocimum lamiifolium* leaves (LMM 2015/05), *Prunus africana* leaves (LMM 2015/03), *Solanum aculeastrum* fruits (LMM 2015/01), *Lippia kituensis* leaves (LMM 2015/04) and *Syzygium cordatum* bark (LMM 2015/02) were collected from Gakoe forest in Gatundu district, Kiambu County, Central region of Kenya. Fresh leaves of *Spinacia oleracea* (LMM 2015/06) were also collected from the local market. These plants were identified using reference material from the University of Nairobi Herbarium. Voucher specimens were deposited at the herbarium. Information about the plants and images are shown in Table 1 and Figure 1 respectively.

Table 1: Medicinal plant parts investigated for antifungal activity

Plant	**Local name**	**Family**	**Medicinal uses**	**Plant part used**
Solanum aculeastrum	Mutongu	Solanaceae	Fruit juice used on wounds	fruits
Prunus africana	'Muiri'	Rosaceae	Leaf extracts inhaled to stop fever and boost appetite	leaves
Lippia kituensis	Muthiriti	Verbenaceae	Leaves extracts used for gastrointestinal and respiratory disorders, antimalarial, anti-inflammatory, sedative and hypersensitive effects	leaves
Ocimum lamiifolium	Mutamaiyu	Labiatae	Leaves used to treat colds, coughs and skin infections; also as an insecticide and disinfectant	leaves
Syzygium cordatum	Mukoe	Myrtaceae	Extracts effective against Diabetes mellitus, Liquid from bark soaked in water used to stop abdominal pains.	bark
Spinacia oleracea	-	Amaranthaceae	Leaves contain chlorophyll which has anticarcinogenic effects, lowers risk of diabetes and improves eyesight by preventing cataracts	leaves

Figure 1: Plants tested for antifungal activity: (A) *Lippia kituensis* leaves, (B) *Prunus africana* leaves, (C) *Syzygium cordatum* bark, (D) *Solanum aculeastrum* fruits, (E) *Ocimum lamiifolium* leaves, (F) *Spinacia oleracea* leaves

3.2 Crude extract preparation

The collected plant parts were air-dried at room temperature, chopped into small pieces using secateurs and ground into powder. Distilled water was used for aqueous extraction. Two hundrend and fifty grams of each ground extract was soaked in 500 ml of distilled water in a glass beaker for five days. The extract was then filtered using Whatman no 1 filter paper (Figure

2). The filtrate was evaporated and dried using a freeze drier (Figure 3) to get powder (Alishtayeh *et al*., 1998). Dichloromethane – methanol (1:1) mixture was used for organic extraction. Two hundrend and fifty grams of each ground extract was soaked in 1 litre of the organic solvents for 48 hours. A rotary evaporator (Figure 4) was used to concentrate the organic extract hence obtaining a semi solid residue for use (Kiswii *et al*., 2014). The resulting products were stored at 4 degrees Celsius to be used for evaluation of antifungal efficacy on aflatoxin producing *A. flavus*.

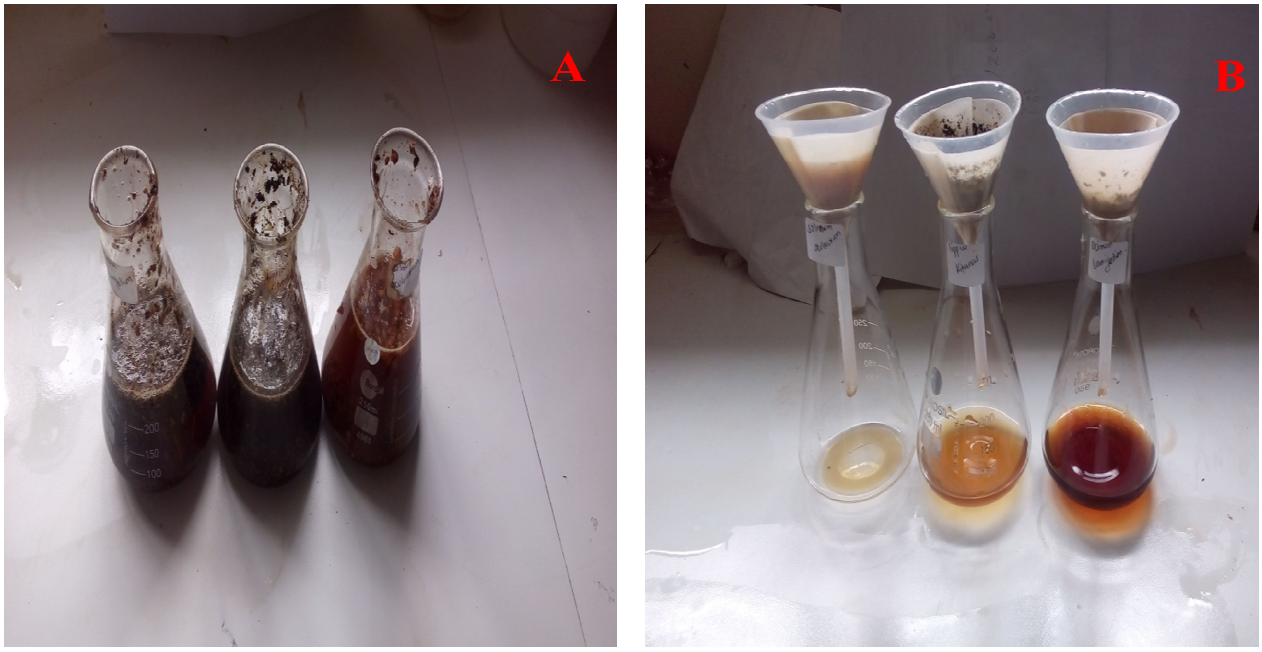

Figure 2: Aqueous extraction procedure: (A) Ground *S. cordatum* extracts soaked in distilled water, (B) Filtration of soaked *S. cordatum* extracts

Figure 3: Freeze drier used for aqueous extraction

Figure 4: Rotary evaporator used for organic extraction

3.3 Microorganism suspension and standardization

Toxigenic *Aspergillus flavus* strains UONV017 and UONV003 used in this study were obtained from the School of Biological Sciences Mycology Culture Collection. Small mycelia of each of the isolates was transferred into sterile PDA plates and incubated for seven days. Spores from the seven days old culture were suspended aseptically in sterile distilled water and standardized to a turbidity of 1 Mc Farland solution (3.0 x10^8 CFU/ml). The antifungal activity and minimum inhibitory concentration were determined using disc diffusion method and broth dilution method respectively (Mahesh and Satish, 2008).

3.4 Determination of inhibition concentration

Disc diffusion technique used to evaluate for antifungal activity of the plant extracts according to National Committee of Clinical and Laboratory Standards (NCCLS, 2007) now CLSI (Hall, 2011). The inoculum was prepared by suspending actively growing toxigenic *A. flavus* cultures in sterile distilled water. The suspensions were then diluted to a turbidity visually comparable to that of 1McFarland standard (3.0 x10^8 CFU/ml). Circular paper discs with a diameter of 6mm were prepared using an office paper perforator from Whatman filter paper No. 3. The discs were placed in a glass bottle and sterilized in an autoclave at 121 degrees Celsius for fifteen minutes. Stock solutions of the plant extracts were prepared using DMSO (100%) water mixture and pure distilled water for the organic and aqueous extracts respectively (Seniya *et al*., 2011). Dilutions of organic and aqueous plant extracts of different concentrations (600 mg/ml, 450 mg/ml, and 300 mg/ml) were prepared in test tubes using sterile distilled water and the plant extracts weighed on a weighing machine. DMSO (100%) was used as the negative control while a fungicide called Apron star 42ws (Syngenta) which is a class III blue active compound with Metaxyl, Difeconazole and Thiamethoxam, was used as the positive control after being diluted

according to the manufacturers guidelines (250 mg/ml). Potato dextrose agar (PDA) was poured aseptically on the surface of sterilized Petri dishes to a thickness of 5 mm. The plates were seeded with the spore inoculums using a pipette with 30 ug of the inoculum which was spread using a sterile glass rod. The discs were impregnated with 15 *ul* of the organic plant extract concentrations using a pipette. 15 *ul* of pure DMSO and 15 *ul* of Apron star (250 mg/ml) were also impregnated on the discs as negative and positive control respectively. The sterile impregnated discs were placed aseptically on inoculated agar surfaces using a pair of sterile forceps. Four discs with similar concentrations were placed at an equal distance from each other in each plate (Nostro *et al*., 2000). The plates seeded with toxigenic fungi and plant extracts were incubated at 25 degrees Celsius for 72 hours. The diameters of the inhibition zones produced around the test material (plant extracts) were measured. Plant extracts that produced a zone of inhibition of 8-11 mm were said to be active. Those with zones above 11 mm were considered very active. Those with zone of inhibition below 8 mm were considered inactive (Mwitari *et al*., 2013). The tests were replicated three times for each material.

3.5 Determination of Minimum Inhibitory Concentration

Broth dilution technique used to determine minimum inhibitory concentration. This was to determine the minimum concentration of the plant extract that would inhibit the growth of toxigenic *A. flavus*. Different concentrations of the extract were prepared and each replicated three times. The extract concentrations were; 100 mg/ml, 50 mg/ml and 25 mg/ml. 5 ml of each concentration of the extract was poured aseptically into a sterile test tube. One ml of the toxigenic *A. flavus* (1Mcfarland standard) was added. One ml of this mixture was poured aseptically into 5 ml of potato dextrose broth (serial dilution) (Kiswii *et al*., 2014). All the tubes were incubated at 27 Degrees Celsius for 72 hours. Observations were made for visible fungal

growth. The lowest dilution without visible growth for each extract was regarded as the minimum inhibitory concentration (MIC).

3.6 Photosensitization

Concentrations of 450 mg/ml and 600 mg/ml of each plant extract were prepared as the working solutions. Toxigenic fungal (*A. flavus*) spores were multiplied by culturing on potato dextrose agar for 4 days at 27 degrees Celsius. The spores from the four day old culture were suspended in sterile distilled water. The turbidity was standardized to 1Mc Farland solution (3×10^8 CFU/ml). The inoculums standardized to Mc Farland solution was serial diluted upto a concentration of 3×10^2 CFU/ml. 2ml of this (3×10^2 CFU/ml) solution was added to 2ml of each extract separately. The mixture was well shaken. Light at a maximum absorption range of the visible light spectrum (420 nm) was provided by a special lamp (Temba *et al*., 2016). The maximum absorption range of the plant extracts was tested using a spectrophotometer and found to be 420 nm. Effects of light irradiated on the photosensitizer (plant extract) for varying time periods (10 mins, 20 mins and 40 mins) were tested on the viability of spores of the toxigenic *A. flavus.* This was done by inoculating 100 ul of each spore photosensitizer solution already irradiated on PDA plates for 72 hours. The colony forming units (CFU) were counted and recorded in comparison with the controls. Controls involved treatments with photosensitizer (plant extracts) but without light (Temba *et al*., 2016). Each treatment was replicated three times. *Prunus africana* and *Spinacia oleracea* organic extracts in solution with toxigenic *A. flavus* are shown in Figure 5 and Figure 6 before and after the light reaction respectively.

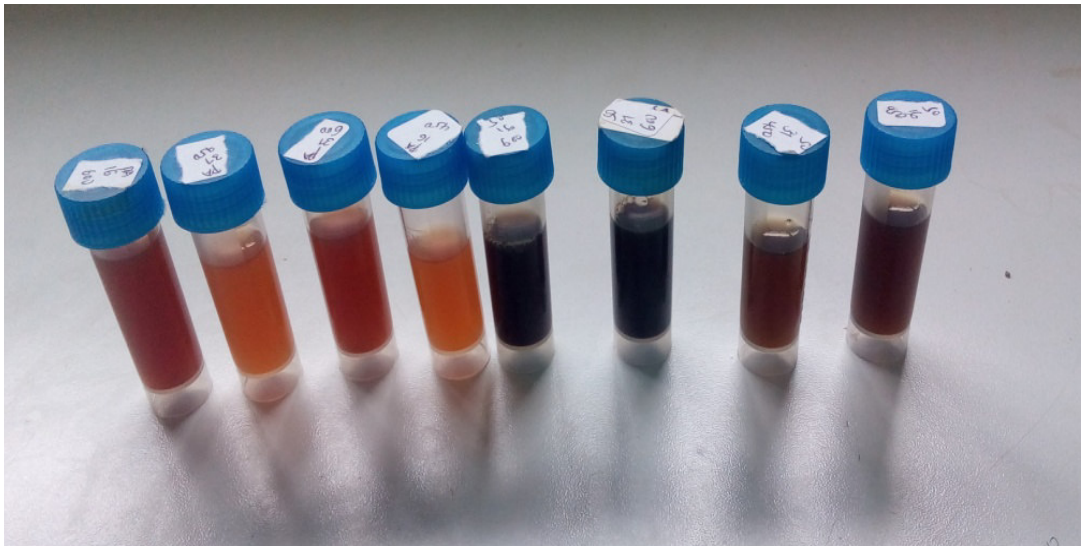

Figure 5: *Prunus africana* and *Spinacia oleracea* in solution with *A. flavus* before light reaction. *P.africana* 600mg/ml againstUONVOO3, 450mg/ml against UONV017, 600mg/ml against UONV017, 450mg/ml against UONV003, *S. oleracea* 600mg/ml against UONV003, 600mg/ml against UONVO17, 450mg/ml against UONV003, 450mg/ml against UONV037.

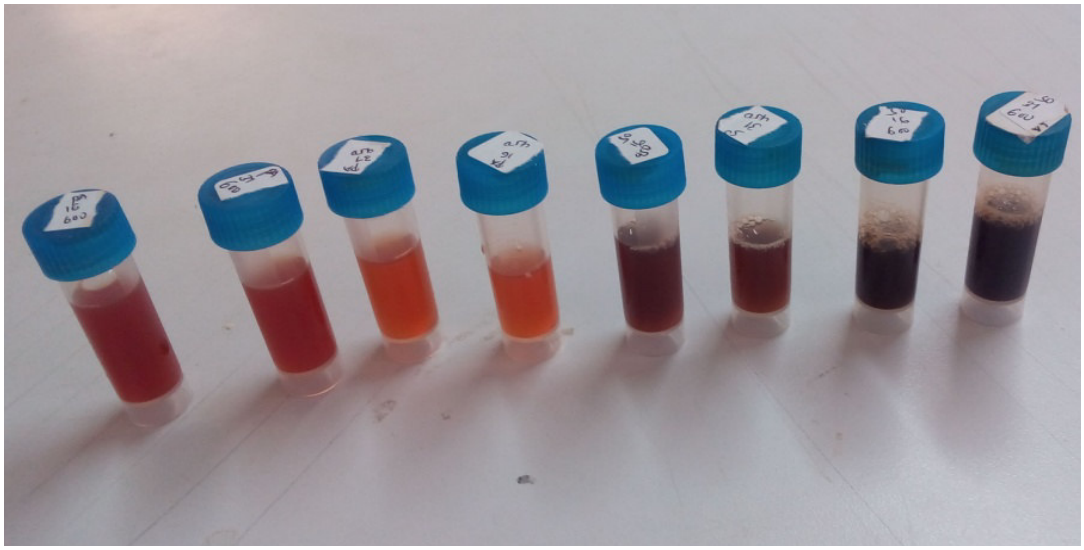

Figure 6: *Prunus africana* and *Spinacia oleracea* in solution with *A. flavus* after light reaction. From left to right: *P.africana* 600mg/ml againstUONVOO3, 600mg/ml against UONV017, 450mg/ml against UONV017, 450mg/ml against UONV003, *S. oleracea* 450mg/ml against UONV003, 450mg/ml against UONV037, 600mg/ml against UONV003, 600mg/ml against UONVO17.

3.7 Phytochemical screening of plant extracts

The six organic and aqueous plant extracts obtained were subjected to phytochemical screening methods according to Opuku *et al*. (2015) and Kiswii *et al*. (2014). This was done to determine the presence of bioactive agents like flavonoids, steroids, terpenoids, saponins, tannins, alkaloids and glycosides. Plant extracts from the stock solution of 800 mg/ml were used for the phytochemical screening. The following methods described were carried out to test for the presence of bioactive compounds.

3.7.1 Test for Tannins

Two ml of each plant extract was mixed with 2 ml ferric chloride ($FeCl_3$). Formation of blue black precipitate indicated positive test for tannins.

3.7.2 Test for saponins

To 1 ml of each extract, 10 ml of distilled water was added and the solution shaken for 15 minutes. Formation of a layer of foam (frothing) 1cm and above indicated positive test for saponins.

3.7.3 Test for flavonoids

To 1ml of each extract, 5 ml of dilute ammonia was added followed by 1 ml of concentrated sulphuric acid (H_2SO_4). Formation of a yellow colour that disappeared on standing indicated positive test for flavonoids.

3.7.4 Test for glycosides

To 1 ml of each extract, 1 ml of glacial acetic acid was added followed by few drops of 5% ferric chloride. Few drops of concentrated H_2SO_4 were added and formation of greenish blue colour indicated positive test for glycosides.

3.7.5 Test for alkaloids

To 2 ml of each extract, 2 ml of concentrated hydrochloric acid was added. Few drops of Mayer's reagent were added and formation of green colour or a white precipitate indicated positive test for alkaloids.

3.7.6 Test for steroids and terpenoids

Two ml of each extract was dissolved in chloroform. Three drops of acetic anhydride were added to the solution followed by two drops of concentrated H_2SO_4. Formation of reddish pinkish coloration indicated positive test for steroids and terpenoids.

3.7.7 Statistical analysis

Data analysis was done using SPSS version 16. Data values were expressed as means ± standard error. Analysis of variance was used and where F was significant ($P \leq 0.05$), comparison of means was done using Tukey's test.

CHAPTER FOUR: RESULTS

4.1 Effect of organic and aqueous medicinal plant extracts on growth of toxigenic *Aspergillus flavus*

Organic and aqueous extracts of the six medicinal plant samples were tested *in vitro* for antifungal activity against two strains of toxigenic *A. flavus*. The crude organic extracts of five out of six of the tested plants exhibited antifungal activity on the growth of toxigenic strains of *A. flavus*. The aqueous extracts did not show significant ($P \leq 0.05$) antifungal activity because all the inhibition diameters were below 8mm. The zones of inhibition were varying among plants and also among different concentrations. Plant extracts with inhibition diameters of 8-11 mm were recorded as active. Those above 11 mm were very active while those below 8mm were considered inactive. Some of the plant extracts had inhibition diameters that had no significant difference hence compared favorably with the standard antifungal control Apron star a class III blue active ingredient (250 mg/ml).

4.1.1 Inhibition of growth of *Aspergillus flavus* UONV003 by different organic plant extracts at different concentrations

The different plant extracts at 600mg/ml significantly ($F=22.558$; $df = 6$; $P < 0.05$) inhibited the growth of toxigenic fungi differently. The results showed that the plant extracts of *S. aculeastrum* (mean = 11.92 ± 0.94 mm) had very active inhibitory activity, those of *S. cordatum* (mean=10.27 ± 0.32 mm), *S. oleracea* (mean = 9.25 ± 0.31 mm), *L. kituensis* (mean = 9.25 ± 0.70 mm), *P. africana* (mean = 8.50 ± 0.72 mm) were also actively inhibitive while those of *O. lamiifolium* (mean = 6.42 ± 0.20 mm) had no inhibitory effect (Table 2).

Inhibition on the growth of *A. flavus* at 450 mg/ml differed significantly ($F = 49.458$; $df = 6$; $P < 0.05$) with variations on the kind of plant used. The results established that extracts from *S. aculeastrum* (mean = 11.00 ± 0.60 mm) had very active inhibitory while *S. cordatum* (mean = 10.25 ± 0.52 mm) had active inhibitory activity. Plant extracts from *S. olarecea* (mean = 7.83 ± 0.79 mm), *L. kituensis* (mean = 6.92 ± 0.34 mm), *O. lamiifolium* (mean = 6.08 ± 0.08 mm) and *P. africana* (mean = 6.00 ± 0.01 mm) had no inhibitory activity (Table 2).

Inhibition on growth of *A. flavus* at 300 mg/ml differed significantly ($F = 24.854$; $df = 6$; $P < 0.05$) with variations in the kind of plants used. Extracts from *S. aculeastrum* (mean = 11.08±0.67 mm) had very active inhibitory activity, *S. cordatum* bark (mean = 9.50 ± 0.68 mm) had active inhibitory activity. Plant extracts from *P. africana* (mean = 3.00 ± 0.90 mm), *O.lamiifolium* (mean = 4.42 ± 0.96 mm), *S. oleracea* (mean = 6.33 ± 0.99 mm) and *L. kituensis* (mean = 6.58 ± 0.70 mm) had no inhibitory activity (Table 2).

The highest concentration of plant extracts (600 mg/ml) in all cases proved the highest antifungal activities. The lowest concentration (300 mg/ml) in all cases apart from *S. aculeastrum* exhibited the lowest antifungal activities. For *S. aculeastrum* the 300 mg/ml concentration resulted in a slightly higher inhibition diameter than the 450 mg/ml concentration which was attributed to the higher viscosity in the higher concentration hence slow diffusion (Table 2).

Table 2: Effect of different organic plant extracts on growth of *Aspergillus flavus* UONV003 at different concentrations

Plants	**Inhibition zones (mm) 600 mg/ml**	**Inhibition zones (mm) 450 mg/ml**	**Inhibition zones (mm) 300 mg/ml**
S. aculeastrum	11.92±0.94ab	11.00±0.60b	11.08±0.67b
S. cordatum	10.27±0.32bc	10.25±0.52b	9.50±0.68bc
L. kituensis	9.25±0.70c	6.92±0.34c	6.58±0.70cd
P. africana	8.50±0.72cd	6.00±0.01c	3.00±0.90e
O. lamiifolium	6.42±0.20d	6.08±0.08c	4.42±0.96de
S. oleracea	9.25±0.31c	7.83±0.79c	6.33±0.99c-e
Positive control 250mg/ml	17.17±0.40a	17.17±0.40a	17.17±0.40a
Sig P<0.05	0.00	0.00	0.00

Numbers are means of twelve replications. One way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same column are not significantly different ($P < 0.05$)

4.1.2 Inhibition of growth of *Aspergillus flavus* UONV017 by different organic plant extracts at different concentrations.

Results showed that at 600 mg/ml organic extracts had significant ($F = 40.055$; $df = 6$; $P < 0.05$) inhibition on growth of *A. flavus*. *Solanum aculeastrum* (mean = 18.50 ± 0.71 mm), *S. cordatum* (mean = 17.00 ± 1.26 mm), *L. kituensis* (mean = 11.08 ± 0.53 mm) and *O. lamiifolium* (mean = 11.42 ± 0.34 mm) had very active inhibitory activity. *Spinacia oleracea* (mean = 10.08 ± 0.53 mm) and *P. africana* (mean = 8.67 ± 0.69 mm) were also actively inhibitive (Table 3).

Inhibition of *A. flavus* at 450 mg/ml also differed significantly ($F = 49.171$; $df = 6$; $P < 0.05$) depending on the different kinds of plants used. Plant extracts from *S. aculeastrum* (mean = 14.42±0.83 mm), *S. cordatum* (mean = 12.00±0.52 mm) had very active inhibitory activity. Plant extracts from *P. africana* (mean = 9.25±0.71 mm), *L. kituensis* (mean = 10.17±0.68 mm) and *S.*

oleracea (mean = 9.00±0.30 mm) also had active inhibitory activity. Plant extracts from *O. lamiifolium* (mean = 6.67±0.26 mm) had no inhibitory activity (Table 3)

Inhibition on growth of *A.flavus* at 300 mg/ml also differed significantly ($F = 46.201$; $df = 6$; $P < 0.05$) depending on the various kinds of plants used. Plant extracts from *S. aculeastrum* (mean = 11.67±0.54 mm) had very active inhibitory activity, *S. cordatum* (mean = 10.67±0.54 mm) and *L. kituensis* (mean = 8.08±0.47 mm) had active inhibitory activity. Plant extracts from *P. africana* (mean = 6.86.83±1.048 mm), *S. oleracea* (mean = 6.33±0.14 mm) and *O. lamiifolium* (mean = 6.00±0.83 mm) had no inhibitory activity (Table 3).

Apart from *P. africana* leaf extracts, all the other extracts had highest inhibitory activity at the highest concentration (600 mg/ml) and the lowest antifungal activity was at the lowest concentration (300 mg/ml) (Table 3).

Comparison on the activity of the organic plant extracts between both strains of *A. flavus* exhibited that *A. flavus* UONV003 was less sensitive than strain *A. flavus* UONV017 because it had smaller inhibition diameters at similar concentrations (Figure 7 and Figure 8).

Table 3: Effect of different organic plant extracts against growth of *Aspergillus flavus* UONV017 at different concentrations

Plants	**Inhibition zones (mm) 600 mg/ml**	**Inhibition zones (mm) 450 mg/ml**	**Inhibition zones (mm) 300 mg/ml**
S. aculeastrum	18.50±0.71ab	14.42±0.83b	11.67±0.54b
S. cordatum	17.00±1.26b	12.00±0.52bc	10.67±0.54bc
L. kituensis	11.08±0.53c	10.17±0.68cd	8.08±0.47cd
P. africana	8.67±0.69c	9.25±0.71d	6.83±1.04d
O. lamiifolium	11.42±0.34c	6.67±0.26e	6.00±0.83d
S. oleracea	10.08±0.36c	9.00±0.30de	6.33±0.14d
Positive control 250mg/ml	22.00±0.63a	22.00±0.63a	22.00±0.63a
(Sig P < 0.05)	0.00	0.00	0.00

Numbers are means of twelve replications. One way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same column are not significantly different (P < 0.05)

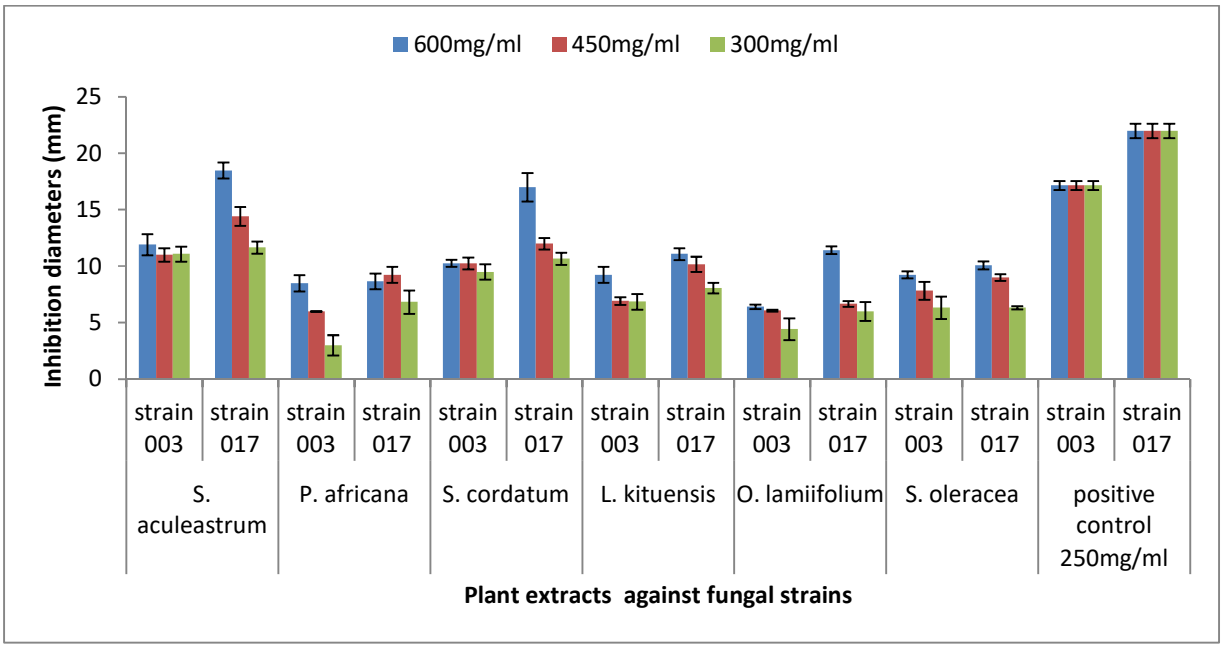

Figure 7: Comparison of inhibition of *Aspergillus flavus* (UONV017) and (UONV003) by organic plant extracts at different concentrations. The bars on top are indicators of standard error of the mean.

4.1.3 Inhibition of growth of *Aspergillus flavus* UONV017 and UONV003 by different aqueous plant extracts at different concentrations

At all the three concentrations, the diameters of zones of inhibition were below 8mm for all plants against both toxigenic *A. flavus* strains hence the aqueous extracts had no significant inhibitory activity. There was significant ($P \leq 0.05$) difference between the antifungal activity of all the aqueous extracts and the standard antifungal control (Apron star 250 mg/ml). Comparison of antifungal activity within different concentrations showed that the highest concentration (600 mg/ml) had the highest antifungal activity followed by the concentration of 450mg/ml; the lowest concentration (300 mg/ml) had no antifungal activity (Table 4 and 5).

Comparison on the activity of the aqueous plant extracts between both strains of *A. flavus* showed that UONV003 was less sensitive than strain UONV017 because it had slightly smaller inhibition diameters at different concentrations. The positive control (Apron star 250 mg/ml) also produced smaller inhibition diameters with strain UONV003 compared to strain UONV017 (Figure 9).

Table 4: Effect of different aqueous plant extracts against growth of *Aspergillus flavus* UONV003 at different concentrations

Plants	**Inhibition zones (mm) 600 mg/ml**	**Inhibition zones (mm) 450 mg/ml**	**Inhibition zones (mm) 300 mg/ml**
S. aculeastrum	5.00±0.67b	4.58±0.80b	3.00±0.90b
S. cordatum	4.00±0.85b	2.75±0.99b	0.00±0.00c
L. kituensis	3.00±0.90b	2.50±0.89b	0.00±0.00c
P. africana	3.50±0.89b	3.50±0.89b	0.00±0.00c
O. lamiifolium	4.50±0.78b	3.08±0.93b	0.00±0.00c
S. oleracea	3.00±0.90b	1.50±0.78b	0.00±0.00c
Positive control 250mg/ml	17.33±0.56a	17.33±0.56a	17.33±0.56a
Sig P < 0.05	0.00	0.00	0.00

Numbers are means of twelve replications. One way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same column are not significantly different (P < 0.05)

Table 5: Effect of different aqueous plant extracts against growth of *Aspergillus flavus* UONV017 at different concentrations

Plants	**Inhibition zones (mm) 600 mg/ml**	**Inhibition zones (mm) 450 mg/ml**	**Inhibition zones (mm) 300 mg/ml**
S. aculeastrum	6.50±0.69b	4.58±0.80b	3.50±0.89b
S. cordatum	6.25±0.62bc	2.50±0.89b	0.00±0.00c
L. kituensis	3.25±0.99c	2.50±0.89b	0.00±0.00c
P. africana	5.50±0.50bc	3.00±0.90b	0.00±0.00c
O. lamiifolium	5.08±0.92bc	3.50±0.89b	0.00±0.00c
S. oleracea	5.00±0.67bc	3.00±0.90b	0.00±0.00c
Positive control 250mg/ml	21.33±0.67a	21.33±0.67a	21.33±0.67a
Sig P < 0.05	0.00	0.00	0.00

Numbers are means of twelve replications. One way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same column are not significantly different (P < 0.05)

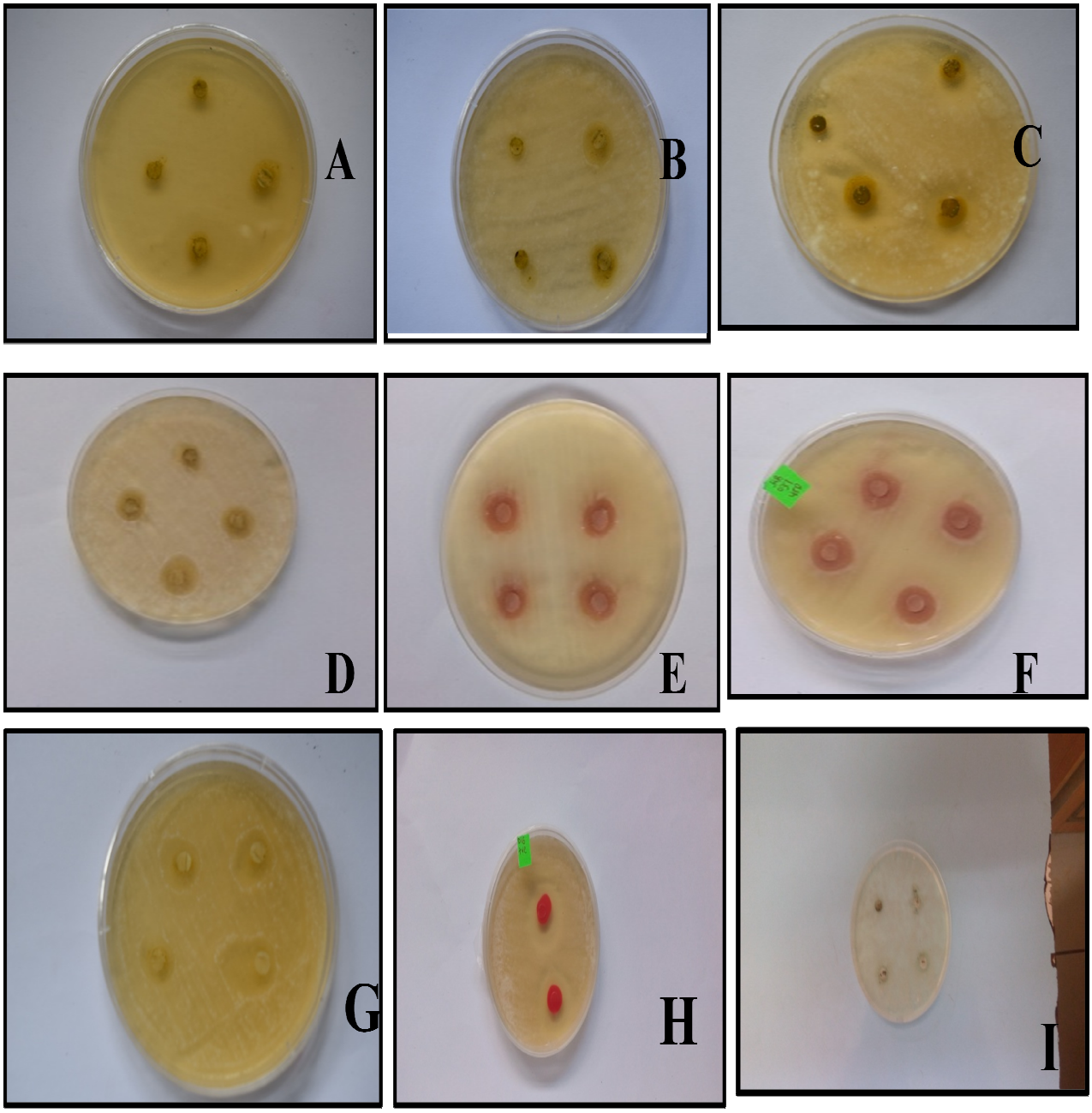

Figure 8: Zones of inhibition caused by organic plant extracts at different concentrations among the two strains of *Aspergillus flavus:* (A) *Solanum aculeastrum* 450 mg/ml on *A. flavus* UONV003 and (B) *A. flavus* UONV017, (C) *Solanum aculeastrum* 600 mg/ml on *A. flavus* UONV017 and (D) UONV003, (E) *Syzygium cordatum* 300 mg/ml on *A. flavus* UONV003 and (F) *A. flavus* UONV017, (G) *Ocimum lamiifolium* 600mg/ml on A. flavus UONV037, (H) positive conrol Apron star 250mg/ml, (I) Negative control Dimethyl Sulphoxide (DMSO) 100%

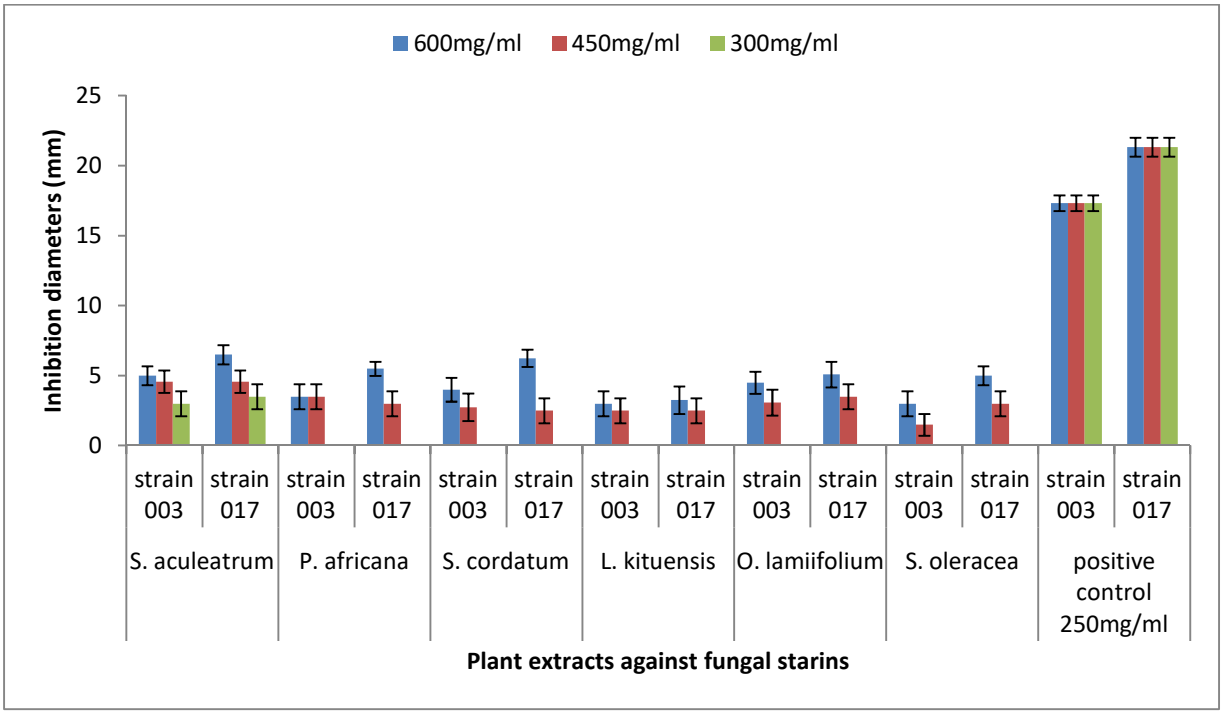

Figure 9: Comparison of inhibition of *Aspergillus flavus* (UONVO17) and (UONV003) by aqueous plant extracts at different concentrations. The bars on top of the graph indicate standard error of the means.

4.1.4 Determination of minimum inhibitory concentrations of the plant extracts

Minimum Inhibitory Concentration was done for the organic plant extracts because they were the ones found to be effective. Different plant extracts had different MIC values at different concentrations. The concentrations used included: 25 mg/ml, 50 mg/ml and 100 mg/ml. Extracts of *S. aculeastrum* had the lowest MIC (25 mg/ml) against *A. flavus* (UONV017). The same *S. aculeastrum* had MIC of 50 mg/ml on *A. flavus* UONV003. *Syzygium cordatum* also had MIC of 50 mg/ml on *A. flavus* (UONV017) and MIC of 100 mg/ml on *A. flavus* (UONV003) (Table 6)

Table 6: Minimum Inhibitory Concentration of organic plant extracts against two toxigenic strains of *A. flavus*

Plants	**MIC mg/ml**	
	***Aspergillus flavus* (UONV003)**	***Aspergillus flavus* (UONV017)**
S. aculeastrum	50	25
P. africana	>100	>100
S. cordatum	100	50
L. kituensis	>100	>100
O. lamiifolium	>100	>100
S. oleracea	>100	>100
Positive control (Apron star)	250mg/ml	250mg/ml

4.2 Evaluation of effect of organic and aqueous medicinal plant extracts in combination with light (photosensitization) on growth of toxigenic *Aspergillus flavus*

Both the aqueous and organic extracts had effective photosensitization activity compared with the controls.

4.2.1 Photosensitization activity of aqueous plant extracts on *Aspergillus flavus* UONV017 at different concentrations and different timings

Interaction between aqueous plants extracts and visible light at 600 mg/ml produced statistically significant effect ($F = 14.338$; $df = 15$; $P \leq 0.05$). Different plants fungi suspensions had different counts of CFU after light treatment at varying time durations. *Solanum aculeastrum* (mean = 2 CFU'S) had the lowest CFU reading at 40 minutes hence the most effective. Other than the controls, *S. oleracea* (mean = 13 CFU's) had the highest CFU reading at 10 minutes hence the

least effective at 600 mg/ml. At 450 mg/ml, interaction between the aqueous extracts and light caused significant ($F = 22.53$; $df = 15$; $P \leq 0.05$) reduction of CFU's. *Solanum aculeastrum* (mean = 3 CFU's) was still the most effective while *O. lamiifolium* (mean = 14 CFU's) was the least effective at 10 minutes (Table 7).

Comparison of photosensitization activity within different time durations proved that treatments kept under light for the highest duration of time (40 minutes) exhibited the lowest CFU counts hence proving the highest inactivation of fungal spores. Treatments that were under light for the shortest time duration (10 minutes) exhibited a higher number of CFU's; samples with no light treatment had the highest CFU count (Table 7).

Table 7: Photosensitization activity of aqueous plant extracts on *Aspergillus flavus* UONV017

Plants	**Colony forming units at 600 mg/ml**					**Colony forming units at 450 mg/ml**			
	10(min)	**20(min)**	**40(min)**	**No light contrl**		**10(min)**	**20(min)**	**40(min)**	**No light contrl**
S. aculeastrum	4.00 ± 1.19b	4.00 ± 1.19 bc	2.00 ±1.19 c	35 ± 2.06a		4.00 ± 0.92b	4.00 ± 0.92bc	3.00 ± 0.92c	35 ± 1.60a
P. africana	5.00 ± 1.19 b	4.00 ± 1.19 bc	3.00 ± 1.19 c	71 ± 2.06a		6.00 ± 0.92b	5.00 ± 0.92bc	5.00 ± 0.92c	71 ± 1.60a
S. cordatum	5.00 ± 1.19 b	4.00 ± 1.19 bc	4.00 ± 1.19 c	41 ± 2.06a		5.00 ± 0.92b	4.00 ± 0.92bc	4.00 ± 0.92c	41 ± 1.60a
L. kituensis	11.00 ± 1.19 b	8.00 ± 1.19 bc	7.00 ± 1.19 c	41 ± 2.06a		13.00 ± 0.92b	11.00± 0.92bc	10.00± 0.92c	41 ± 1.60a
O. lamiifolium	12.00 ± 1.19 b	8.00 ± 1.19 bc	6.00 ± 1.19 c	62 ± 2.06a		14.00 ± 0.92b	11.00± 0.92bc	10.00± 0.92c	62 ± 1.60a
S. oleracea	13.00 ± 1.19 b	10.00 ± 1.19bc	7.00 ± 1.19c	54 ± 2.06a		12.00 ± 0.92b	11.00±0. 92bc	10.00± 0.92c	54 ± 1.60a

Numbers are means of three replications. Two way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same row within each concentration are not significantly different (**P < 0.05**)

4.2.2 Photosensitization activity of aqueous plant extracts against *Aspergillus flavus* UONV003 at different concentrations and different timings

Statistically significant ($F = 5.033$; $df = 15$; $P \leq 0.05$) interaction existed between aqueous plants extracts and light in their activity against *A. flavus* at 600 mg/ml. *Syzygium cordatum* (mean = 3 CFU'S) had the lowest CFU reading at 40 minutes hence the most effective. *Lippia kituensis* (mean = 16 CFU's) had the highest CFU reading at 10 minutes hence the least effective. At 450 mg/ml, interaction between aqueous extracts and light was also significant ($P < 0.05$). *Solanum aculeastrum* (mean = 3 CFU's) was the most effective while *L. kituensis* (mean = 17 CFU's) was the least effective at 10 minutes (Table 8)

Photosensitization activity within different time durations was compared and it proved that treatments kept under light for the highest duration of time (40 minutes) exhibited the lowest CFU counts hence proving the highest inactivation of fungal spores. Treatments that were under light for the shortest time duration (10 minutes) exhibited a higher number of CFU's; samples with no light treatment had the highest CFU count (Table 8).

Table 8: Photosensitization activity of aqueous plant extracts on *Aspergillus flavus* UONV003

Plants	**Colony forming units at 600 mg/ml**					**Colony forming units at 450 mg/ml**			
	10(min)	**20(min)**	**40(min)**	**No light contrl**		**10(min)**	**20(min)**	**40(min)**	**No light contrl**
S. aculeastrum	5.00 ± 1.95b	5.00 ± 1.95bc	4.00 ± 1.95c	43 ± 3.38a		5.00 ± 1.51b	4.00 ± 1.51bc	4.00 ± 1.51c	43 ± 2.62a
P. africana	6.00 ± 1.95b	5.00 ± 1.95bc	4.00 ± 1.95c	72 ± 3.38a		5.00 ± 1.51b	4.00 ± 1.51bc	4.00 ± 1.51c	72 ± 2.62a
S. cordatum	5.00 ± 1.95b	4.00 ± 1.95bc	3.00 ± 1.95c	34 ± 3.38a		5.00 ± 1.51b	4.00 ± 1.51bc	3.00 ± 1.51c	34 ± 2.62a
L. kituensis	16.00± 1.95b	12.00± 1.95bc	8.00 ± 1.95c	44 ± 3.38a		17.00 ± 1.51b	14.00 ± 1.51bc	12.00± 1.51c	44 ± 2.62a
O. lamiifolium	13.00± 1.95b	11.00± 1.95bc	8.00 ± 1.95c	56 ± 3.38a		15.00 ± 1.51b	11.00 ±1.51bc	10.00± 1.51c	56 ± 2.62a
S. oleracea	14.00± 1.95b	14.00± 1.95bc	11.00 ± 1.95c	58 ± 3.38a		16.00 ± 1.51b	13.00 ±1.51bc	10.00± 1.51c	58 ± 2.62a

Numbers are means of three replications. Two way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same row within each concentration are not significantly different (**P < 0.05**)

4.2.3 Photosensitization activity of organic plant extracts on *Aspergillus flavus* UONV017 at different concentrations and different timings

Organic extracts at both concentrations displayed significant ($F = 8.448$; $df = 15$; $P \leq 0.05$) photosensitization activity. *Syzygium cordatum* (mean = 4 CFU'S) had the lowest CFU reading at 40 minutes hence the most effective at a concentration of 600 mg/ml. *Ocimum lamiifolium* (mean = 19 CFU's) had the highest CFU reading at 10 minutes hence the least effective at 600mg/ml. At 450 mg/ml, there was also significant ($F = 22.326$; $df = 15$; $P \leq 0.05$) photosensitization activity. *Syzygium cordatum* (mean = 7 CFU's) was the most effective at 40 minutes (Table 9)

The comparison of photosensitization activity within different time durations showed that treatments kept under light for the highest duration of time (40 minutes) exhibited the lowest CFU counts hence proving the highest inactivation of fungal spores. Treatments that were under light for the shortest time duration (10 minutes) exhibited a higher number of CFU's hence the photosensitization effect was significant; samples with no light treatment had the highest CFU count (Figure 14).

Comparison between photosensitization effect between aqueous and organic extracts proved that both extracts were effective against toxigenic *A. flavus* (UONV017) because there was significant reduction of CFU in comparison with the controls. Aqueous extracts had a lower number of CFU's than the organic extracts. The higher concentration (600 mg/ml) proved to have greater reduction of CFU compared to the 450 mg/ml concentration though both were effective (Figure 10 and 11).

Table 9: Photosensitization activity of organic plant extracts on *Aspergillus flavus* UONV017

Plants	**Colony forming units at 600mg/ml**					**Colony forming units at 450mg/ml**			
	10(min)	**20(min)**	**40(min)**	**No light contrl**		**10(min)**	**20(min)**	**40(min)**	**No light contrl**
S. aculeastrum	11.00±1.64b	11.00±1.64b	8.00±1.64c	62±2.85a		24.00±2.63b	12.00±2.63c	9.00 ± 2.63c	62±4.56a
P. africana	10.00±1.64b	10.00±1.64b	8.00 ±1.64c	76±2.85a		41.00±2.63b	23.00±2.63c	14.00±2.63c	76±4.56a
S. cordatum	10.00±1.64b	8.00±1.64b	4.00 ±1.64c	51±2.85a		11.00±2.63b	8.00±2.63c	7.00 ±2.63c	51±4.56a
L. kituensis	16.00±1.64b	12.00±1.64bc	8.00 ±1.64c	44±2.85a		22.00±2.63b	12.00±2.63c	11.00±2.63c	48±4.56a
O. lamiifolium	19.00±1.64b	12.00±1.64b	9.00 ±1.64c	48±2.85a		23.00±2.63b	16.00±2.63c	14.00±2.6c	72±4.56a
S. oleracea	11.00±1.64b	10.00±1.64bc	9.00 ±1.64c	47±2.85a		14.00±2.63b	12.00±2.63c	9.00±2.63c	47±4.56a

Numbers are means of three replications. Two way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same row within each concentration are not significantly different (**P < 0.05**).

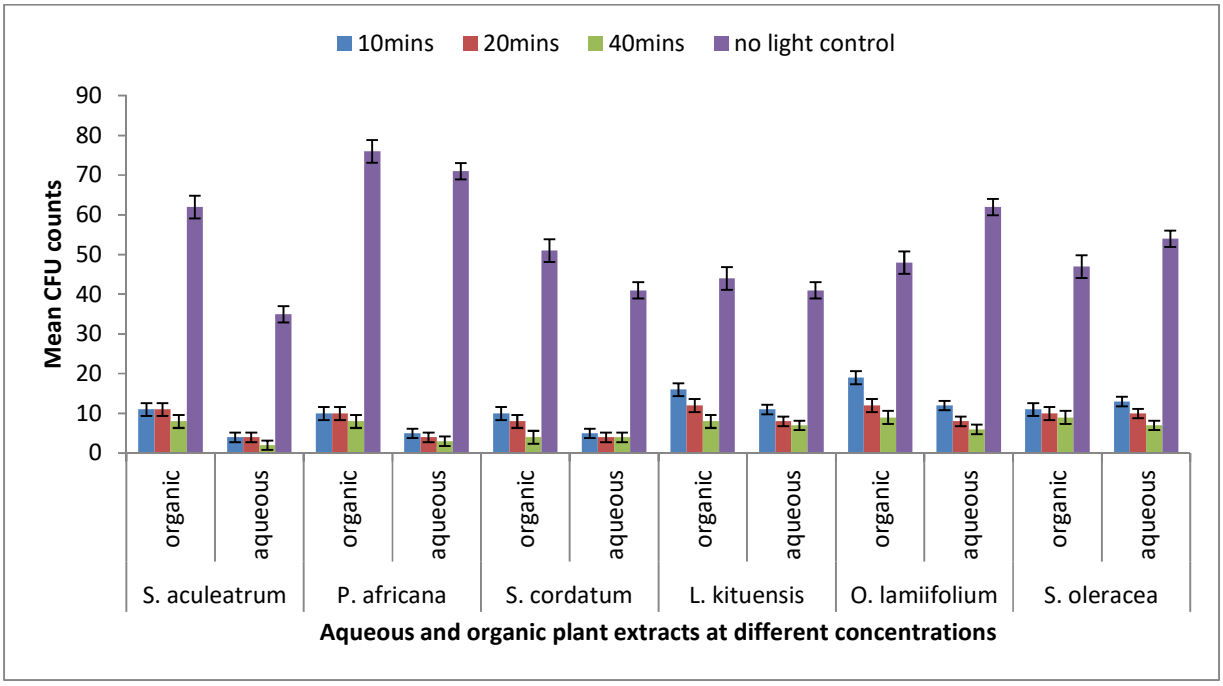

Figure 10: Effect of photosensitization on organic and aqueous plant extracts on *Aspergillus flavus* (UONV017) at 600 mg/ml within different time durations. The bars on top of the graph indicate standard error of the means.

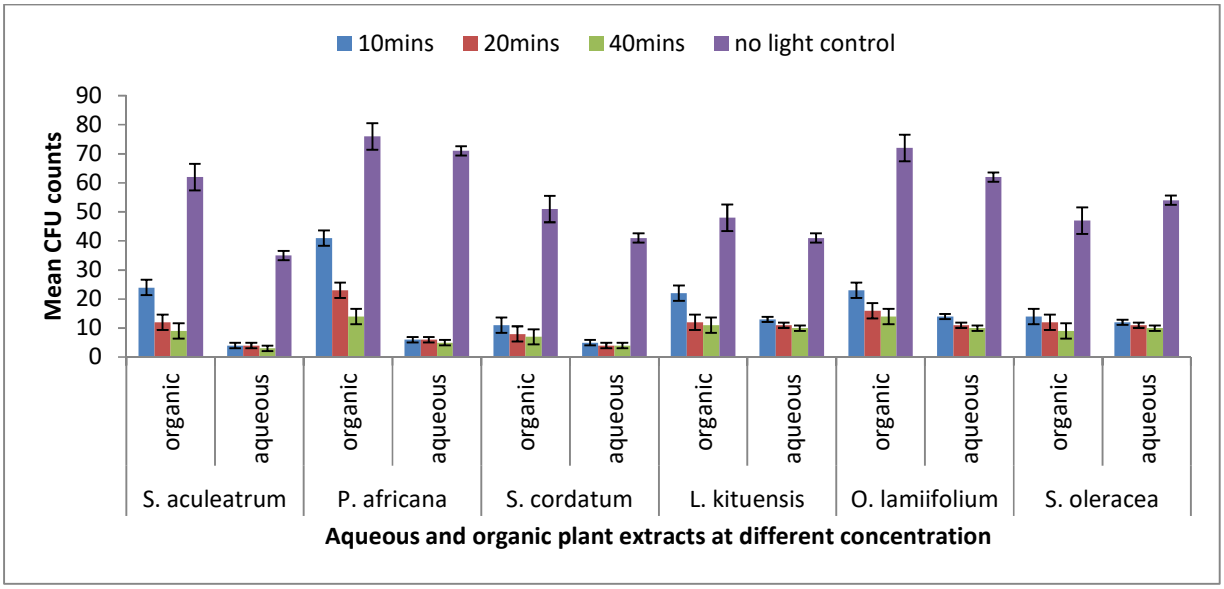

Figure 11: Effect of photosensitization by organic and aqueous plant extracts on *Aspergillus flavus* (UONV017) at 450 mg/ml within different time durations. The bars on top of the graph indicate standard error of the means.

4.2.4 Photosensitization activity of organic plant extracts against *Aspergillus flavus* UONV003 at different concentrations and different timings

Interaction between organic plants extracts and light was statistically significant ($F = 8.105$; $df = 15$; $P \leq 0.05$) at a concentration of 600 mg/ml. *Solanum aculeastrum* (mean = 9 CFU'S) had the lowest CFU reading at 40 minutes hence the most effective. *Lippia kituensis* (mean = 23 CFU's) had the highest CFU reading at 10 minutes hence the least effective at 600mg/ml. At 450 mg/ml, organic extracts exhibited significant ($F = 11.291$; $df = 15$; $P \leq 0.05$) photosensitization activity. *Solanum aculeastrum* (mean = 9 CFU's) was still the most effective at 40 minutes (Table 10).

Comparison between photosensitization effect between aqueous and organic extracts proved that both extracts were effective against toxigenic *A. flavus* (UONV003) because there was significant reduction of CFU in comparison with the controls. Aqueous extracts had a lower number of CFU's than the organic extracts. The higher concentration (600 mg/ml) proved to have greater reduction of CFU compared to the 450 mg/ml concentration though both were effective (Figure 12 and 13).

Comparison of photosensitization activity within different time durations showed that treatments kept under light for the highest duration of time (40 minutes) exhibited the lowest CFU counts hence proving the highest inactivation of fungal spores. Treatments that were under light for the shortest time duration (10 minutes) exhibited a higher number of CFU's; samples with no light treatment had the highest CFU count (Figure 14)

Table 10: Photosensitization activity of organic plant extracts on *Aspergillus flavus* UONV003

Plants	**Colony forming units at 600 mg/ml**					**Colony forming units at 450 mg/ml**			
	10(min)	**20(min)**	**40(min)**	**No light contrl**		**10(min)**	**20(min)**	**40(min)**	**No light contrl**
S. aculeastrum	12.00±1.84b	12.00±1.84bc	9.00±1.84c	69±3.18a		26.00±2.59b	13.00±2.59c	9.00±2.59c	69±4.48a
P. africana	18.00±1.84b	17.00±1.84bc	11.00±1.84c	100±3.18a		34.00±2.59b	20.00±2.59c	15.00±2.59c	100±4.48a
S. cordatum	15.00±1.84b	14.00±1.84bc	10.00±1.84c	60±3.18a		18.00±2.59b	15.00±2.59c	12.00±2.59c	60±4.48a
L. kituensis	23.00±1.84b	12.00±1.84bc	14.00±1.84c	64±3.18a		27.00±2.59b	13.00±2.59c	11.00±2.59c	74±4.48a
O. lamiifolium	12.00±1.84b	10.00±1.84bc	11.00±1.84c	74±3.18a		13.00±2.59b	10.00±2.59c	10.00±2.59c	52±4.48a
S. oleracea	12.00±1.84b	12.00a±1.84bc	10.00±1.84c	52±3.18a		11.00±2.59b	11.00±2.59c	10.00±2.59c	47±4.48a

Numbers are means of three replications. Two way ANOVA was used for analysis and means separated by Tukey's test. Numbers followed by the same letters in the same row within each concentration are not significantly different (**P < 0.05**)

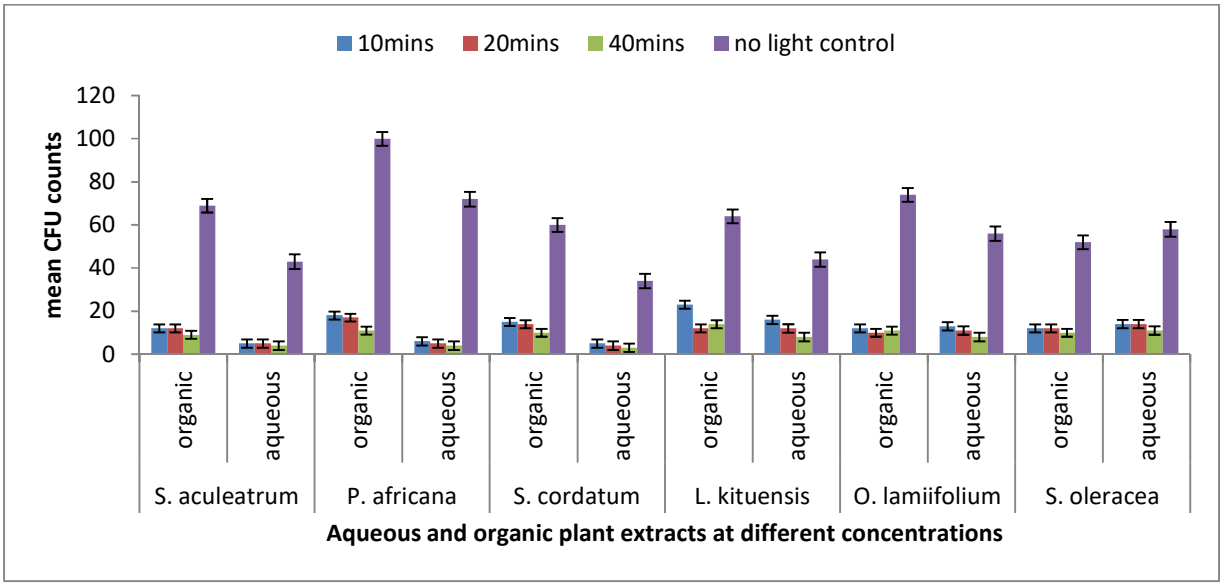

Figure 12: Comparison of photosensitization effect between organic and aqueous plant extracts at 600 mg/ml on *Aspergillus flavus* (UONV003) within different time durations. The bars on top of the graph indicate standard error of the means.

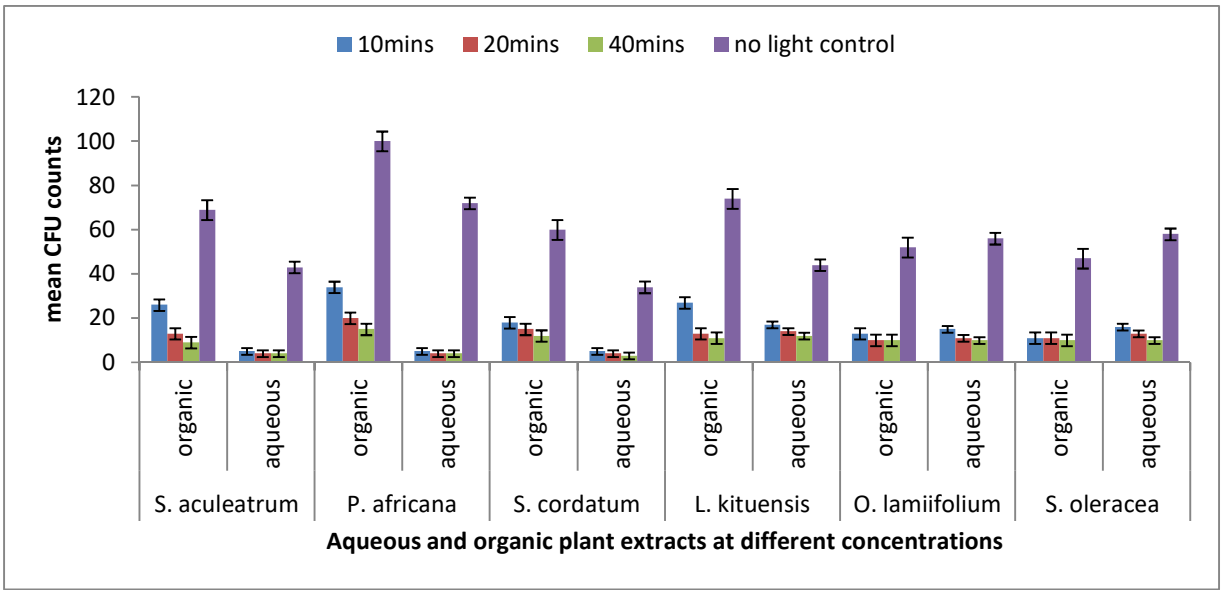

Figure 13: Comparison of photosensitization activity between organic and aqueous plant extracts at 450 mg/ml on *Aspergillus flavus* (UONV003) within different time durations. The bars on top of the graph indicate standard error of the means.

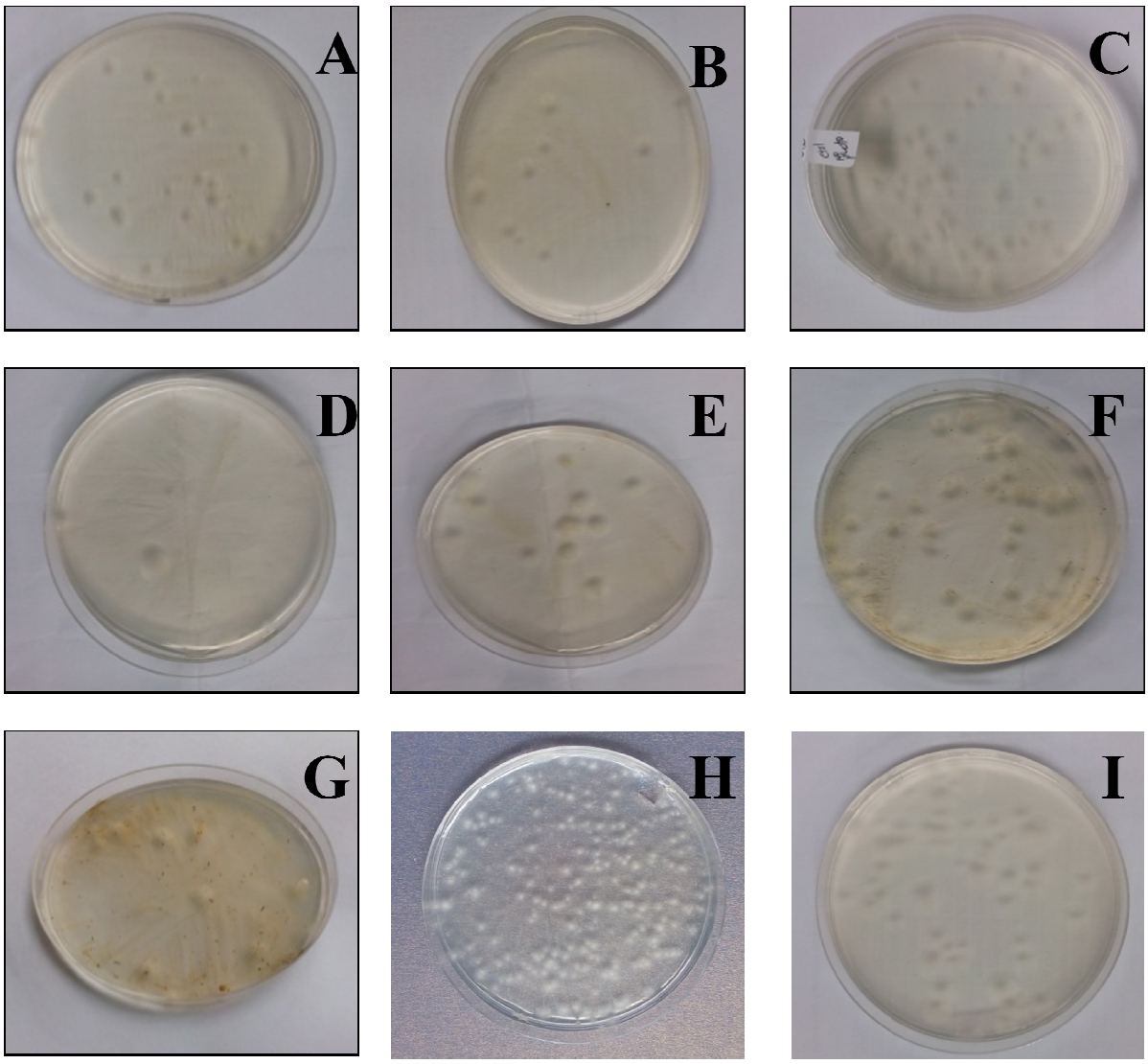

Figure 14: Comparison of photosensitization (CFU's) activity of some of the plant extracts with no light controls at different concentrations and different time durations: (A) *Spinacia oleracea* 600 mg/ml on A. flavus UON017 (20 mins), (B) *Solanum aculeastrum* 600 mg/ml on *A. flavus* UON017 (20mins), (C) *Solanum aculeastrum* control on *A. flavus* UON017, (D) *Syzygium cordatum* 600 mg/ml on *A. flavus* UON017 (40 mins), (E) *Syzygium cordatum* 450 mg/ml on *A. flavus* UON003 (40 mins), (F) *Syzygium cordatum* control on *A. flavus* UON003, (G) *Prunus africana* 600 mg/ml on *A. flavus* UON003 (40 mins), (H) *Spinacia oleracea* control on *A. flavus* UONV017, (I) *Prunus africana* control on *A. flavus* UON003

4.3 Phytochemical screening of selected medicinal plant extracts

The phytochemical screening of the six medicinal plant extracts showed the presence of several bioactive compounds in both organic and aqueous forms. All the plants had a minimum of two bioactive compounds. The organic extract of *S. oleracea, L. kituensis and S. aculeastrum* exhibited presence of all the bioactive compounds tested. The bioactive agents they contained are: saponins, flavonoids, terpenoids, tannins, alkaloids and glycosides (Table 11). The key below the table gives codes and names of the medicinal plants tested.

Table 11: **Frequency of bioactive compounds in plant extracts**

		FREQUENCY	PERCENT	CUMULATIVE PERCENT
PLANTS	*Prunus africana*	5	9.8	9.8
	Lippia kituensis	7	13.7	23.5
	Solanum aculeastrum	10	19.6	43.1
	Syzygium cordatum	9	17.6	60.8
	Spinacia oleracea	11	21.6	82.4
	Ocimum lamiifolium	9	17.6	100
	TOTAL	**51**	**100**	
BIOACTIVE COMPOUNDS	Saponins	8	15.7	15.7
	Tannins	9	17.6	33.3
	Flavonoids	11	21.6	54.9
	Alkaloids	10	1.96	74.5
	Glycosides	8	15.7	90.2
	Terpenoids and steroids	5	9.8	100
	TOTAL	**51**	**100**	
FORMULATION	Organic	31	60.8	60.8
	Aqueous	20	39.2	100
	TOTAL	**51**	**100**	

Key

Code	**Plant**	
01	*Ocimum lamiifolium*	+: positive
02	*Lippia kituensis*	-: negative
03	*Solanum aculeastrum*	Aqu: Aqueous
04	*Syzygium cordatum*	Org: Organic
05	*Spinacia oleracea*	
06	*Prunus africana*	

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Microorganisms inclusive of some fungal strains are the major causes of food losses in fields and markets. Production of mycotoxins by these microorganisms leads to loss of human and animal lives (Temba *et al*., 2015). This study revealed that the selected medicinal plant extracts had antifungal activity against toxigenic *A. flavus.* The organic plant extracts assayed indicated antifungal activity which varied depending on concentration and plant species. Some of the extracts had antifungal activity against strain UONV003 that was not significantly different with the standard antifungal control Apron star 250 mg/ml. Of all the six organic plant extracts, five had significant ($P \leq 0.05$) antifungal activity at 600 mg/ml, three had significant ($P \leq 0.05$) antifungal activity at 450 mg/ml and two had significant ($P \leq 0.05$) antifungal activity at the lowest concentration of 300 mg/ml. Activity of the extracts against strain UONV017 also displayed significantly good results. Out of the six plants, five had significant ($P \leq 0.05$) antifungal activity at 600 mg/ml and also at 450 mg/ml. At 300 mg/ml only three plants had significant ($P \leq 0.05$) antifungal activity. At 600 mg/ml the antifungal activity of *S. aculeastrum* and *S. cordatum* had no significant difference with the standard antifungal control Apron star. This supports the significance of crude plant extracts in lowering risk of toxigenic *A. flavus* hence lowering aflatoxin contamination (El Nagerabi *et al*., 2013). The effectiveness of most plant extacts was directly proportional with the concentration which parallels a study by Kiswii *et al*., 2014. However, the aqueous plant extracts did not show significant antifungal activity.

The success of finding specific bioactive compounds in plant extracts is highly dependent on the solvent used for extraction. In this study Dichloromethane: methanol (1:1v/v) was used for the organic extraction while distilled water was used for the aqueous extraction. Organic extracts had

significant antifungal activity while aqueous extracts had no significant antifungal activity. This could be related to a study that indicated that aqueous (water) extracts have low extracting capacity compared to methanol. Concentration of the active ingredients is also lower in water than in organic solvents. The study indicates that most active ingredients dissolve better in organic solvents than in water (Kalidindi *et al*., 2015). Another study carried out using organic and aqueous neem leaf extracts against four *Aspergillus* species including *A. flavus*, *A. terreus*, *A. fumigatus* and *A.niger* indicated that inhibition caused by organic extracts was higher than the inhibition caused by aqueous extracts (Mahmoud *et al*., 2011)

Preliminary phytochemical screening indicated presence of various bioactive compounds in the plant extracts at varying percentages. The agents were; tannins, flavonoids, alkaloids, glycosides, steroids and saponins. The bioactive compounds detected in this study have been shown to cause antimicrobial activities in other studies through various mechanisms (Shobowale *et al*., 2010, Zhang *et al*., 2009, Handali *et al*., 2013). Increase in the concentration of bioactive compounds increases the antifungal activity as reported by (Zhaoxiu *et al*., 2000). This supports the increase in antifungal activity caused by increase in plant extract concentration in our study and the greater antifungal activity of organic extracts which had a higher percentage (60.8%) of bioactive compounds, compared to the aqueous extracts (39.2%).

The plant (fruits) extracts of *S. aculeastrum* had the highest antifungal activity against both strains of toxigenic *A. flavus.* This agrees with the results of (Koduru *et al*., 2006) where organic and aqueous fruit extracts of *S. aculeastrum* were tested against fungi and bacteria and they had good antimicrobial activity. The organic extracts had much better activity compared to the aqueous extracts. Methanol and acetone extracts inhibited fungal growth while aqueous extracts did not. *Solanum aculeastrum* in this study contained bioactive compounds like tannins,

saponins, alkaloids, steroids, glycoside and flavonoids according to the phytochemical screening. These could be the possible cause of the antifungal activity. The glycosides, alkaloids and saponins in *S. aculeastrum* fruits have been associated with anticancer (skin and cervix), anti inflammatory and anti cholesterol activities (Omosa *et al*., 2016). The active compounds in the plant can hence be produced in large masses and isolated then used against the toxigenic *A. flavus*.

Syzygium cordatum bark extracts had significant antifungal activity against both *A. flavus* strains. This agrees with (Steenkamp *et al*., 2007) where *S. cordatum* bark (organic extract) exhibited antifungal activity. In this study, *S. cordatum* contains saponins, tannins, terpenoids, alkaloids and steroids. These as supported by (Oliveira *et al*., 2007) were the causes of antifungal activity. Other *Syzygium* species extracts like *S. jambolanum* seeds have proved antifungal activity (Chandrasekaran *et al*., 2004). *Syzygium cumini* alcoholic extracts also have proved antifungal activity against *Candida* sp. (Oliveira *et al*., 2007) hence supporting the antifungal activity of the *Syzygium* sp.

Organic extracts of *P. africana, L. kituensis, S. oleracea* and *O. lamiifolium* had antifungal activity against both strains of *A. flavus*. They all had varying levels of bioactive compounds which were attributed to the varying levels of antifungal activity and this justifies the use of these plant extracts in ethnomedicine. The percentage of bioactive compounds (9.8%) in *P. africana* was the lowest and this could be attributed to the low antifungal activity by this extract. Organic extracts of *P. africana* against *A. flavus* UONV017 had higher antifungal activity at 450mg/ml than at 600 mg/ml. This could have been caused by thickness in the higher concentration which interfered with diffusion. *Spinacia oleracea* extracts had the highest percentage of bioactive compounds (21.6%). Despite having the highest percentage of bioactive compounds, it did not

prove the highest level of antifungal activity. This could be attributed to presence of the bioactive compounds in small amounts. Studies by (Chapagain *et al*., Iqbal *et al*., 2012Amabye & Said, 2015) have proved that bioactive compounds in these plant extracts cause them to have antifungal activity.

Prunus africana methanol leaf extracts have been proven non-toxic according to a study carried out by (Nabende *et al*., 2015). This makes it a safe alternative to use in control of *A. flavus* in food and feed stuff. *Prunus laurocerus* which is in the same genus as *P. africana* has proved antifungal activity against several fungi strains including *Aspergillus sp*. Organic extracts of the leaf extract had more antifungal activity compared to the aqueous extracts (Sahan, 2011). This supports the results in this study where organic *P. africana* extracts had antifungal activity against *A. flavus*. *Prunus africana* leaves extract in this study according to phytochemical analysis contained five bioactive compounds namely; tannins, saponins, flavonoids, alkaloids and glycosides. These could be attributed to the antifungal activity hence justification on use of *P. africana* leaves in ethnomedicine. Organic extracts of *P. africana* against *A. flavus* UONV017 had higher antifungal activity at 450 mg/ml than at 600 mg/ml. This could have been caused by higher viscosity in the higher concentration (600 mg/ml) which interfered with diffusion hence a small diameter of zone of inhibition.

Lippia kituensis in this study contained tannins, saponins, glycosides, alkaloids and flavonoids and this is supported by (Kosgei, 2014) where these bioactive compounds in *L. kituensis* leaves caused its activity against parasites. In the same study the *L. kituensis* leaf extracts showed no toxicity against vero cells at 500 ug/ml hence the extract is considered safe for use. Organic leaf extracts of other species of Lippia like *Lippia alba* have exhibited antifungal activities against *Candida sp*. and *Cryptococcus sp*. (Oliveira *et al*., 2014).

Spinacia oleracea exhibited some antifungal activity against both strains of toxigenic *A. flavus*. *Spinacia oleracea* being a consumable and nutritious vegetable makes its efficacy significant. The organic extracts had greater antifungal activity compared to the aqueous extracts. This parallels a study that proved that organic *S. oleracea* leaf extracts had stronger antimicrobial activity compared to the aqueous extracts (Iqbal *et al*., 2012). Aqueous and ethanol extracts of *S. oleracea* leaves have also been proved to exhibit antifungal activity on *A. niger* and *S. cerevisae* (Bader *et al*., 2016). *Spinacia oleracea* has antioxidants which are able to bind toxigenic molecules and inactivate them.The antioxidants include; carotenoid, peptides and some phenolic compounds (Bader *et al*., 2016). The phytochemical screening also revealed that *S. oleracea* leaf extracts contain tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids and steroids. Presence of these bioactive compounds and antioxidants could be attributed to the antifungal activity of *S. oleracea*.

Ocimum lamiifolium organic leaf extracts had significant antifungal activity. The phytochemical analysis revealed presence of saponins, tannins and alkaloids. The bioactive compounds in the other extracts used in this study were in greater varieties compared to those of *O. lamiifolium* and this could be the cause of the lower antifungal activity. The extraction methods used in this study may not have maximized on extracting all useful bioactive compounds in *O. lamiifolium*. In other studies however, methanol leaf extracts of *O. lamiifolium* exhibited some antimicrobial activity against several bacterial species. Several phenolic compounds were also found in the extracts and attributed to the antimicrobial activity (Amabye and Said, 2015).

Efficacy of plant extracts is dependent on solvent used for extraction. In this study, organic extracts exhibited significant antifungal activity while the aqueous extracts did not. The organic extracts had a higher percentage of bioactive compounds (60.8%) than the aqueous extracts

(39.2%). This supports earlier findings that organic leaf extracts had stronger antimicrobial activity compared to aqueous extracts (Shobowale *et al*., 2013). The reason for this sequence in higher antifungal activity in organic than aqueous extracts is because bioactive compounds that cause the antimicrobial activity dissolve more easily in organic than aqueous solvents (Iqbal *et al*., 2012). Different solvents have varying levels of solubility for different bioactive compounds (Shobowale *et al*., 2013).

The antifungal activity was directly proportional to the concentration of the plant extracts apart from two instances which were attributed to viscosity. This observation supports findings in a study carried out by (Mahmoud *et al*., 2011) and (Kiswii *et al*., 2009). This could be explained by the fact that the high concentration contains a higher percentage of the bioactive compounds.

In the phytochemical analysis in this study, tannins, saponins and alkaloids were detected in all the plant extracts. Tannins have been reported to prevent microorganism development by causing unavailability of useful proteins to the organism and precipitation of the microbial protein (Shabowale *et al*., 2013) in (Sodipo *et al*., 1991). Saponin rich extracts have proved inhibition on several phyto pathogenic fungi species like *Fusarium sp., Alternaria sp., Colletotrichum sp*. and *Verticillium sp*. (Chapagain *et al*., 2007). Increase in saponins concentration increases the antifungal activity as reported by (Handali *et al*., 2013). This supports the increase in antifungal activity caused by increase in plant extract concentration in our study.

Alkaloids extracted from seeds have proved antifungal activity against five plant pathogenic fungi which include; *Fusarium sp., Alternaria sp., Bipolaris sp., Exserohilum sp.* and *Sclerotinia sp*. (Zhang *et al*., 2009). Alkaloids and phenols extracted from black pepper also proved antifungal activity against thirteen fungal species isolated from dermal infections. The antifungal

activity increased with an increase in the concentration of the bioactive compounds as reported by (Shanmugapriya *et al*., 2012).

Flavonoids, terpenoids, steroids and glycosides were present in only some of the plant extracts in this study. Flavonoids isolated from *Mononthotaxis litorralis* exhibited antifungal activity against three species of mycotoxigenic fungi of maize namely; *Fusarium sp., Aspergillus sp*. and *Penicilium sp*. The antifungal activity was dependent on the concentration of the flavonoids (Chepkirui *et al*., 2014). Terpenoids have also been reported to inhibit activity of yeast and dermatophytes through cell wall destruction (Miron *et al*., 2014). The activity of terpenoids is through inactivation of cellular enzymes. Terpenoids are reported to cause disruption of membranes and cellular integrity hence causing death of the microbes (Jinikuti and Archana., 2013). A steroid was tested against two *Aspergillus sp*., *Fusarium sp*. and *Candida sp*. Water and alcohol extracts were used and antifungal activity was higher in the steroid from alcohol extract compared to that of water extract (Subhisha and Subramoniam, 2015). A steroidal glycoside has also showed antifungal activity against human pathogenic fungi, dermatophytes, filamentous species and yeasts as reported by (Favel *et al*., 2005).

The studies above parallel our findings that bioactive compounds in plants are the key to antifungal activity. The higher the concentration of bioactive compounds, the higher the antifungal activity.

Organic plant extracts were tested for minimum inhibitory concentration since they had significant antifungal activity. *Solanum aculeastrum* that had the highest antifungal activity recorded the lowest MIC value of 25 mg/ml for strain UONV017 and 50 mg/ml for strain UONV003. *Syzygium cordatum* also had a low MIC of 50 mg/ml against strain UONV017. This

is supported by (Kiswii *et al*., 2009) where the extract with the highest antifungal activity had the lowest MIC value. The low MIC could be attributed to the enhanced antifungal activity.

Strain UONV003 of *A. flavus* proved to be more resistant compared to *A. flavus* UONV017 in terms of inhibition diameters and also MIC though both are toxigenic strains. This could be explained by the fact that pathogenicity varies between different strains of *A. flavus* and strains with higher pathogenicity may exhibit higher resistance. This is supported by a study that was carried out to test pathogenicity and toxigenicity of ground nut *A. flavus* strains. Results showed that there was variation in pathogenicity within the same toxigenic strains (ZhaoXiu *et al*., 2010)

In this study, photosensitization which is a technique for inactivating pathogenic microorganisms was tested. It involves hitting of a photosensitizer by light of a specific wavelength which makes the photosensitizer reactive thereby killing the toxigenic cells (Jong *et al*., 2013). The technique has been reported as a safe and a potential control of mycotoxigenic fungi (Temba *et al*., 2016). However, very few photosensitisers have been approved clinically for use against toxigenic microbes hence need for researchers to identify photosensitisers. The photosensitization activity of six medicinal plant extracts was tested in this study. Aqueous and organic extracts from the different plant parts were used including *S. oleracea* which is a consumable vegetable.

For the organic extracts against strain UONV017, *S. cordatum* bark was the most inhibitive at 600 mg/ml and 400 mg/ml with only (mean = 4 CFU) and (mean = 7 CFU) respectively compared to the no light control that had (mean = 51 CFU). *Syzygium cordatum* which had significant inhibition even in the aqueous form against both strains have been reported to have several antimicrobial abilities (Steenkamp *et al*., 2007). *Solanum aculeastrum* was the second most effective against both strains in the organic form and the most effective in the aqueous form

against both strains. Colony Forming Units counts of the aqueous forms against strain UONV017 were (mean = 2 CFU's) and (mean = 3 CFU's) at 600 mg/ml and 450 mg/ml respectively. This was a low CFU count compared to the no light control which was (mean = 35 CFU's). *Solanum aculeastrum* against strain UONV003 had a CFU count of (mean = 4 CFU's) in both 600 mg/ml and 400 mg/ml which was low compared to the control (mean = 43 CFU). *Solanum aculeastrum* posses several antimicrobial activities and of significance to this study, the fruit extracts possess antifungal activity against *Aspergillus spp*. (Koduru *et al*., 2006).

Both aqueous and organic extracts of the other four plants; *O. lamiifolium, S. oleracea, P. africana* and *L. kituensis* had significant reduction of spores as they had low CFU counts compared to the no light control against both strains. All the named plants have been reported to possess several antimicrobial activities (Iqbal *et al*., 2012; Oliveira *et al*., 2014; Sahan, 2011; Mequanint *et al*., 2011).

The photo degrading effect of plant extracts in this study is supported by a study where plant extracts that affect the central nervous system were tested for photo protection and photosensitization. They were tested at wavelengths ranging from 280 nm to 436 nm. The plants showed photo protection effect at low concentration and photosensitization effects at the higher concentrations (Bol'shakova *et al*., 1996). This also parallels this study where the photosensitization effect was directly proportional to the concentration of the plant extracts. This is explained by the fact that higher concentrations have high number of bioactive compounds which are activated by light to inactivate toxigenic microbes.

Photosensitization effect is attributed to the contents of the plant extracts which have strong absorption at high wavelength range (Bol'shakova *et al*., 1996). Plant extracts in this study all

exhibited the presence of several components (phytochemicals). These phytochemicals have proved antifungal activity and they have also been proven as photosensitisers. Secondary plant compounds with polyphenols have been reported to cause photosensitization in grazing animals exposed to UV or visible light. This occurs in animal cells after the animal feeds on plants with light absorbing molecule which become reactive with the animal cell (Quinn *et al*., 2014). Though damage of animal cells is a negative effect, in humans in this study, ingestion of *S. oleracea* then natural exposure to solar energy can lead to binding of aflatoxigenic molecules caused by *A. flavus* in the body.

Disinfection of water using the solar energy and plant extracts has been proved. Photodynamic activity was attributed to the presence of quinines and anthraquinones which generate singlet oxygen killing the microorganisms in the water. Two mililitres of plant extract per one litre of the polluted water was exposed to the solar energy for one hour to allow complete inactivation of the coli forms (Sunda *et al*., 2008). This parallels this study where plant extracts in combination with toxigenic molecules are exposed to light for a specific duration leading to reduced fungal growth.

In this study, the time of exposure of the *A. flavus* and plant extract under light was indirectly proportional to the CFU counts; this indicates that more light exposure led to more spore knock out. This parallels a study by (Temba *et al*., 2016) where increase in light dose increased the rate of spore knock out. In this study also, spore reduction was higher at the higher plant extract concentration of 600 mg/ml compared to the 450 mg/ml. In the study by (Temba *et al*., 2016), rate of spore knock out increased with the extract concentrations upto a certain level where the rate of spore knock out decreased with the highest concentration. This was attributed to the high fluid turbidity which may have caused reduced light penetration and transmission. In this study

therefore, the extract concentrations were at a suitable turbidity which did not cause much inhibition of light penetration and inhibition.

Extracts used in this study, have not been tested for photosensitization in another study. However, the extracts already have proved antimicrobial specifically antifungal activity. The plants also contain bioactive compounds of which some of the compounds have proved photosensitization effects. Alkaloids were tested for their photobiotic activity using *Chlamydomonas sp*. Toxicity of the compounds after exposure to UV light was determined by the cells ability to form colonies and mutagenicity was evident after the reaction (Schimmer *et al*., 1991).

Both aqueous and organic extracts were effective photosensitisers with no significance difference in their activity. This is supported by a study by (Temba *et al*., 2016) where both organic and aqueous extracts were effective though the aqueous extracts had better activity.

The findings of this study could fulfill the need of new antifungal methods due to unavailability of effective antifungal agents against toxigenic *A. flavus*, resistance of the fungi to the available methods and the shortcomings of these methods (Razzaghi and Rai, 2013). The plants in the selected region were correlated with the biodiversity of the region and they are accessible for research and new developments. Bioactive compounds are present in the plants; this is of key importance because bioactive compounds in higher plants are biodegradable and selectively toxic as reported by (Razzaghi and Rai, 2013). Toxicity levels of the plants used could be tested to ensure safety in use.

5.2 Conclusions

In this study, the selected medicinal plant extracts were found to possess antifungal activity against growth of toxigenic *A. flavus.* Five out of the organic extracts had significant antifungal activity. This makes a good background for research on aflatoxins because aflatoxins also dissolve better in organic extracts compared to aqueous extracts. The extracts were found to contain bioactive compounds which were the specific causes of antifungal activity. Isolation of these useful bioactive compounds using the phytochemical results is of ultimate importance. The bioactive compounds can then be produced in larger quantities for use in the control of the toxigenic fungi. The study challenges for screening of more medicinal plants for identification of more useful bioactive compounds with antimicrobial activity and purification of the already identified ones, as this portion of all medicinal plants in Central, Kenya have shown high quantities of bioactive compounds with antimicrobial activity. Only 10% of all traditional medicine has been evaluated pharmacologically. The bioactive compounds in this study also acted as good photosensitisers in both aqueous and organic forms because they led to a lower number of colony forming units (CFU) compared to the non treated controls. This could be an indicator that the light energy was strong enough to activate the bioactive compounds in the extracts even in the aqueous form. The visible light in the process of photosensitization was able to enhance the antifungal properties of the selected medicinal plant extracts.

5.3 Recommendations

1. The bioactive compounds should be isolated using the guidance of phytochemical results from the tested plant extracts from which simple formulations can be developed after purification and standardization as new antifungal agents against toxigenic *A. flavus*.

2. Ways of modifying the consumable plants found to have the bioactive agents should be developed so the plants can be produced in larger masses for sufficient use in antifungal activity against toxigenic *A. flavus*.

3. The consumable plant in this study *S. oleracea* can be ground and mixed with grains then dried under the sun which will lower aflatoxin contamination in storage.

4. Of all the plants used, those whose toxicity levels are unknown should be tested before use to ensure safety.

REFERENCES

Adjou, E. S., Kouton, S., Dahouenon-Ahoussi, E., Soumanou, M. M. and Sohounhloue, D. C., 2013. Effects of essential oils from fresh leaves of *Ocimum gratissimum L*. on Mycoflora during storage of peanuts in Benin. *Mycotoxin Research*, 29 (1), 29–38.

Adongo, O.S., 2013. *Medicinal Plants of Chuka community in Tharaka nithi county, Kenya and some of their selected Essential Elements* (Doctoral dissertation, KENYATTA UNIVERSITY).

Al-Fatimi, M., Wurster, M., Schröder, G. and Lindequist, U., 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology,* 111 (3), 657–66.

Al-fawwaz, A.T. and Khaled A. A., 2016. Antibacterial and antifungal effect of some natural extracts and their potential use as photosensitizers. *European Scientific Journal,* 12 (6)

Aliero, A.A. and Afolayan, A.J., 2006. Antimicrobial activity of *Solanum tomentosum*. *African Journal of Biotechnology*, *5*(4), 369.

Ali-Shtayeh, M. S., Yaghmour, R. M., Faidi, Y. R., Salem, K. and Al-Nuri, M. A., 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area." *Journal of Ethnopharmacology,* 60 (3), 265–71.

Amabye, T. G. and Said, M., 2015. In vitro antimicrobial efficacy of fractions from Demakese (*Ocimum lamifolium*) leaves extract from Mekelle Tigray, Ethiopia. *Natural Products Chemistry and Research* 2015

Anjorin, S. T., Makun, H. A. and Iheneacho, H. E., 2008. Effect of *Lippia* multiflora leaf extract and *Aspergillus flavus* on germination and vigour indices of *Sorghum bicolor*

[L](Moench). *International Journal of Tropical Agriculture and Food System,* 2 (1), 130–34.

Basappa, S.C., 2009. *Aflatoxins: formation, analysis and control*. Alpha Science International.

Bergquist, S., 2006. *Bioactive compounds in baby spinach (Spinacia oleracea)* (Vol. 2006, No. 62).

Bhatia, A. L. and Jain, M., 2004. *Spinacia oleracea* protects against Gamma radiations: a study on glutathione and lipid peroxidation in mouse liver. *Phytomedicine,* 11 (7), 607–15.

Bhatnagar, D., Cary, J. W., Ehrlich, K., Yu, J. and Cleveland, T. E., 2006. Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development. *Mycopathologia*, 162 (1), 155–66.

Bii, C., Korir, K. R., Rugutt, K. J. and Mutai, C., 2010. The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections. *Journal of Medicinal Plants Research*, 4 (11), 995–998.

Boisio, M. L., Esposito, M. and Merlo. F., 1993. [Separation and identifying features of the cardiac aglycones and glycosides of *Nerium oleander* flowers by thin-layer chromatography]. *Minerva Medica*, 84(11), 627–632.

Bol'shakova, I. V., Lozovskaia, E. L. and Sapezhinskiĭ, I. I., 1996. Photosensitizing and photoprotective properties of extracts from groups of medicinal plants. *Biofizika,* 42 (4), 926–32.

Carris, Lori M., Christopher, R. Little, and Carol, M. Stiles., 2012. Introduction to fungi. *The Plant Health Instructor*, 2012.

Chalannavar, R. K., Baijnath, H. and Odhav, B., 2011. Chemical constituents of the essential oil from *Syzygium cordatum* (Myrtaceae). *African Journal of Biotechnology,* 10 (14), 2741.

Chanda, S. and Baravalia, Y., 2010. Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms. *African Journal of Biotechnology*, 9 (21), 3210–3217.

Chandrasekaran, M., and Venkatesalu, V., 2004. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *Journal of Ethnopharmacology,* 91 (1), 105–8.

CDC., 2004. Outbreak of aflatoxin poisoning– Eastern and Central provinces, Kenya, January–July 2004.Morb. Mortal Weekly. Rep. 53, 790-793

Chapagain, B. P., Wiesman, Z. and Tsror, L., 2007. In vitro study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. *Industrial Crops and Products,* 26 (2), 109–15.

Chelala, C. A. and Margolin, P., 1983. Bactericidal photoproducts in medium containing riboflavin plus aromatic compounds and MnCl2. *Canadian Journal of Microbiology,* 29 (6), 670–75.

Clara, C., Matasyoh, J. C., Wagara, I. N. and Nakavuma, J., 2014. Antifungal activity of flavonoids isolated from *Monanthotaxis littoralis* against mycotoxigenic fungi from maize. *Am. J. Chem. Appl,* 1 (4), 54–60.

Cotty, P. J., 1990. Effect of atoxigenic strains of *Aspergillus flavus* on aflatoxin contamination of developing cottonseed. *Plant Disease,* 74(3), 233–235.

Cotty, P. J., Bayman, P., Egel, D. S. and Elias, K. S. (1994). "Agriculture, aflatoxins and *Aspergillus*." In *The Genus Aspergillus*, 1–27.

Cotty, P.J. and Jaime-Garcia, R., 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, *119*(1), pp.109-115.

Dambolena, J. S., Zunino, M. P., López, A. G., Rubinstein, H. R., Zygadlo, J. A., Mwangi, J. W., Thoithi, G. N., Kibwage, I. O., Mwalukumbi, J. N. and Kariuki, S. T.,2010. Essential oils composition of *Ocimum basilicum L.* and *Ocimum gratissimum L.* from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*. *Innovative Food Science and Emerging Technologies*, 11(2), 410–414.

de Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I. and Levenfors, J. J., 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology,* 96 (3), 461–69.

de Menezes, H. D., Rodrigues, G.B., de Pádua Teixeira, S., Massola, N. S., Bachmann, L., Wainwright, M. and Braga, G.UL., 2014. In Vitro Photodynamic Inactivation of Plant-Pathogenic Fungi *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* with Novel Phenothiazinium Photosensitizers. *Applied and Environmental Microbiology,* 80 (5), 1623–32.

Diener, U. L., Cole, R. J., Sanders, T. H., Payne, G. A., Lee, L. S. and Klich, M. A., 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annual Review of Phytopathology*, 25 (1), 249–270.

Dorner, J. W.,2004. Biological control of aflatoxin contamination of crops. *Toxin Reviews,* 23(2-3), 425–450.

Dowling, F. S.,1997. Fumonisin and its toxic effects. *Cereal Foods World,* 42, 13- 15

Duraipandiyan, V. and Ignacimuthu, S., 2011. Antifungal activity of traditional medicinal plants from Tamil Nadu, India. *Asian Pacific Journal of Tropical Biomedicine,* 1(2), 204–215.

Ehrlich, K.C., 2015. Non-aflatoxigenic Aspergillus flavus to prevent aflatoxin contamination in crops: advantages and limitations. *Global health issues of aflatoxins in food and agriculture: Challenges and opportunities*, p.25.

El-Nagerabi, S. A. F., Elshafie, A. E., AlKhanjari, S. S., Al-Bahry, S. N. and Elamin, M. R., 2013. The potential of Baobab (*Adansonia digitata L.*) extracts as biocontrol on the growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus*. *Journal of Food Research,* 2, (3), 93.

Favel, A., Kemertelidze, E., Benidze, M., Fallague, K. and Regli, P.,2005. Antifungal activity of steroidal glycosides from *Yucca gloriosa L. Phytotherapy Research,* 19 (2), 158–61.

FAO, 2004. United Nations Food and Agricultural Organization, evaluation of certain mycotoxins in food: fifty-sixth report of the joint FAO/WHO expert committee on food additives, World Health organization, Geneva.

Goldblatt, L. A., 1971. Control and removal of aflatoxin. *Journal of the American Oil Chemists' Society,* 48(10), 605–610.

Gurib-Fakim, A., 2008. Plant resources of tropical Africa: Medicinal plants 1. *PROTA Foundation, Wageningen, Netherlands*, 11 (1), 321–22.

Hall, G. S., 2011. *Interactions of Yeasts, Moulds, and Antifungal Agents: How to Detect Resistance*. Springer Science and Business Media.

Handali, S., Moghimipour, E., Sadaghi-Nejad, B., Ameri, A., Ramezani, Z. and Azemi, M. E., 2013. *In vitro* screening of anti-Candida activity of saponins extracted from *Glycyrrhiza glabra* and *Quillaja saponaria*. *Asian Journal of Pharmaceutical and Clinical Research,* 1 (2), 160–62.

Harborne, J. B., 1998. *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis*. Springer science and Business media.

Hasan, M. F., Das, R., Khan, A., Hasan, M. S. and Rahman, M., 2009. The determination of antibacterial and antifungal activities of *Polygonum hydropiper (L.)* root extract. *Advanced Biology Research,* 3 (1–2), 53–56.

Hell, K., Mutegi, C. and Fandohan, P., 2010. Aflatoxin control and prevention strategies in maize for Sub-Saharan Africa. *Julius-Kühn-Archiv*, (425), p.534.

Hell, K. and Mutegi, C., 2011. Aflatoxin control and prevention strategies in key crops of Sub-saharan Africa. *African Journal of Microbiology Research*, 5 (5), 459–466.

Herzallah, S.M., 2013. Aflatoxin B1 residues in eggs and flesh of laying hens fed aflatoxin B1 contaminated diet. *American Journal of Agricultural and Biological Sciences*, *8*, pp.156-161.

Hostettmann, K., Marston, A., Maillard, M. and Wolfender, J.-L., 1996. Search for molluscicidal and antifungal saponins from tropical plants. In *Saponins Used in Traditional and Modern Medicine*, 117–128.

Hua, S. S. (2013). "Biocontrol of *Aspergillus flavus* by *Pichia anomala*." *Book Chapter* 2, 1067–72.

Hua, S. S. T., Grosjean, O.-K. and Baker, J. L.,1999. Inhibition of aflatoxin biosynthesis by phenolic compounds. *Letters in Applied Microbiology,* 29 (5), 289–91.

Hua, S.S.T., Baker, J. L. and Flores-Espiritu, M., 1999. Interactions of saprophytic yeasts with a nor mutant of *Aspergillus flavus* applied and environmental microbiology, 65, 2738–2740.

Hugo J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I. and Levenfors, J. J., 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology,* 96 (3), 461–469.

Hussein, H. S. and Brasel, J. M., 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, 167 (2), 101–134.

Iqbal, M., Ghous, T., Khan, A. N. and Akhtar, K., 2012. Evaluation of antimicrobial activity of extracts of fresh and spoiled *Spinacia oleracea* against some mammalian pathogens. *African Journal of Microbiology Research,* 6 (29), 5847–51.

Ingram, V. J., Loo, J., Dawson, I., Muchugi, A., Duminil, J., Awono, A., Asaah, E., Tchoundjeu, Z. and Sunderland, T., 2015. Ensuring the future of the Pygeum ree (*Prunus africana*).

Jasprica, I., Smolčić-Bubalo, A., Mornar, A. and Medić-Šarić, M., 2004. Investigation of the flavonoids in croatian propolis by thin-layer chromatography. *JPC-Journal of Planar Chromatography-Modern TLC,* 17(2), 95–101.

Jinukuti, M. G. and Giri, A., 2013. Antimicrobial activity of phytopharmaceuticals for prevention and cure of diseases. *Ann. Phytomed*, 2 (2), 28–46.

Jong, W. W., Tan, P. J., Kamarulzaman, F. A., Mejin, M., Lim, D., Ang, I., Naming, M., Yeo, T. C., Ho, A. S. H. and Teo, S. H., 2013. Photodynamic activity of plant extracts from Sarawak, Borneo. *Chemistry and Biodiversity,* 10 (8), 1475–86.

Kaigongi, M. M., Dossaji, S. F., Nguta, J. M., Lukhoba, C. W. and Musila, F. M., 2014. Antimicrobial activity, toxicity and phytochemical screening of four medicinal plants traditionally used in Msambweni district, Kenya. *Journal of Biology, Agriculture and Healthcare,* 4(28).

Kalidindi, N., Thimmaiah, N. V., Jagadeesh, N. V., Nandeep, R., Swetha, S. and Kalidindi, B., 2015. Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn. leaves., *Journal of Food and Drug Analysis,* 23(4), 795–802.

Kariba, R. M., 2000) Antifungal activity of extracts from selected Kenyan medicinal plants, n.p

Kariuki, J. M., Kariuki, S. T., Muchiri, D. R. and Njoka, E.,2016. Field evaluation of naturally occurring mosquito repellents in Mt. Kenya region, Kenya. *International Journal of Biodiversity and Conservation,* 8 (2), 55–59.

Kiama, S. G., 2014. Evaluation of antimicrobial activity and toxicity of *Vernonia hymenolepis* (A. Rich) traditionally used for toothache in Kenya. University Of Nairobi.

Kiswii, M. T., Monda, E. O., Bii, C. and Alakonya, A. E., 2014. Efficacy of selected medicinal plants from Eastern Kenya against *Aspergillus flavus*,

Klich, M. A., 2007. *Aspergillus flavus*: The major producer of aflatoxin. *Molecular Plant Pathology*, 8 (6), 713–722.

Ko, S-H., Park, J-H., Kim, S-Y., Lee, S. W., Chun, S-S. and Park, E., 2014. Antioxidant effects of Spinach (*Spinacia oleracea L.)* supplementation in hyperlipidemic rats. *Preventive Nutrition and Food Science,* 19 (1), 19–26.

Koduru, S., Grierson, D. S. and Afolayan, A. J., 2006. Antimicrobial activity of *Solanum aculeastrum*. *Pharmaceutical Biology,* 44 (4), 283–286.

Kosgei, C. J., 2014. Phytochemical screening, cytotoxicity studies and larvicidal activity of hexane extract of *Lippia kituiensis* against *Rhipicephalus appendiculatus*. *Journal of Biomedical and Pharmaceutical Research,* 3 (4).

Krogh, P., 2013. *Control of Mycotoxins: Special Lectures Presented at the Symposium on the Control of Mycotoxins Held at Göteborg, Sweden, 21—22 August 1972*. Elsevier.

Lizárraga-Paulín, E.G., Moreno-Martínez, E. and Miranda-Castro, S.P., 2011. *Aflatoxins and their impact on human and animal health: An emerging problem*. INTECH Open Access Publisher.

Lizárraga-Paulín, E. G., Lara-Sagahón, A. V., Moreno-Martínez, E., Torres-Pacheco, I. and S. P. Miranda-Castro, S. P., 2013. *Novel Methods for Preventing and Controlling Aflatoxins in Food: A Worldwide Daily Challenge*. Intech Open Access Publisher.

Lucca, A. J., Carter-Wientjes, C., Williams, K. A. and Bhatnagar, D., 2012. Blue light (470 Nm) effectively inhibits bacterial and fungal growth. *Letters in Applied Microbiology* ,55 (6), 460–66.

Machida, M., Yamada, O. and Gomi, K., 2008. Genomics of *Aspergillus oryzae*: Learning from the history of Koji mold and exploration of its future. *DNA Research,* 15 (4), 173–83.

Machida, Masayuki and Katsuya Gomi., 2010. *Aspergillus: Molecular Biology and Genomics*. Horizon Scientific Press, 2010.

Magan, N. and Aldred, D., 2007. Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology,* 119 (1), 131–39.

Mahesh, B. and Satish, S., 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences,* 4 (5), 839–843.

Mahmoud, D. A., Hassanein, N. M., Youssef, K. A. and Zeid, A., 2011. Antifungal activity of different Neem leaf extracts and the nimonol against some important human pathogens. *Brazilian Journal of Microbiology*, 42 (3), 1007–16.

Martins, H. M., Martins, M. L. and Bernardo, F. A., 2000. Interaction of strains of non-toxigenic *Aspergillus flavus* with *Aspergillus parasiticus* on aflatoxin production. *Brazilian Journal of Veterinary Research and Animal Science,* 37 (6), 101–10.

Maxwell, S.M., Apeagyei, F., De Vries, H.R., Mwanmut, D.D. and Hendrickse, R.G., 1989. Aflatoxins in breast milk, neonatal cord blood and sera of pregnant women. *Journal of Toxicology: Toxin Reviews*, *8*(1-2), pp.19-29.

Mburu, B.K., Kung'u, J.B. and Muriuki, J.N., 2015. Climate change adaptation strategies by small-scale farmers in Yatta District, Kenya. *African Journal of Environmental Science and Technology*, *9*(9), pp.712-722.

Mdee, L. K., Masoko, P. and Eloff, J. N., 2009. The activity of extracts of seven common invasive plant species on fungal phytopathogens. *South African Journal of Botany,* 75 (2), 375–379.

Mequanint, W., Makonnen, E. and Urga, K., 2011. *In vivo* anti-inflammatory activities of leaf extracts of *Ocimum lamiifolium* in mice model. *Journal of Ethnopharmacology,* 134 (1), 32–36.

Miron, D., Battisti, F., Silva, F. K., Lana, A. D., Pippi, B., Casanova, B., Gnoatto, S., Fuentefria, A., Mayorga, P. and Schapoval, E. E. S., 2014. Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. *Revista Brasileira de Farmacognosia,* 24 (6), 660–67.

Mortimer, P. H. and Ronaldson, J. W., 1983. Fungal-Toxin-Induced Photosensitization. *Handbook of Natural Toxins* (1), 361–419.

Munkvold, G. P., 2003. Cultural and genetic approaches to managing mycotoxins in maize. *Annual review of phytopathology*, *41*(1), 99-116.

Musabayane, C. T., Mahlalela, N., Shode, F. O. and Ojewole, J. A. O., 2005. Effects of *Syzygium cordatum* (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology,* 97 (3), 485–490.

Mwitari, P. G., Ayeka, P. A., Ondicho, J., Matu, E N. and Bii, C. C., 2013. Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: *Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus,* plos one 8 (6), e65619

Nabende, P. N., Karanja, S. M., Mwatha, J. K. and Wachira, S. W., 2015. Anti-proliferative activity of *Prunus africana*, *Warburgia stuhlmannii* and *Maytenus senegalensis* extracts in breast and colon cancer cell lines. *European Journal of Medicinal Plants,* 5 (4), 366.

Ngindu, A., Johnson, B. K., Kenya, P. R., Ngira, J. A., Ocheng, D. M. and Nandwa, H., 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet,* 1(8285), 1346 - 1348.

Njuguna, C., 2010. Making Kenyan maize safe from deadly aflatoxins. *Science in Africa*.

Nostro, A., Germano, M. P., D'angelo, V., Marino, A. and Cannatelli, M. A., 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology,* 30 (5), 379–384.

Program, National Toxicology. (2011). NTP 12th Report on Carcinogens. *Report on Carcinogens: Carcinogen Profiles/US Dept. of Health and Human Services, Public Health Service, National Toxicology Program* 12 (2011)

Odhiambo, J. A., Siboe, G. M., Lukhoba, C. W. and Dossaji, S. F., 2009. Antifungal activity of crude extracts of selected medicinal plants used in combinations in lake Victoria basin, Kenya. *Plant Product Research Journal,* 13, 35–43.

Ogunlesi, M., Okiei, W., Ofor, E. and Osibote, A. E., 2009. Analysis of the essential oil from the dried leaves of *Euphorbia hirta Linn* (Euphorbiaceae), a potential medication for Asthma. *African Journal of Biotechnology,* 8 (24).

Okoth, S., Nyongesa, B., Ayugi, V., Kang'ethe, E., Korhonen, H. and Joutsjoki, V., 2012. Toxigenic potential of *Aspergillus* species occurring on maize kernels from two agro-ecological zones in Kenya. *Toxins*, 4(11), 991-1007.

Oliveira, G. T. de., Ferreira, J. M. S., Rosa, L. H., de Siqueira, E. P., Johann, S. and Lima, L. A. R. d. S., 2014. *In vitro* antifungal activities of leaf extracts of *Lippia alba* (Verbenaceae)

against clinically important yeast species. *Revista Da Sociedade Brasileira de Medicina Tropical,* 47 (2), 247–50.

Oliveira, G. F. de., Furtado, N. A. J. C., Filho, A. A. da S., Martins, C. H. G., Bastos, J. K. and Cunha, W. R., 2007. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) Leaves Extract. *Brazilian Journal of Microbiology*, 38 (2), 381–84.

Omosa, L. K., Midiwo, J. O., Masila, V. M., Gisacho, B. M., Munayi, R., Chemutai, K. P., Elhaboob, G., Saeed, M. E. M., Hamdoun, S. and Kuete, V., 2016. Cytotoxicity of 91 Kenyan indigenous medicinal plants towards human CCRF-CEM leukemia cells. *Journal of Ethnopharmacology,* 179, 177–96.

Omwenga, O. E., Mariita, R. M., Alaro, L. and. Okemo, P. O., 2011. Evaluation of methanolic extracts of six medicinal plants used by herbal practitioners in Central province-Kenya. *International Journal of Pharmaceutical Sciences and Research*, 2 (4), 867–874.

Onaran, A. and Sağlam, H. D., 2016. Antifungal activity of some plant extracts against different plant pathogenic fungi, n.d.

Opoku, F. and Akoto, O., 2015. Antimicrobial and phytochemical properties of *Alstonia boonei* Extracts. *Organic Chemistry: Current Research* 1 (137), 2161-0401

Ormond, A. B. and Freeman, H. S., 2013. Dye sensitizers for photodynamic therapy. *Materials,* 6 (3), 817–40.

Paranagama, P. A., Abeysekera, K. H. T., Abeywickrama, K. and Nugaliyadde, L., 2003. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemon grass) against *Aspergillus flavus* Link. Isolated from Stored Rice. *Letters in Applied Microbiology,* 37 (1), 86–90.

Parekh, J. and Chanda, S., 2008. In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African Journal of Biotechnology,* 7 (23).

Park, C., Yeo, J-D., Park, M-H., Park, J-B. and Lee, J-H., 2010. Effects of riboflavin photosensitization on Daidzein and its photosensitized derivatives. *Journal of Food Science,* 75(8), 659–66.

Paulo, S., 2007. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. *Braz. J. Microbiol*, 38, 2–5.

Pilau, M. R., Alves, S. H., Weiblen, R., Arenhart, S., Cueto, A. P. and Lovato, L. T., 2011. Antiviral activity of the *Lippia graveolens* (Mexican Oregano) essential oil and its main compound carvacrol against human and animal viruses. *Brazilian Journal of Microbiology,* 42 (4), 1616–24.

Pitt, J. I., 2000. Toxigenic fungi and mycotoxins. *British Medical Bulletin,* 56 (1), 184–92.

Prabhakar, K., Kumar, L. S., Rajendran, S., Chandrasekaran, M., Bhaskar, K. and Khan, AK. S., 2008. Antifungal activity of plant extracts against *Candida* species from oral lesions. *Indian Journal of Pharmaceutical Sciences,* 70 (6), 801.

Probst, C., Bandyopadhyay, R., Price L. E, and Cotty, P. J., 2011. Identification of atoxigenic *Aspergillus flavus* isolates to reduce aflatoxin contamination of maize in Kenya. *Plant Disease*, 95 (2), 212–218.

Probst, C., Schulthess, F. and Cotty, P. J., 2010. Impact of *Aspergillus* section *Flavi* community structure on the development of lethal levels of aflatoxins in Kenyan maize (*Zea mays).* *Journal of Applied Microbiology*, 108 (2), 600–610.

Quinn, J. C., Kessell, A. and Weston, L. A., 2014. Secondary plant products causing photosensitization in grazing herbivores: their structure, activity and regulation. *International Journal of Molecular Sciences,* 15 (1), 1441–65.

Ramesh, J., Sarathchandra, G. and Sureshkumar, V., 2013. Survey of market samples of food grains and grain flour for Aflatoxin B1 contamination. *Int. J. Curr. Microbiol. Appl. Sci*, *2*(5), pp.184-188.

Razzaghi-Abyaneh, M. and Rai. M., 2013. *Antifungal Metabolites from Plants*. Springer

Reddy, K. R. N., Farhana, N. I. Salleh, B. and Oliveira, C. A. F., 2010. Microbiological control of mycotoxins: present status and future concerns. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology,* 2, 1078–86.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K. and Chinou, I., 2010. Chemical composition and antimicrobial activity of the essential oils of four Ocimum Species growing in Tanzania. *Food Chemistry*, 119(1), 311–316.

Rustom, I.Y., 1997. Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. *Food chemistry*, *59*(1), pp.57-67.

Sahan, Y., 2011. Effect of Prunus Laurocerasus L. (cherry Laurel) Leaf extracts on growth of bread spoilage fungi. *Bulgarian Journal of Agricultural Science,* 17 (1), 83–92.

Sandosskumar, R., Karthikeyan, M., Mathiyazhagan, S., Mohankumar, M., Chandrasekar, G. and Velazhahan, R., 2007. Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B1 by the medicinal plant *Zimmu (Allium sativum L. × Allium cepa L.*). *World Journal of Microbiology and Biotechnology*, 23 (7), 1007–1014.

Satcher, D. (2000). “Food safety: A growing global health problem.” *JAMA,* 283 (14), 1817–1817.

Schaechter, M., Ingraham, J. L. and Neidhardt. F. C., 2006. *Microbe.* ASM press.

Schimmer, O. and Kühne, I., 1991. Furoquinoline alkaloids as photosensitizers in *Chlamydomonas reinhardtii*. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 249 (1), 105–10.

Schmale, D.G. and Munkvold, G.P., 2009. Mycotoxins in crops: A threat to human and domestic animal health. The plant health instructor, *3*, pp.340-353.

Sen, A. and Batra, A., 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach L*. *International Journal of Current Pharmarcy Research,* 4, (2), 67–73.

Seniya, C., Trivedia, S. S. and Verma, S. K., 2011. Journal of Chemical and Pharmaceutical Research. *Journal Chemistry,* 3 (6), 330–36.

Shanmugapriya, K., Saravana, P. S. Payal, H., Mohammed, P. and Williams, S. B., 2012. Antioxidant potential of Pepper (*Piper nigrum Linn*.) leaves and its antimicrobial potential against some pathogenic microbes. *Indian Journal of Natural Products and Resources,* 3 (4), 570–77.

Shinha, K. K. and Bhatnagar, D., 1998. *Mycotoxins in Agriculture and Food Safety*. CRC Press.

Shobowale, O. O., Ogbulie, N. J., Itoandon, E. E., Oresegun, M. O. and Olatope, S. O. A., 2013. Phytochemical and antimicrobial evaluation of aqueous and organic extracts of *Calotropis procera* leaf and latex. *Nigerian Food Journal,* 31 (1), 77–82.

Shukla, R., Kumar, A., Prasad, C. S., Srivastava, B. and Dubey, N. K., 2008. Antimycotic and antiaflatoxigenic potency of *Adenocalymma alliaceum* Miers. on fungi causing biodeterioration of food commodities and raw herbal drugs. *Int. Biodeteration & Biodegradation,* 62, 348-351

Sigei, E. C., Muturi, M. and Bii, C., 2015. Antifungal activities of *Camellia sinensis* crude extract, mixture with milk, on selected pathogenic and mycotoxic fungi. *Journal of Medicinal Plants Research,* 9 (42), 1070–80.

Simon, J. E., Koroch, A. R,. Acquaye, D., Jefthas, E., Juliani, R. and Govindasamy, R., 2007. Medicinal crops of Africa.

Sodipo, O. A., Akanji, M. A., Kolawole, F. B. and Odutuga, A. A., 1991. Saponin is the active antifungal principle in *Garcinia Kola*, heckle seed. *Biosci. Res. Commun,* 3, 171.

Stashenko, E., Ruiz, C., Munoz, A., Castaneda, M. and Martinez, J., 2008. Composition and antioxidant activity of essential oils of *Lippia origanoides* HBK grown in Colombia. *Natural Product Communications,* 3 (4), 563–66.

Steenkamp, V., Fernandes, A. C. and Van Rensburg, C. EJ., 2007. Screening of Venda Medicinal Plants for Antifungal Activity against *Candida albicans. South African Journal of Botany*, 73 (2), 256–258.

Subhisha, S. and Subramoniam, A., 2005. Antifungal activities of a steroid from *Pallavicinia lyellii*, a liverwort. *Indian Journal of Pharmacology,* 37 (5), 304.

Sunda, M., Rosillon, F. and Taba, K. M., 2008. Contribution to the water disinfection study by photosensitization with plants extracts. *European Journal of Water Quality= Journal Européen d'Hydrologie,* 39 (2), 199–209.

Tardivo, J. P., Giglio, A. D., de Oliveira, C. S., Gabrielli, D. S., Junqueira, H. C., Tada, D. B., Severino, D., Turchiello, R. de- F. and Baptista, M. S., 2005. Methylene blue in photodynamic therapy: from basic mechanisms to clinical applications. *Photodiagnosis and Photodynamic Therapy,* 2 (3), 175–91.

Temba, B. A., Fletcher, M. T., Fox, G. P., Harvey, J. JW. and Sultanbawa, Y., 2016. Inactivation of *Aspergillus flavus* spores by *Curcumin*-mediated photosensitization. *Food Control,* 59, 708–13.

Thembo, K. M., Vismer, H. F., Nyazema, N. Z., Gelderblom, W. C. A. and Katerere, D. R., 2010. Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. *Journal of Applied Microbiology*, 109 (4), 1479–1486.

Thippeswamy, S., Mohana, D. C., Abhishek, R. U. and Manjunath, K., 2014. Inhibitory activity of plant extracts on aflatoxin B1 biosynthesis by *Aspergillus flavus. Journal of Agricultural Science and Technology,* 16 (5),1123–32.

Towers, G. H. N., Page, J. E. and Hudson, J. B., 1997. Light-mediated biological activities of natural products from plants and fungi. *Current Organic Chemistry,* 1, 395–414.

Tzeng, D., and DeVay, J. E., 1989. Biocidal activity of mixtures of methionine and riboflavin against plant pathogenic fungi and bacteria and possible modes of action. *Mycologia*, 404–12.

Unnevehr, L., and Grace, D., (Eds.) 2013. *Aflatoxins: Finding solutions for improved food safety* (Vol. 20). Intl Food Policy Res Inst.

Van Egmond, H. P., Schothorst R. C. and Jonker, M. A., 2007. Regulations relating to mycotoxins in food. *Analytical and Bioanalytical Chemistry,* 389 (1), 147–157.

Wagacha, J. M., and Muthomi, J. W., 2008. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International journal of food microbiology*, *124*(1), 1-12.

Waliyar, F., Osiru, M., Ntare, B.R., Kumar, K.V.K., Sudini, H., Traore, A. and Diarra, B., 2014. Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, *8*(2), 245-252.

Wambugu, S. N., Mathiu, P. M., Gakuya, D.W., Kanui, T. I., Kabasa, J. D. and Kiama, S. G., 2011. Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya. *Journal of Ethnopharmacology,* 137 (2), 945–55.

Watson, R. R., and Preedy, V. R., 2009. *Bioactive Foods in Promoting Health: Fruits and Vegetables*. Academic Press.

Yard, E.E., Daniel, J.H., Lewis, L.S., Rybak, M.E., Paliakov, E.M., Kim, A.A., Montgomery, J.M., Bunnell, R., Abudo, M.U., Akhwale, W. and Breiman, R.F., 2013. Human aflatoxin exposure in Kenya, 2007: a cross-sectional study. *Food Additives & Contaminants: Part A*, *30*(7), pp.1322-1331.

Yin, Y., Yan, L., Jiang, J. and Ma, Z., 2008. Biological control of aflatoxin contamination of crops. *Journal of Zhejiang University Science B,* 9 (10), 787–92.

Yu, J., Cleveland, T. E., Nierman, W. C. and Bennett, J. W., 2005. *Aspergillus flavus* genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *Revista Iberoamericana de Micología,* 22 (4), 194–202.

Yu, J., Payne, G. A., Campbell, B. C., Guo, B. Z., Cleveland, T. E., Robens, J. F., Keller, N. P., Bennett, J. W. and Nierman, W. C., 2008. Mycotoxin production and prevention of

aflatoxin contamination in food and feed. *The Aspergilli: Genomics, Medical Aspects, Biotechnology, and Research Methods,* 26, 457–472.

Zain, M. E., 2011. "Impact of mycotoxins on humans and animals." *Journal of Saudi Chemical Society*, 15 (2), 129–144.

Zhang, J-W., Gao, J-M., Xu, T., Zhang, X-C., Ma, Y-T., Jarussophon, S. and Konishi, Y., 2009. Antifungal activity of alkaloids from the seeds of *Chimonanthus praecox*. *Chemistry and Biodiversity,* 6 (6), 838–45.

ZhaoXiu, T., Li GuangXing, J-RC., YuMei, K. and Wen, Z. Z., 2000. Toxigenicity and pathogenicity of groundnut *A. flavus* strain. *Fujian Journal of Agricultural Sciences,* 15 (2), 19–22.

APPENDICES

Appendix 1: Maximum acceptable levels of aflatoxin for selected countries (Aflatoxin B, unless otherwise stated) (FAO., 2002)

Country	Limit (ug/kg)	Foods
United kingdom	2	Nuts, dried figs and their products
	5	As above but intended for further processing
United states	20	Total aflatoxins in all foods
	0.5	Aflatoxin M1 in whole milk, low fat milk and skim milk
Australia	20	All foods except peanut products
	15	Peanut products
India	30	All foods
Japan	10	All foods
China	50	Rice, peanut, maize, sorghum, beans, wheat, barley, oats

Appendix 2: One way ANOVA: Zones of inhibition of organic plant extracts at 600mg/ml against strain UONV017

ANOVA

Diameters of zone of inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1423.083	6	237.181	40.055	.000
Within Groups	420.417	71	5.921		
Total	1843.500	77			

Appendix 3: One way ANOVA: Zones of inhibition of organic plant extracts at 600mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	545.615	6	90.936	22.588	.000
Within Groups	285.833	71	4.026		
Total	831.449	77			

Appendix 4: One way ANOVA: Zones of inhibition of organic plant extracts at 450mg/ml against strain UONV017

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1194.654	6	199.109	49.171	.000
Within Groups	287.500	71	4.049		
Total	1482.154	77			

Appendix 5: One way ANOVA: Zones of inhibition of organic plant extracts at 450mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	786.135	6	131.022	49.858	.000
Within Groups	186.583	71	2.628		
Total	972.718	77			

Appendix 6: One way ANOVA: Zones of inhibition of organic plant extracts at 300mg/ml against strain UONV017

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1384.404	6	230.734	46.201	.000
Within Groups	354.583	71	4.994		
Total	1738.987	77			

Appendix 7: One way ANOVA: Zones of inhibition of organic plant extracts at 300mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1145.212	6	190.869	24.854	.000
Within Groups	545.250	71	7.680		
Total	1690.462	77			

Appendix 8: One way ANOVA: Zones of inhibition of aqueous plant extracts at 600mg/ml against strain UONV017

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1510.750	6	251.792	38.969	.000
Within Groups	458.750	71	6.461		
Total	1969.500	77			

Appendix 9: One way ANOVA: Zones of inhibition of aqueous plant extracts at 600mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1049.385	6	174.897	21.888	.000
Within Groups	567.333	71	7.991		
Total	1616.718	77			

Appendix 10: One way ANOVA: Zones of inhibition of aqueous plant extracts at 450mg/ml against strain UONV017

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1861.788	6	310.298	35.012	.000
Within Groups	629.250	71	8.863		
Total	2491.038	77			

Appendix 11: One way ANOVA: Zones of inhibition of aqueous plant extracts at 450mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1312.365	6	218.728	24.608	.000
Within Groups	631.083	71	8.888		
Total	1943.449	77			

Appendix 12: One way ANOVA: Zones of inhibition of aqueous plant extracts at 300mg/ml against strain UONV017

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2507.154	6	417.859	250.715	.000
Within Groups	118.333	71	1.667		
Total	2625.487	77			

Appendix 13: One way ANOVA: Zones of inhibition of aqueous plant extracts at 300mg/ml against strain UONV003

ANOVA					
Diameters of zone of inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1659.385	6	276.564	167.353	.000
Within Groups	117.333	71	1.653		
Total	1776.718	77			

Appendix 14: Two way ANOVA: Photosensitization effect of organic plant extracts at 600mg/ml against strain UONV017 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	742.602	5	148.520	18.311	.000
Timings	14281.706	3	4760.569	586.919	.000
Plants * Timings	1027.794	15	68.520	8.448	.000
a. R Squared = .982 (Adjusted R Squared = .970)					

Appendix 15: Two way ANOVA: Photosensitization effect of organic plant extracts at 600mg/ml against strain UONV003 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	1267.157	5	253.431	25.019	.000
Timings	18142.794	3	6047.598	597.021	.000
Plants * Timings	1231.439	15	82.096	8.105	.000
a. R Squared = .982 (Adjusted R Squared = .971)					

Appendix 16: Two way ANOVA: Photosensitization effect of organic plant extracts at 450mg/ml against strain UONV017 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	5332.089	5	1066.418	51.234	.000
Timings	14634.687	3	4878.229	234.363	.000
Plants * Timings	6506.036	14	464.717	22.326	.000
a. R Squared = .975 (Adjusted R Squared = .959)					

Appendix 17: Two way ANOVA: Photosensitization effect of organic plant extracts at 450mg/ml against strain UONV003 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	3696.000	5	739.200	36.824	.000
Timings	16246.000	3	5415.333	269.768	.000
Plants * Timings	3399.933	15	226.662	11.291	.000
a. R Squared = .970 (Adjusted R Squared = .950)					

Appendix 18: Two way ANOVA: Photosensitization effect of aqueous plant extracts at 600mg/ml against strain UONV017 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	783.741	5	156.748	37.125	.000
Timings	10762.711	3	3587.570	849.688	.000
Plants * Timings	908.089	15	60.539	14.338	.000
a. R Squared = .988 (Adjusted R Squared = .980)					

Appendix 19: Two way ANOVA: Photosensitization effect of aqueous plant extracts at 600mg/ml against strain UONV003 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	943.519	5	188.704	16.542	.000
Timings	10179.933	3	3393.311	297.466	.000
Plants * Timings	861.200	15	57.413	5.033	.000
a. R Squared = .966 (Adjusted R Squared = .945)					

Appendix 20: Two way ANOVA: Photosensitization effect of aqueous plant extracts at 450mg/ml against strain UONV017 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	1004.824	5	200.965	78.638	.000
Timings	10075.372	3	3358.457	1.314E3	.000
Plants * Timings	864.528	15	57.635	22.553	.000
a. R Squared = .992 (Adjusted R Squared = .987)					

Appendix 21: Two way ANOVA: Photosensitization effect by aqueous plant extracts at 450mg/ml against strain UONV003 (CFU's)

Tests of Between-Subjects Effects					
Dependent Variable:Number of CFU,s					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plants	1106.380	5	221.276	32.207	.000
Timings	9990.928	3	3330.309	484.735	.000
Plants * Timings	928.839	15	61.923	9.013	.000
a. R Squared = .980 (Adjusted R Squared = .967)					