ANTIMICROBIAL PROPERTIES AND PHYTOCHEMICAL COMPOSITION OF *SOLANUM NIGRUM* COMPLEX (BLACK NIGHT SHADE)

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Microbiology of the department of biological sciences, University of Eldoret

NOVEMBER, 2013

DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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DEDICATION

To my husband, Eng. Samwel Mwaura

ABSTRACT

Solanum nigrum complex is a group of plants used as indigenous vegetables and also a source of traditional medicine in Kenya and other parts of the world. There are many species in the *S. nigrum* complex which include *Solanum villosum, Solanum scabrum, and Solanum nigrum* among others. This study determined the antibacterial properties, antifungal properties and phytochemical composition of some species in the *S. nigrum* complex. Samples were collected from Eldoret, Kisumu, Kakamega, and Bungoma. The plants were dried in dark room and then ground. Crude extracts were prepared from the plants using methanol. The antifungal and antibacterial properties were performed using the samples. The fungi used were *Fusarium culmorum, Fusarium avenaceum and Fusarium moniliforme.* The bacterial species used were *Staphylococcus aureus, Salmonella typhi, Pseudomonas aureginosa, Proteus mirabilis, Shigella spp, Pseudomonas syringae, Escherichia coli* and *Bacillus subtilis*. Minimum inhibition concentration (MIC) of the samples against active microbes was determined. Phytochemical composition of the extracts was analysed. T –test was used to analyse the significance of the activity indexes of the extracts against the different groups of the microbes. The samples collected were found to belong to the species *S. nigrum, S. scabrum* and *S. villosum* which all belong to the *S. nigrum* complex. The samples showed a considerable antibacterial and antifungal activity against the tested microbes. The highest antibacterial activity was 29.00 mm which was shown by sample KO4L (Bungoma less bitter) which belongs to *S. villosum* species; when tested against *S. aureus*. The antibacterial and antifungal activity was dose dependent with MIC values as low as 0.09g/ml exhibited by sample K06L against all tested bacteria. The antimicrobial activity was associated with the wide array of phytochemical compounds seen in the samples that included tannins, saponins, flavonoids, steroids, terpenes, phenolic compounds, and cardiac glycosides. A pure crystal isolated from sample K06L of the *S. nigrum* Mill species also exhibited a considerable level of antimicrobial activity with the highest activity index of 14.00 mm observed against *S. typhi*. It was concluded that the *S. nigrum* complex is rich in many phytochemical compounds like saponins, flavonoids, steroids, glycosides, terpenoids among others which make the plant useful in inhibition of many microbes. It can also be used in treatment of plant fungal infections and also act as a food preservative. Further research on the plants was therefore recommended in order to come up with more beneficial application of *S. nigrum* complex in the medical field for improvement of human health.

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ACRONYMES AND ABBREVIATIONS

3-ADON -3-acetyldeoxynivalelon

DON- Deoxynivalelon

GIT - Gastro intestinal tract

MHA - Muller Hilton Agar

MIC - Minimum inhibition concentration

SDA - Sabouraud dextrose agar

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Solanum nigrum complex comprise of both native and bred solanum species used as vegetables and source of fruits in Kenya and other parts of the world (Schippers, 2000). Both indigenous (wild types) and modified (hybrid) varieties that have been cultivated. (Peter *et al.,* 2009). These plants are believed to have a high nutritional value (Schippers, 2000). The leaves are eaten as vegetable in most parts of the world while the ripe seeds are also edible (Edmonds and Chweya, 1997).

There are a number of species in the *S. nigrum* complex which include *S. nigrum, S. villosum, S. scabrum, Solanum americana, Solanum burkankii, and Solanum schenopodioides* among others. All these species highly resemble each other closely and these pose a problem in the taxonomy of the species, they are therefore all considered to belong to the *S. nigrum* complex. Species in the *S. nigrum* complex exhibit considerable genetic variation, both florally and vegetatively. These variations occur in different populations of the same species. Sometimes the character may be genetically controlled in one variant, but phenotypically plastic in another (Edmonds and Chweya, 1997). This group of plants display a great amount of phenotypic variation, particularly in their vegetative features such as plant habits, leaf size and form, and stem winging. In addition, senescence of this plant is usually accompanied by smaller and fewer flowers and berries than usual, while the gene for anthocyanin pigmentation in flowers seems to be dependent on light intensity and temperature for its expression. It is often difficult to define the limits within which such features are genetically fixed (Edmonds, 1997).

Other than being used as vegetables, *S. nigrum* complex also forms an important part of traditional medicine in Africa. In Kenya, unripe fruits are used to soothe toothache. They are also squeezed on babies' gums to ease pain during teething. Leaves are used to treat stomach-ache and extracts from leaves and fruits are used to treat tonsillitis (Edmonds and Chweya, 1997).

1.2 Statement of the problem

Bacteria and fungi are a major challenge in both medical and agricultural fields; they cause fatal infections to humans, animals and plants. People have been using the conventional medicine made of artificial chemicals to manage and treat these infections. Some of these treatments are supposed to be used for a very long time for example treatment of tuberculosis and opportunistic infections due to human immunodeficiency virus (HIV). Most of these synthetic drugs are also too expensive and unaffordable to most people in the third world countries. They have also been proved to have long term side effects like development of allergies and cancer after long term use. Most of these conventional medicines have also lost their efficacy due to development of drug resistant. Given that plants in the *S. nigrum* complex are edible and universally acceptable as vegetables, there is need to establish their antimicrobial potential with a view of using them as herbal medicine for management of various diseases.

1.3 Justification of the study

Research shows that herbal remedies have been used successfully and they pose fewer side effects as compared to the synthetic medicines. Species in the *S. nigrum* complex have been used for treatment of microbial and non microbial diseases successfully in the traditional medicine. There are very many different species and variants of Solanum that are grown in the country and are used traditionally both as a vegetable and as traditional

medicine. This research is important because it is aimed at establishing the specific medicinal value as well as the best species or variant of the *S. nigrum* complex for use as herbal medicine.

1.4 Research objectives

1.4.1 Main objective

- To determine the antimicrobial properties and phytochemical composition of some of the species in the *S. nigrum* complex.

1.4.2 Specific objectives

1. To identify the different species and variants of the *S. nigrum* complex in Eldoret, Kisumu, Kakamega and Bungoma districts.
2. To screen for antibacterial and antifungal property in crude extracts from some of the species in the *S. nigrum* complex
3. To determine the phytochemical composition of the plant extracts

1.5. Research hypothesis

- There are few species belonging to the *S. nigrum* complex that are found in Eldoret, Kisumu, Kakamega and Bungoma districts.
- There is no variation in the antibacterial and antifungal property in the different species or variants of *S. nigrum* complex.
- There are no phytochemical compounds in the extracts of *S. nigrum* complex.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Solanum nigrum* complex

2.1.1 Synonyms

Some of the local names used include the following: European Black Nightshade "black nightshade", Duscle, Garden Nightshade, Hound's Berry, Petty Morel, Wonder Berry, Small-fruited black nightshade or popolo.

2.1.2 General physical characteristics of the *S. nigrum* complex

Black nightshade is a fairly common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats. It has a height range of 30 to 120 cm, the leaves are alternate, dark green, soft, rather thin, and often riddled with bug holes like those of amaranth, The size of the leaves is quite variable, while the shape is moderately so, ranging from ovate to lanceolate to diamond-shaped, ovate to heart-shaped, with wavy or large-toothed edges; both surfaces hairy or hairless; petiole 1 to 3 cm long with a winged upper portion (Edmonds, 1983). The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers. The berry is mostly 6 to 8 mm diameter, dull black, purple-black, red or orange in colour (Edmonds 1997; Olet *et al.,* 2005; Jacoby *et al.,* 2003). A photograph of one of the species in the complex is shown in Figure 2.0.1 below.

Figure 2. 1: A photograph of *S. Nigrum*

(**Source: Author, 2012)**

2.1.3 Taxonomy

The taxonomic classification of the African black night shade has posed a difficulty among taxonomists. There is a taxonomic confusion surrounding *S. nigrum* complex and its component species (Edmonds and Chweya 1997), presumably because of the historical factors, phenotypic plasticity, genetic variation, the existence of a polyploidy series, and the possibilities of interspecific hybridization (Edmonds, 1977). Different species and varieties of these plants have been identified but there is no clear cut in the difference between the various species. This has led to many different synonyms and hence all the species in this genus have been classified as the *S. nigrum* complex. (Olet *et al.,* 2005).

Some of the species in this genus include; *S. villosum, S. nigrum, S. scabrum, S. americana, S. burkankii, S. schenopodioides, S. sarrachoides, S. grossidentatum, S. hirsutum and Solanum florulentum* and *S. tarderemotum* (Olet *et al.,* 2005).

A study done by Berinyuy *et al.,* (2002) showed that *S. scabrum* is the most important indigenous leafy vegetable and is widely cultivated in Africa and Asia. However, according to Olet *et al.,* (2005), it is likely that *S. grossidentatum, S. villosum and S. sarrachoides* do not occur in Uganda

The list of most studied species include; *S. americanum, S. nigrum* Mill*, S. scabrum, S. villosum, S. physalifoliun, S. chenopodioides, S. retroflexum, S. sarachoides* and *S. physalifolium var nitidabaccatum* (Edmonds and Chweya, 1997).

However Schippers (2000) stated that there are other taxa in Africa which are very poorly known and may present good species and need a taxonomic revision. These taxas are *S. floruletum, S. grossidentatum, S. hirsutum and S. tanderemotum*. There are several species with black berries, but the most popular are those with orange berries belonging to *S. villosum*. This group of species is often erroneously referred to as *S. nigrum*, a poisonous plant from Europe that is not usually grown in Africa (AVRDC 2003).

2.1.4 Species of the *S. nigrum* complex found in Kenya

According to Olet (2004), Solanum species that are found in Kenyan vegetable gardens include *S. nigrum* Mill, *S. macrocarpon, S. scabrum* and *S. villosum. S. nigrum, S. scabrum* and *S. villosum* are black night shades but *S. macrocarpon* is an egg plant. Some of the species of the black night shade commonly found in Kenya include the following

According to Henderson (1974), Edmonds (1983), Symon (1981), Bukenya (1996), *S. scabrum* is associated to the following characteristics: The Plants is erect, glabrescent to subglabrous, with sparse eglandular-headed hairs; lateral. Branches are sparse and

usually spreading horizontally. Stems are prominently and dentately winged. The Leaves are usually ovate, occasionally lanceolate, large, 10.0 cm to 12.0 cm long x 6.0 cm to 8.0 cm broad. The margins are entire to sinuate, lobes absent, apices acute to obtuse. The inflorescence is simple or forked, lax and often extended cymes, 6 to 14-flowered; the peduncles are 3.0-6.0 cm long fruiting when erect. Calyces are approximately around 1.9 mm to 3.5 mm long while the sepals are usually reflexed away from mature berry. Flowers are stellate, white, occasionally tinged purple, with yellow/green basal star, 7 mm - 9 mm radius. The berries are usually broadly ovoid, deeply purple with opaque cuticles, 15 mm - 17 mm broad, remaining on plant and adhering to erect pedicels at maturity.

The Plants belonging to *S. scabrum* are subglabrous to villous annuals, up to 50 cm high. The Leaves are usually rhombic to ovatelanceolate, 2.0-7.0 cm long x 1.5-4.0 cm broad with margins entire to sinuate-dentate.

The inflorescences are simple, umbellate to slightly lax solitary cymes; the peduncles are erect, 4mm to 13 mm during fruiting. The pedicels are often longer, deflexed in fruit. The Calyces are 1.2-2.2 mm long, slightly accrescent, deflexed or adhering to base of mature berry; the sepals are triangular lobes. The corollas are stellate, white with translucent to yellow basal star, 4-8 mm radius, 3-5 times as long as calyx. Berries are usually longer than wide, occasionally globose, red, orange or yellow, 6-10 mm broad, falling from calyces when ripe. The seeds are approximately 1.6-2.2 mm long, usually visible through translucent cuticles and are usually 30 to 45per berry (Henderson, 1974; Randell and Symon, 1976; Edmonds, 1984a; Bukenya, 1996)

Solanum nigrum Mill has plants which are subglabrous to villous annuals with a maximum height of up to 70 cm high, they are covered with simple multicellular hairs

with glandular or eglandular heads. The stems are decumbent to erect. The leaves ovate, ovate-lanceolate, ovate-rhombic to lanceolate, they are 2.5-7.0 cm long x 2.0 to 4.5cm broad, with the margins being entire to sinuate-dentate. The inflorescences are simple, lax and often extended cymes, 3 to 10-flowered; the pedicels are much shorter, recurved in fruit. The calyces are 1.2-2.5 mm long, slightly accrescent, deflexed or adhering to base of mature berry, the sepals are usually ovate. Corollas are stellate, white with translucent basal star, 4mm to 7mm radius, usually 1.5-3 times as long as calyx. Anthers are yellow, 1.5 to 2.5 mm long. The berries are usually broadly ovoid, dull purple to blackish or yellowish-green, 6-10 mm broad and they are found to remain on plants or fall down from calyces when ripe. The seeds are 1.7-2.4 mm long, 26 to 96 per berry (Edmonds, 1984; Henderson, 1974; Randell and Symon, 1976; Symon, 1981; Bukenya, 1996).

2.2 Microbes

2.2.1 Bacteria

These are microscopic organisms which are normally associated with causing disease in both plants and animals. They exist as prokaryotic cells either inside an organism or free living.

There are two broad categories of bacteria based on their ability to retain the gram stain after rinsing with alcohol in the Gram staining technique. These categories include:

- Gram positive bacteria
- Gram negative bacteria

2.2.1.1 Gram positive bacteria

These are bacterial cells which retain the purple colour of the gram stain after rinsing with alcohol. This is because of their thick murein layer in the cell wall. They include *S. aureus, Streptococcus feacalis*, etc.

Staphylococcus aureus are Gram positive bacterial which usually occur as normal flora on the body of humans. They have been isolated on the nasal cavity, the skin, oral cavity and the gastro intestinal tract.

Other than occurring as a beneficial normal flora, some variants of *S. aureus* are potential pathogens to man (www.textbookofbacteriology.net). According to Kenneth (2011), *S. aureus* is associated with a variety of supportive (pus forming) infections in humans thereby causing skin lesions such as boils. It also causes more serious infections such as pneumonia, mastitis, meningitis and urinary tract infections. *S. aureus* is also associated with a variety of hospital acquired (nosocomial) infections.

Upon ingestion in contaminated foods, *S. aureus* causes food poisoning by releasing enterotoxins in food and toxic shock syndrome by releasing super-antigens into the blood streams (Kenneth, 2011).

Some of the virulence factors of *S. aureus* include membrane damaging toxins to lyse the host cells, surface factors that inhibit phagocytic engulfment, surface proteins that promote bacterial attachment onto the host cell, among others (Kenneth, 2011).

Bacillus subtilis is a Gram positive bacterium which exists in the gut as a normal commensal. It can also be found in the soil whereby the soil act as a reservoir after faecal materials get in contact with the soil, but the main habitat for these bacteria is the GI (gastro intestinal) system. *B. subtilis* is a rod shaped endospore forming bacteria. It is

therefore able to withstand extreme environmental conditions. (Madigan and Martinko (ed), 2005).

The bacteria do not cause infection to normal cells; it is known to cause disease in severely immuno-compromised patients and can be used as a probiotic in healthy patients (Ryan and Ray (ed) (2004). It is not known to cause food poisoning (Ryan and Ray (2004) but some strains can produce proteolytic enzyme called subtilisin and therefore cause spoilage of protein foods, ropiness in stored bread and bakers dough (Noirot, 2007). Due to presence of endospores this food spoilage cannot be controlled by boiling or cooking.

This bacteria is not a serious pathogen, it has been reported to cause bacteraemia on cancer patients as a nosocomial infection but is not associated with the subsequent deaths of the infected patients (Ryan and Ray, 2004).

2.2.1.2 Gram negative bacteria

These are bacterial cells which do not retain the purple colour of the Gram stain. They therefore appear pink after counterstaining with safranin in the gram staining procedure. This group of bacteria include *E. coli, Pseudomonas, Shigella,* etc.

Shigella is an enteric pathogen of humans. Species on this genus include. *S. boydii, S. dysenteriae, S. flexneri* and *S. sonnei.* It causes a disease called shigelosis or *shigella* dysentery which is characterised by diarrhoea, fever, nausea, vomiting, stomach cramps and flatulence, bloody stool with mucus. It invades the human host through the faecal – oral route after feeding on infected foods. It is one major cause of epidemics in areas where people live confined in congested areas like refugee camps (WHO, 2012).

This pathogen causes infection in the caecum and rectal mucosa by destruction of the epithelial cells producing a protein. Some strains produce enterotoxins like the Shiga

toxin which are associated with haemolytic uremic syndrome (Hale *et al.,* (1996). This toxin facilitates the pathogen's penetration into the host cells and subsequent lysis of host cell (Ram *et al.,* 2008).

Escherichia coli are Gram negative bacteria which occur on the human body as a normal flora. The bacteria get its way to the gastro intestinal tract of the humans through foods or water directly. The bacteria adhere to the mucous walls overlaying the large intestine and are therefore used as indicator organisms for faecal contamination of water. However some pathogenic strains are found in the intestines after ingestion of contaminated foods resulting to serious gastroenteritis. According to Tarun *et al* (2011), *E. coli* is considered one of the most frequent causes of most common bacterial infections including bacteremia, cholangitis, cholecystitis, UTI (urinary tract infections), and traveller's diarrhoea. It is also associated with other clinical infections such as neonatal meningitis and pneumonia (Tarun *et al.,* 2011).

This is a species of Gram negative free living bacteria naturally found in the soil or water. *Pseudomonas spp* species have been isolated in soil, water, plants and animal tissues. *P. aureginosa* is the most common pseudomonal species. *Pseudomonas* species are clinically significant as an opportunistic pathogen, often causing nosocomial infections. Rarely does Pseudomonas affect healthy cells; it only affects the cells whose immune system has been compromised in one way or another. This pathogen has been known to cause serious diseases and also it exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents (Selina, 2012).

Pseudomonas bacteria, other than causing human and animal diseases, are also pathogenic to plants (Kenneth, 2012).*Pseudomonas spp* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteraemia, bone and

joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immune-suppressed. It has been reported to cause a fatality rate of nearly 50% to infected patients and is the fourth most commonly isolated pathogen accounting for 10.1% of all hospital acquired infections (Kenneth, 2012).

Pseudomonas syringae is a Gram negative bacterium of the Pseudomonas genus that is strictly a plant pathogen. It infects a wide range of plant species including tomatoes and beans where it causes spots on the leaves and the fruits. Usually, the bacterium is favoured by wet and cold seasons (Hirano and Upper 2000).

Pseudomonas syringae causes plant disease by producing protein toxins to destroy the plant cell membrane and therefore facilitate entry of the pathogen into the host cell (Anzai *et al*, 2000). Some of the toxins facilitate entrance of the pathogen into the host cell by inducing the opening of the plant stoma to permit entry of the pathogen. Other toxins are associated with attachment and surfactant activity while others target the metabolic pathways in the host (Melotto *et al*, 2006).

Proteus spp is a pathogenic Gram negative bacteria which occur naturally and is widely distributed in soil and water. These bacteria form part of the normal flora in the gut of humans and animals. However, like *Pseudomonas*, it does not cause infections on health cells but affects cells whose immunity has been compromised. *P. mirabilis* is the most common and most virulent species causing 90% of all Proteus infections in humans (Frenod, 2006).

Proteus is usually associated with infections of the urinary systems where it produces high levels of urease enzyme which facilitate hydrolysis of urea to ammonia and thus make the urine more alkaline leading to formation of crystals that obstruct the renal

system thereby causing kidney stones (Frenod, 2006). *Proteus* bacteria can also cause wound infections, pneumonias, and septicaemia, mostly in hospitalized patients.

Salmonella species are Gram negative human and animal pathogenic bacteria. Human pathogenic species in this genus are *S. typhi* and *S. typhimurium*. *Salmonella* species causes diseases like gastroenteritis, salmonellosis, typhoid fever and paratyphoid fever in humans (Frenod, 2006).

The routes of infection are unwashed fruits, vagetables and nuts, uncooked meats and eggs, and contaminated water. These infections are usually treated using conventional antibiotics but according to some clinicians, some patients do not require antibiotics.

Control of salmonella is by giving salmonella vaccines for paultry and animals; only typhoid fever vaccines are available to humans, but there is no vaccine available for salmonellosis (Frenod, 2006).

2.2.2 Fungi

Fungi are members of large group of eukaryotic organisms which includes both the microscopic organisms (yeasts) and macroscopic organisms (moulds). The main distinguishing characteristic of fungi is the presence of chitin as their cell wall material as compared to plants which have cellulose and bacteria which have murein (Zabriskie and Jackson, 2000).

Fungi are important in the ecosystem in that they assist in the breakdown of dead organic material thereby continuing the cycle of nutrients in the ecosystem. Some fungi referred to as mycorrhizae live symbiotically with higher plants thereby assisting the plant in the absorption of water and essential minerals from the soil (Zabriskie and Jackson, 2000).

Fungi also cause a number of plant and animal diseases: in humans, the diseases caosed by fungi include ringworm and athlete's foot. Due to the fact that fungi are chemically and genetically similar to animals than other organisms, fungal diseases are very difficult to treat. Plant diseases caused by fungi include rusts, smuts, and leaf, root, and stem rots, and may cause severe damage to crops (Bruns 2006).

Some pathogenic fungi also cause food poisoning when they produce mycotoxins in grains and serials which are stored in humid conditions (Rodriguez *et al.,* 2008).

2.2.2.1 Fusarium culmorum

This is a fungus that is plant pathogenic and a causal agent of seedling blight, foot rot, ear blight, common root rot and other diseases of cereals, grasses, and a wide variety of monocots and dicots (Rodriguez *et al.,* 2008).

Fusarium culmorum is also capable of producing mycotoxins in plants. The most common mycotoxins of *F. culmorum* in wheat are deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON) which are harmful to human and animal health. (Marasas *et al*., 1984)

The toxin occur when the cereals are stored under humid conditions, the microbe grows and produce toxins which affect humans and animal health upon consumption.

2.2.2.2 Fusaruim moniliforme

Fusarium moniliforme is an important pathogen of many cultivated crops. In maize, it causes seeding blight and stalk, ear rots (Desjardins, 2006). *F. Moniliforme* is a filamentous, ascomycetous fungus which is pathogenic to a variety of animals and

plants (Thiel *et al.,* 1991). *F. moniliforme* produces gibberela ear rot, kernel rot, stalk rot, seedling blight, seed rot, wilt and stunt (Thiel *et al.,* 1991).

Fusarium moniliforme produces mycotoxins which cause *leucoencephalomalacia* and hepato-toxicity in horses, pulmonary oedema in pigs and liver cancer in rats, and has been correlated with oesophageal cancer in humans. The causative agents are thought to be consumption of food contaminated with a family of compounds produced by these fungi.

2.2.2.3 Fusarium avenaceum

Fusarium avenaceum is a globally distributed fungus commonly isolated from soil and a wide range of plants. Severe outbreaks of crown and stem rot of the flowering ornamental, lisianthus (*Eustoma grandiflorum*), have been attributed to *F. avenaceum.* It is a major component of fusarium head blight disease in cereals and fusarium ear rot in maize (Desjardins, 2006). It has also been reported in barley where it inhibit germination of malting grains (Rodriguez *et al.,* 2008)

2.3 Phytochemical compounds

Phytochemical compounds are secondary metabolites produced by plants as a result of evolutionary adaptation to their environment; these compounds have been seen to accord the plant advantages that help the plant to survive against disease infection by microorganisms, and also resist destruction by herbivores and man (Ogunwenmo *et al.,* 2007). Many of these phytochemical compounds especially alkaloids are produced as exudates or latex when plants are bruised, cut or damaged and have been proved useful to humans as antibiotics or antifungal medicine (Simpson and Ogorzaly, 2000; Audesirk *et al.,* 2006). They have also been proved to be immune-modulative (Okuda, 2005). The

compounds are responsible for the successful application of plants in herbal medicine. These compounds are used as ingredients of many medicines of plant origin due to their broad spectrum of biological activities, including anti-bacterial, anti-inflammatory, anti-tumorous, purgative, astringent, anti-viral antioxidant and antifungal (Agarwal *et al.,* 2000; Manojlovic *et al.,* 2002; Manojlovic *et al.,* 2008).

Some of the common phytochemical compounds are discussed below:

2.3.1 Saponins

They are responsible for the soap like foaming of *S. nigrum* during boiling (Harbone, 1973). They are detected by their ability to haemolyse red blood cells. They are often bitter to taste and can reduce plant palatability in application as human food or animal feeds. Saponins have antimicrobial effect and they therefore protect the plants against microbial infection (Foerster, 2006). They are often associated with life threatening animal toxicity.

Saponins have been scientifically proven to be effective in treatment of syphilis and other venereal diseases. Their medicinal properties range within their antibacterial, antifungal, anti inflammatory, antiviral (Al-Bayati and Al-Mola, 2008), and anti-protozoan (Cheeke, 1998) activities.

The saponins react with the cholesterol molecule of the protozoans thereby causing lysis of the pathogenic cells. Their mode of action against bacteria is associated with their membranolytic properties and also lowering the surface tension of the extracellular medium thereby interfering with the attachment of the pathogen to host cells.

According to Dong *et al.,* (2005), Saponins have been shown to have anticancer effects by reacting with cholesterol rich membranes of cancer cells and arresting cell

proliferation at mitotic phase of the cell cycle. They are also used in controlling the amount of low density cholesterol in the blood (Assiak *et al.,* 2001)

A study done by Victor *et al.,* (2005) demonstrated that saponins have anti-oedema and immune-regulatory effects.

2.3.2 Terpenes

According to Ghoshal *et al.,* (1996), terpenes have anti-amoebic characteristics when tested *in vitro.* They also have been proved to have antibacterial effects, antifungal effects (Rana *et al.,* 1997) and anti HIV (Fujioka *et al.,* 1994) effects. These effects are associated to their ability to disrupt the membranes of the microbes by their lipophilic compounds. Terpenes are also considered to block the action of cancer causing factors (carcinogens) and may inhibit hormone related cancers such as ovarian cancers (Sam and Chandak, 2008)

2.3.3 Phenolic compounds

These are phytochemical compounds which contain one or more phenol groups. Hydroxylated phenols have been found to be toxic to microorganisms and their relative activity increases with increasing level of oxidation (Scalbert, 1991)**.** Phenols and polyphenols protect the plants from chemical damage and they have been proved to perform the same function in humans. Polyphenols from tea are thought to protect against stomach cancer.

The anti-oxidative potential of plant phenols to prevent cell damage suggests that they have antimicrobial, anticancer, anti-inflammatory effects and mild anti-hypertensive properties (Del-Rio *et al.,*, 1997; Okwu, 2004).

2.3.4 Glycosides (cardiac glycosides)

These are phytochemical compounds which are associated with the characteristic bitter taste of unripe fruits and berries. It is also responsible for preventing the decay of damaged plant tissues (Okudu, 2005).

Purified cardiac glycosides are administered as injections or tablets to inhibit active transport of potassium ions (k^+) and sodium ions (Na^+) through membranes thereby strengthening the heart muscles and the power of systolic concentration. This helps in the management of congestive heart failure and regulation of heart beat (Clifford *et al.,* 1973; Leverin and McMatron, 1999) hence the name cardiac glycosides.

2.3.5 Steroids

Plant steroids are referred to as phytosteroids. They are similar to the animal steroids but they only differ in the functional groups attached to the main structure (Hobbs, 2005). These steroids are important starting materials for synthesis of drugs.

They are also used to treat reproductive complications, promote fertility in women and facilitate easy delivery for pregnant. They also help in promoting libido in men (Victor *et al.,* 2005). They have industrial application in the production of artificial hormone e.g. oestrogen-like substances and therefore used as contraceptive (Victor *et al.,* 2005).

Their antibacterial effect has an important application in treatment of stomach related ailments (Balch and Balch, 2000). It has also been shown to have analgesic and anti-inflammatory effects. It is also used in decreasing serum cholesterol levels (William, 2008)

2.3.6 Flavonoids

Flavonoids constitute one of the most common categories of phytochemical compounds in higher plants. Many flavonoids are present as flower pigments in most angiosperm families. However they are also present in all parts of the plant. Flavonoids are divided into six subgroups namely chalcones, flavones, flavonol, flavanone, xanthocyanins and isoflavonoids. They are responsible for colouration of fruits, vegetables or herbs. They are found in high concentrations in tea, fruits and soy beans.

Their health promoting effects include anti-inflammatory, anti-viral, anti-cancer, anti-oxidant, and anti-allergic effects (Balch and Balch, 2000). Studies in epidemiology have illustrated that heart diseases are highly reduced by flavonoid intake. It is also confirmed that flavonoids reduce the oxidation of low density lipoproteins thereby reducing the risk of development of anthrosclerosis. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Isoflavones can also reduce blood cholesterol and can help to prevent osteoporosis. They are also used to ease menopausal symptoms (Chen *et al.,* 2001).

2.3.7 Tannins and Phlobatanins

These are polyphenolic compounds of plant origin specifically known by their ability to precipitate proteins. Tannins are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms like bacteria and fungi and protect the tree. They have antibacterial effect by inhibiting adhesion of the microbial cells to the potential host cells (Victor *et al.,* 2005). They also inhibit microbial enzymes and cell membrane transport proteins of the microbial cells. These compounds are used as purgative and also in treatment of asthma, cough and other respiratory diseases (Burkil, 1994).

They are toxic to filamentous fungi, yeasts and bacteria (Scalbert *et al.,* 2005). A study done by Nonak *et al.,* (1990) showed that tannins have an ability to inhibit viral reverse transcriptase.

According to Gertrudes (2006) the consumption of tannins as green teas and wines prevents different illnesses and inhibits viral reverse transcriptase. Condensed tannins bind to cell walls of ruminal bacteria thereby preventing bacterial growth and protease activity (Jones *et al.,* 1994). They also inhibit insect growth on plants (Buttler, 1998).

They have a pharmaceutical application in their use as antidotes for poisoning by alkaloids depending on their capacity to form insoluble tannates (Dharmananda, 2003). According to Yu *et al.,* (2000), tannins are responsible for anti-diarrhoeal activity by denaturing the proteins through formation of tannate, thereby causing the intestinal mucosa more resistant and reduce secretion (Degenhardt, 2000).

2.3.8 Alkaloids

Alkaloids are useful phytochemical compounds. Examples of alkaloids include isopteropodine, pteropopine and isomitraphylline which help the white blood cells to fight harmful microorganisms and cell debris, and rynchophylline which improve cardiac conditions by reducing blood pressure, increasing circulation and inhibiting the accumulation of arteriosclerosis plaque and blood clots (Jeffery and Harborne, 2000).

Some alkaloids have antimicrobial property based on their ability to disrupt the nucleic acid of the pathogens. Others function by disrupting the cell wall material of the bacterial cells (Ogwenwenmo *et al.,* 2007). Other alkaloids function as anti-cancer by arresting the protein synthesis of the cell proliferation process through disintegration of the spindle protein of cancer cell during cell division (Sneden, 2005)

Indole alkaloids are used in treatment of leukaemia disease. A research done by Okwu (2004) showed that alkaloids have antibacterial, antifungal and anti inflammatory effects. They have also been proved to act as anti-hypersensitive agents (Sofowora, 1993).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study area

Samples were collected from farmers in Eldoret (Ziwa), Bungoma (Kamukuywa), Kakamega (Butsotso and Kabras) and Kisumu (Ahero and Kano). The choice of the study area was based on the fact that the plants are highly cultivated and used in these regions. Content extraction, antibacterial and antifungal properties were done in the Biotechnology laboratory of Egerton University. Phytochemical assays were done at the Chemistry laboratory at Egerton University.

3.2 Sample collection and identification

A survey was done by consulting with the farmers and the Agricultural extension officers from the ministry of Agriculture in order to determine the number of species and varieties of *S. nigrum* complex present in the region.

One sack of each of the varieties was collected from farmers in the field. A total of ten different populations were collected from the various regions. The plants were labelled according to the local names used by the farmers in the field pending identification. One set of each sample was pressed and taken to a taxonomist at Egerton University for identification. The specific names of the plants were identified for the different plant populations collected. Photos of the plants, the berries and the seeds were also taken to assist in the classification. Pressed samples of the plants are stored at the herbarium in University of Eldoret under reference number SN23/12.

3.3 Preparation of plant sample for crude extraction

The samples were dried in a dark room up to a constant weight for a period of one month, turning the plants up and down daily to ensure they dried evenly without fungal growth or spoilage. The samples were then pulverized using a super mixer grinder and the powdered plants were parked in well labelled paper bags and stored at room temperature.

3.4 Methanol purification for extraction of plant compounds

Industrial methanol was purified by controlled distillation at 60 to 65 ^{o}C. The purified methanol was collected in a clean dry flask and stored for future use.

3.5 Preparation of crude extracts for antimicrobial tests

Powdered samples (500 g) were soaked overnight in 1400 ml of distilled methanol and filtered. The residue was re-extracted three times with 500 ml of methanol and the filtrates combined. The combined filtrates were concentrated using a rotary evaporator with the water bath temperature maintained at 70 °C to prevent thermal decomposition of labile compounds. The rotations were maintained at 80 rpms. A photograph of the rotary everporator unit is shown in figure 3.0.1.

The samples were then removed and placed in clean dry bottles and allowed to settle for 24 hours. They formed distinct solid and aqueous phases. The aqueous phase was due to some traces of water found in methanol. The two phases were separated by decantation for some of the samples and filtration for the other samples

The solid samples were allowed to dry in a fume cupboard to a constant weight. The liquid samples were dried with anhydrous sodium sulphate in order to remove any water

present. They were then concentrated in a rotary evaporator and then placed in a fume cupboard until dried completely.

The solid samples were all similar in appearance and appeared as dark solids. The aqueous phase samples were not drying easily; they took longer time before attaining a constant weight.

Figure 3. 1: The rotary evaporator unit

(Source: Author, 2012)

The liquid samples were then dried to a constant weight. Sample K04L did not dry to a solid; instead a constant weight was achieved while the sample was still at semi solid state. This sample also formed crystals. Sample K06L also formed some crystals of the same shape and appearance as those isolated from sample K04L. They were insoluble in organic solvents but highly soluble in water. These crystals were isolated and cleaned using methanol after trying a number of other organic solvents. The sample K06L was then dried to a constant weight. Sample K06L formed a brown and oily substance with small slender crystals which were clearly seen under the microscope (Figure 3.0.2).

Figure 3. 2: Crystals isolated from sample K06L

(Source:Author, 2012)

Samples K01L, K02L, K03L and K05L had the same appearance; they all dried to form hard black oily solids after drying to a constant weight. Sample K07L remained liquid even after drying to a constant weight. The liquid sample was oily and highly viscous. It was dark brown in colour (honey-like appearance). Sample K08L formed a dark brown to black oily solid. Small crystals were seen on the sample; appearing as small straight lines. Sample K09L formed a brown oily solid with small cuboidal crystals appearing in the solid. K10L sample formed a black oily liquid with a shiny surface after drying to a constant weight; it did not form a solid.

The entire process of sample preparation is summarised in the schematic diagram below (Figure 3.3).

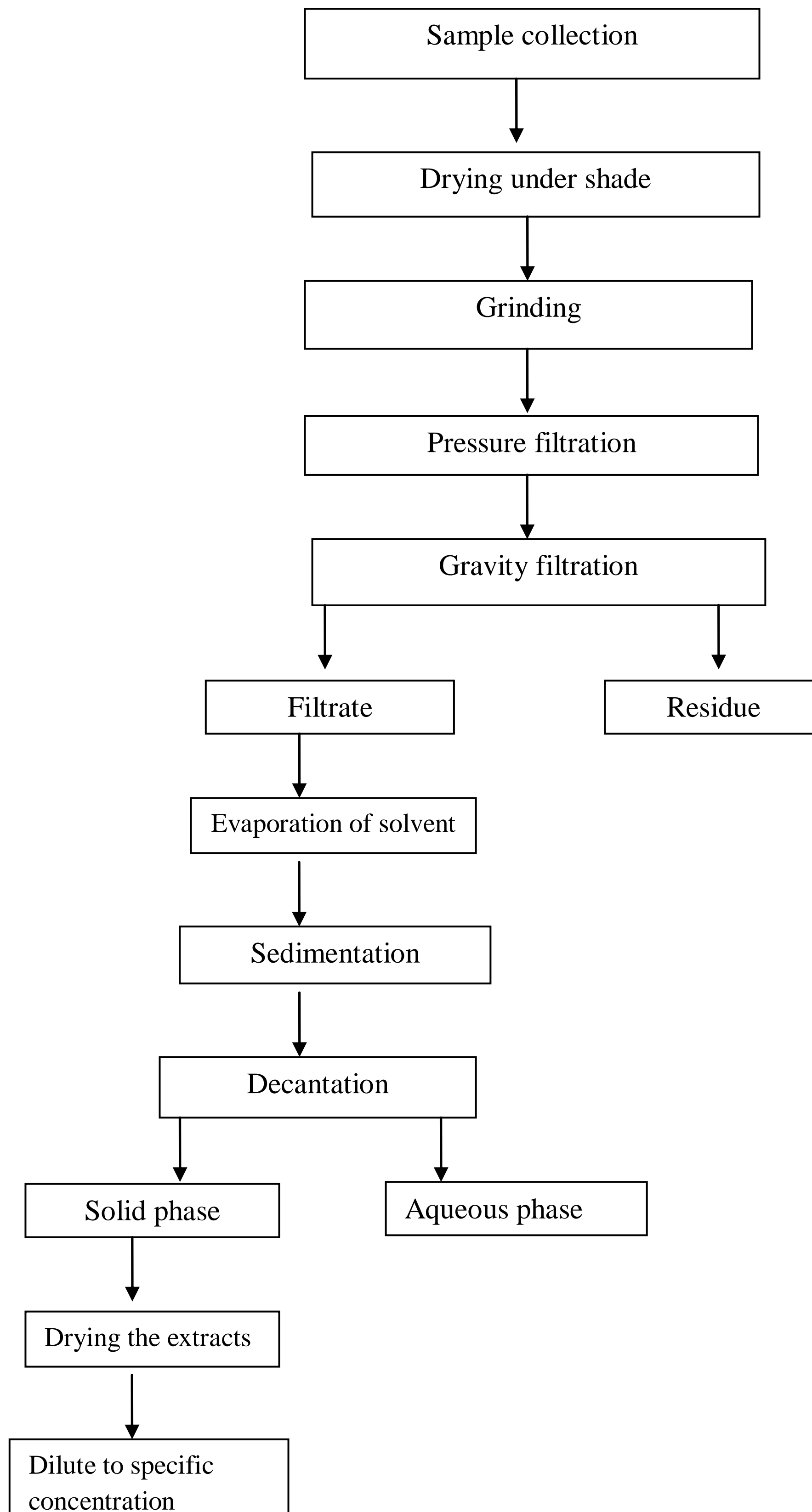

Figure 3. 3: Summary of sample preparation to produce crude extracts

3.6 Screening for antifungal and antibacterial activity

3.6.1 Preparation of the sample for antimicrobial screening

The samples K01S – K05S were weighed to an approximate mass of 50 mg and dissolved in 2 ml of distilled methanol to make an approximate concentration of 25 mg/ml. Samples K06 – K10S were prepared at approximate concentration of 100 mg/ml. The exact weights arrived at were recorded and the specific concentration of the samples calculated as indicated in Table 1.

Table 3. 1: Preparation of extracts from *S. nigrum* complex for antimicrobial assay

Sample	K01 S	KO2 S	KO3 S	K04 S	K05 S	K06 S	K07 S	K08 S	K09 S	K10 S
Volume of (mm) methanol	2	2	2	2	2	1	1	1	1	1
Sample mass (mg) ($*10^{-2}$)	5.06	5.00	5.04	5.03	5.00	10.15	10.15	10.06	10.00	10.12
Concentration (mg/ml)	25.3	25.0	25.2	25.15	25.0	101.5	101.5	100.6	100.0	101.2

KEY

KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 – Eldoret indigenous (Ziwa), K07 – Kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret AMP

3.6.2 Test microorganisms

Eleven test microorganisms were used in antimicrobial sensitivity tests. They included the following:

1. Gram positive bacteria –*S. aureus*, *B. subtilis* and *Proteus ssp*
2. Gram negative bacteria – *E. coli*, *Shigella ssp*, *S. typhi*, *Pseudomonas ssp* and *P. syringae*
3. Fungi – *F. culmorum*, *F. avenaceum* and *F. moniliforme*

3.6.3 Preparation of Mc Farland solution

This is a standard solution that is used to determine the correct concentration of inoculum for antimicrobial tests. 1% solution of (w/v) of anhydrous barium chloride solution was prepared. 1% solution (v/v) of sulphuric acid (H_2SO_4) was also prepared. The two solutions were then mixed using the ratio of 0.05:9.95 for barium chloride and sulphuric acid solutions respectively to obtain a standard McFarland solution with approximate bacterial concentration of 1.0×10^8 cells per ml (McFarland, 1907).

3.6.4 Preparation of the inoculums

The microbes were subcultured to revive the organisms from the stock culture. Nutrient Broth and sterile distilled water were used to prepare broth cultures of bacterial and fungal test organisms respectively. The nutrient broth media was prepared according to the manufacturers' instructions (American Public Health Association, 1917). The autoclaved media and distilled water were aseptically transferred to sterile capped test tubes of about 6ml each. Pure isolates of subcultured bacteria and fungi colonies were aseptically transferred to the respective broth media and the concentration of the

inoculum adjusted to make an approximate cell concentration of 1.0 x 10^8 cells /ml (Baris *et al.,* 2006) which is equal to McFarland 0.5 turbidity standard.

3.7 Antimicrobial activity tests

3.7.1 Screening for antibacterial and antifungal property

Antimicrobial activity of the methanol extracts was determined by Disc diffusion assay method as described by Rojas *et al.,* (2006) and Moshi *et al.,* (2006).

3.7.1.1 Disc diffusion method

Muller Hilton Agar and Sabouraud Dextrose Agar (SDA) were prepared for bacteria and fungi respectively according to the manufacturers' instructions (Aliero and Afolayan, 2005). The media was autoclaved and then the media was allowed to cool in a 45°C to 50°C water bath. The freshly prepared and cooled media was poured into flat-bottomed Petri dishes (90mm in diameter) placed on a level, horizontal surface in laminar flow to give a uniform depth of approximately 4 mm. The agar media was allowed to cool and solidify at room temperature. About 0.2ml of the test inoculum was evenly spread on the surface of the solidified agar media using a sterile cotton swabs.

Sterile 5 mm-Whatmann No. 1 filter paper discs were used in the disc diffusion method. A sterile flat glass surface was partitioned using a marker pen and the regions labelled according to the samples. The discs were placed on the glass in the labelled partitions and 10µl of the corresponding samples were dispensed on the discs using micro-dispenser, changing the tips after every sample. The discs were then allowed to dry in laminar flow hood for 4 – 5 hours after which the discs were placed at the middle of the labelled inoculated plates.

The treated plates were stored in a refrigerator at 4°C for 24 hours to allow sufficient diffusion of the samples into the media and then transferred to incubator at 37°C for 24 hours and 72 hours for bacterial and fungal cultures respectively. The test was carried out in duplicates. Antimicrobial activities were determined by measuring the diameters of zones of inhibition (Activity index) in millimetres.

The means and standard deviations (±SD) of the diameters of zones of growth inhibitions for the treatments were determined using Microsoft Excel software.

3.7.1.2 Controls

Negative control was prepared by dispensing 10µl methanol on blank discs and allowed to dry. Chloramphenical discs (30µg/disc) and nystatin discs (25µg/disc) were used as a positive control for bacterial cultures and fungal cultures respectively.

Some inoculated plates were prepared without any disc as a proof for viability of the inoculums. The plates were put in the same conditions as the plates with test samples and then their activity indexes were determined and recorded.

3.8 Minimum Inhibition Concentration (MIC) assay for antimicrobial tests

3.8.1 Sample preparation

The samples which were used for screening were diluted at specific ratios. A 100% sample, which is a sample with concentration equivalent to the sample used for screening was prepared by dissolving 250mg of each sample in 10ml of methanol in separate bottles to make a concentration of 25mg/ml.

The other dilutions made were 90%, 80%, 70%, 60%, 50% and 40% which were prepared by mixing portion of the 100% with distilled methanol in appropriate ratios to make the required concentrations (Table 2).

10μl of each sample of the prepared dilutions was placed on sterilised 6mm diameter discs prepared from Whatmann filter paper on a well partitioned flat glass which was labled accordingly. This was done in a laminar flow hood and the discs were left there for 4-5 hours to allow for drying in order to ensure there was no residual methanol in the discs.

Table 3. 2: Dilution of samples for MIC assay

Sample dilutions	Volume of 100% sample (ml)	Volume of methanol (ml)	Volume of sample made (ml)	Concentration Mg/ml	Mass dispensed (mg)
100%	2	0	2	25.0	0.250
90%	1.8	0.2	2	22.5	0.225
80%	1.6	0.4	2	20.0	0.200
70%	1.4	0.6	2	17.5	0.175
60%	1.2	0.8	2	15.0	0.150
50%	1.0	1.0	2	12.5	0.125
40%	0.8	1.2	2	10.0	0.100

3.8.2 Antifungal and antibacterial MIC assay

Sabouraud Dextrose Agar (SDA) and Muller Hilton agar plates were prepared for fungal and bacterial cultures respectively. The appropriate microbes for MIC assay were selected as those which showed inhibition zone values of at least 10mm. The microbes were spread on media using sterile cotton swabs. The discs were then placed on the

middle of the inoculated plates and the plates were appropriately labelled. Each test for MIC assay was done in triplicate. The cultures were placed in the fridge at 4 ^{o}C for 24 hours in order to allow the samples to spread into the media. The cultures were then removed and placed in the incubator at temperature of 37 ^{o}C for 24 hours and 72 hours for bacteria and fungi growth respectively.

The cultures were then removed, the inhibition zones were measured using a ruler in millimetres and recorded. The mean and the standard deviation of the diameters were calculated and recorded. The lowest concentration which showed a minimum activity index was considered as the MIC value.

3.9 Determination of the phytochemical composition

Chemical tests were carried out on the methanol crude extracts of *S. nigrum* complex leaves using standard procedures to identify the constituents as described by Sofowora (1993) and Edeoga *et al.,* (2005). Presence of the compounds was identified by specific colour changes. The results were reported as (+) for presence and (-) for absence.

3.9.1 Terpenoids (Salkowski test)

0.2 g of the extract of the sample was mixed with 2ml of chloroform and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results (Edeoga *et al.,* 2005).

3.9.2 Alkaloids

About 0.2 g of the extracts was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the presence of alkaloids (Sofowora, 1993).

3.9.3 Tannins

Small quantity of extract was mixed with water heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. Positive tests were confirmed by a characteristic dark green or blue – green colouration (Edeoga *et al.,* 2005).

3.9.4 Phenolic compounds

5% aqueous iron III chloride was added in a small amount of the sample and warmed in a water bath. An orange colour confirmed presence of phenolic compounds (Sofowora, 1993).

3.9.5 Flavonoids

A small amount of the sample was dissolved in sodium hydroxide followed by aqueous hydrochloric acid. White fumes were observed and colour of the mixture changed dark green and then brown, confirming presence of flavonoids (Sofowora, 1993).

3.9.6 Phlobatanins

A small amount of the sample was dissolved in distilled water and then filtered. The filtrate was then boiled in 2% HCl a red precipitate indicates presence of phlobatanins (Edeoga *et al.,* 2005).

3.9.7 Sterols

The sample was treated with 50% acetic anhydride in sulphuric acid and then heated on a Bunsen flame. Colour change to brown then green indicated presence of sterols.

3.9.8 Saponins

To a small amount of the extract dissolved in methanol, few drops of olive oil was added and shaken vigorously. Formation of an ice emulsion confirmed a positive test of saponins (Edeoga *et al*, 2005).

3.9.9 Steroids

To a small amount of the sample dissolved in methanol, acetic anhydride was added followed by aqueous sulphuric acid. Colour change to brownish green indicated presence of steroids (Shanmugavali *et al.,* 2009).

3.9.10 Cardiac glycosides (Keller-Killiani test)

Acetic acid was added to a small amount of the extract followed by Iron III chloride. Concentrated sulphuric acid was carefully added on the sides of the tube without shaking to form two layers. Observation of a brown ring at the interphase showed a positive test (Edeoga *et al.,* 2005).

3.10 Data analysis

T-test was used to determine the level of significance between the antimicrobial results of the extracts at different sample concentrations. The efficacy of the samples against gram negative bacteria and Gram positive bacteria was also compared, and the antimicrobial activity of the solid phase samples was compared to that of the aqueous phase samples. Statistical Package for Social Sciences (SPSS) was used for this analysis.

CHAPTER 4

RESULTS

4.1 Species of *S. nigrum* complex collected

The samples were collected in different places. Samples K02, K04 and K05 were collected from Bungoma, K03 and K09 from Kakamega, K01 and K07 from Kisumu, K08 and K10 were collected from Eldoret. Each of the samples had different morphological characteristics (Figures 4.1 – 4.3). These morphological characteristics included flower colour which was either yellow or white, colour of berries which were either black or orange when ripe, leaf size; some leaves were wider while others were narrow, size of berries; some were smaller (about 5 mm diameter) while others were larger (20 mm in diameter), shape of leaves; some had smooth edges while others had serrated edges. The figures 5 to 7 represent photographs of some of the variants collected from Bungoma district.

Figure 4. 1: *Solanum scabrum* (Bungoma Agriculture sample – K05)

(Source: Author, 2012)

Bungoma agriculture sample K05 is the modified variety with the seeds supplied by the Kenya seed company. The berries are wide (up to 20 mm diameter); they are usually

black in colour. The leaves are more rounded and wide with smooth edges as (Figure 4.2). The stems and petiole are also wide and highly succulent.

The other variant collected was locally referred to as Bungoma bitter. This is an indigenous variety. The name is associated to the characteristic bitter taste of the leaves. The leaves are more elongated with longer leaf tip as compared to the Bungoma Agriculture variant. The main distinguishing feature of this variant is the colour and size of the berries; ripe berries of the Bungoma bitter variant are black in colour, just like the modified variety, but in this case, they are small (approximately 5mm in diameter).

Figure 4. 2: ***Solanum nigrum*** **Mill (Bungoma bitter_K02)**

(Source: Author, 2012)

The Bungoma less bitter variant is characterized by the relatively less bitter taste of the leaves when used as vegetables. The leaves are far slender with serrated edges (Figure 4.3). The main distinguishing characteristic is the colour of the berries. The berries are usually green when unripe and then they turn orange while ripe. These berries are usually sweet and edible when ripe. They have a diameter of approximately 5mm.

Figure 4. 3: ***Solanum villosum*** **(Bungoma less bitter-K04)**

(Source: Author, 2012)

There were two samples collected from Kisumu. One was Kisumu Agricultire (K07) which is a cultivated variety and the seeds are bought from the modern agrovets, being supplied by the Kenya seed company. This variety is the modified type and not indigenous, thus its locally refered to as the agriculture variety because the seeds were introduced to the local people by the Agricultural extension officers.

The main distinguishing feature of this variant is the large size of the berries (approximately 20mm in diameter), the berries are green when unripe and black when ripe. The leaves are wide and unlike the Bungoma agriculture variety, they have pointed petioles. These leaves are less bitter and according to the farmer, they do not necessarily require boiling and discarding the water before frying during cooking. These leaves are highly succulent (Appendix 1).

The indigenous variety from Kisumu is reffered to as the Kisumu Ahero (Sample K01). This sample highly resemble the sample Bungoma less bitter (K04). The berries are

small; red when ripe and green whem unripe. These seeds are edible and sweet. The seeds are processed from ripe berries locally by the farmers and accprding to the farmers, they have a shelf life of up to two years before they start loosing viability. The leaves are narrow and have serrated edges. According to the farmers, these leaves are bitter and milk is added in the leaves during cooking in order to reduce the bitterness (Appendix 1).

Samples collected from kakamega were two, the bred variant (Kakamega Butsotso-K03) was the most common and most popular in this region according to the farmers. It is highly commercialised in this region and highly marketable because it is said to be not bitter. The leaves are broad and highly succulent. The plants are usually cultivated under irrigation during the dry seasons. The berries are wide and black in colour, and the leaves have rounded petioles, thereby highly resembling the Bungoma agriculture sample (K05). The indigenous variety has narrow leaves and is considered to be bitter. It is therefore less popular and only available as a weed which grows during the rainy season, it is rarely cultivated in this region. This is the sample reffered to as Kakamega indigenous (K09). This sample has high resemblance to the Bungoma bitter sample.

Samples collected from Eldoret were Eldoret AMP (K10) , Eldoret Ziwa-A1 (K08) and Eldoret indigenous (K06). Samples K10 and K08 are both the bred varieties, with broad leaves and broad black berries, the only difference being the fact that the sample K10 had more rounded leaves and the sample K08 had the leaves with more pointed petioles. Sample K06 was the indigenous variety with narrow leaves and small berries. The colour of the berries was black when ripe and green when unripe. This sample highly resemble the Bungoma bitter sample. It is also cultivated just like the bred variants in the Eldoret region and is equally highly marketable (Appendix 1).

The samples were found to belong to three different species namely *S. nigrum, S. villosum* and *S. scabrum*. The *S. villosum* species is characterised by the colour of ripe berries ranging from yellow through orange to red while those of *S. nigrum* Mill vary from green through purple to black (Edmonds and Chweya 1997). There were both interspecific variation and intraspecific variations. The difference in variants from the same species can be associated to the kind of climate, type of soil (soil structure and texture) and presence of different water soluble compounds in the soil (Edmonds and Chweya, 1997), it can also be associated to phenotypic plasticity, genetic variation, the existence of a polyploidy series, and the possibilities of interspecific hybridization (Edmonds, 1997). A summary of the classification of the collected samples into species is shown below (Table 4.1).

Table 4. 1: A summary of the samples collected and the corresponding species

Species	Samples
S. nigrum Mill	K02 – Bungoma bitter
	K09 – Kakamega indigenous
	K01 - Kisumu Ahero
S. scabrum	K03 – Kakamega Butsotso
	K05 – Bungoma agriculture
	KO7 – Kisumu agriculture
	K08 – Eldoret ziwa (A1)
S. villosum	K04 – Bungoma less bitter
	K06 – Eldoret indigenous

4.2 Percentage yield of crude extracts from the different samples

After extracting and drying the samples to a constant weight, the percentage yields were determined and recorded. The sample K04 had the highest percentage yield of 15.01% while sample K10 had the least percentage yield of 6.14%. The rest of the samples had the percentage yields ranging between these two values (Table 1.2).

Table 4. 2: Percentage yields of extracts from *S. nigrum* complex

Samples	K01	K02	KO3	K04	K05	K06	K07	K08	K09	K10
Plant dry mass used (g)	174.37	500	500	500	500	500	200	350	350	500
Mass of aqueous phase (g)	9.76	7.12	9.27	8.26	11.13	15.76	12.05	10.08	9.43	15.24
Mass of solid phase (g)	12.40	25.30	34.40	66.80	59.50	23.66	17.39	11.79	19.38	15.45
Total mass of crude extract (g)	22.16	32.42	43.67	75.06	70.63	39.42	29.44	21.87	28.81	30.69
Percentage yield	**12.70%**	**6.48%**	**8.73%**	**15.01%**	**14.12%**	**7.88%**	**14.72%**	**6.24%**	**8.23%**	**6.14%**

Key: K01= Kisumu Ahero, K02 =Bungoma bitter, K03= Kakamega Butsotso, K04 =Bungoma less bitter, K05 = Bungoma Agriculture, K06=Eldoret indigenous (Ziwa), K07 = Kisumu Agriculture, K08 = Eldoret Ziwa (A1), K09 = Kakamega indigenous, K10 = Eldoret AMP

4.3 Antibacterial activity of the *Solanum nigrum* complex samples

4.3.1 Antibacterial activity of the solid phase samples

The *S. nigrum* complex samples were tested against eight different bacterial species manely; *S. typhi, E. coli, S. aureus, P. mirabilis, P. aureginosa, P. syringae, B. subtillis*, and *Shigella ssp*. Methanol was used as a negative control but the discs were allowed to dry alongside the samples. The plain discs containing methanol showed no inhibition because the entire methanol was evaporated. This was used to account for any residual methanol in the sample. Figure 4.4 shows one of the plates for antibacterial screening of the *S. nigrum* complex samples (Figure 4.4).

Figure 4. 4: A plate showing inhibition zones around the discs

(Source: Author, 2012)

The solid samples were screened for activity against eight different species of bacteria namely; *S. typhi, E. coli, S. aureus, P. mirabillis, P. aureginosa, P. syringae, B. subtillis*, and *Shigella ssp*. The samples K01S to K05S were screened at a concentration of 25 mg/ml while samples K06S to K10S were screened at a higher concentration of 100 mg/ml.

The solid phase samples showed a considerable amount of antibacterial activity. All the five samples were active against *S. typhi*, *P. aureginosa* and *E. coli. Shigella spp* showed resistance against the samples K01S, K02S, K03S, K04S and K05S. However, the standard antibiotic had a small inhibition of 9.50 mm against *Shigella ssp*. Other bacteria which showed total resistance against the five samples are *S. aureus*, *P. syringae, B. subtilis* and *P. Mirabilis* (Table 4a).

For samples K06S to K10S, only *P. aureginosa* and *B. subtilis* showed total resistance. Sample K08S showed no inhibition against *P. aureginosa, E. coli, P. Syringae, S. aureus and B. Subtilis.* The rest of the samples showed activity against all the other tested bacteria with sample K07S showing the highest activity of 10.50 mm against *E. coli*. *Shigella spp* was also resistant to Sample K06S (Tables 4.3).

Table 4. 3: Inhibition zones for the antimicrobial activity of the solid samples at 25 mg/ml (K01 – K05S)

Bacteria species	**Samples**					**[a] STANDARD**
	Inhibition zones (mm)					
	K01S	K02S	K03S	K04S	K05S	
Shigella ssp	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.50 ± 0.70
S. typhi	8.00 ± 1.41	8.00 ± 1.41	8.00 ± 1.41	8.00 ± 1.41	8.00 ± 1.41	22.50 ± 0.70
S. aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	44.00 ± 1.41
P. aureginosa	6.50 ± 0.07	6.50 ± 0.07	6.50 ± 0.07	6.50 ± 0.07	6.50 ± 0.07	6.50 ± 0.70
E. coli	6.50 ± 0.70	6.50 ± 0.70	6.50 ± 0.70	6.50 ± 0.70	0.00 ± 0.00	46.00 ± 1.41
P. syringae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	35.00 ± 0.00
B. subtilis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	47.50 ± 3.53
P. mirabilis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.00 ± 1.41

KEY

a - Chloramphenical, KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture

Table 4. 4: Inhibition zones of antibacterial activity of solid samples at 100 mg/ml (K06S – K10S)

Bacterial species	Samples					[a] STANDARD
	Inhibition zones (mm)					
	K06S	K07S	K08S	K09S	K10S	
Shigella ssp	0.00 ± 0.00	6.50 ± 0.71	6 .00 ± 0.00	8.50 ± 0.71	9.00 ± 0.00	9.50 ± 0.70
S. typhi	7.00 ± 1.41	6.50 ± 0.71	6.50 ± 0.71	7.00 ± 1.41	9.00 ± 0.00	22.50 ± 0.70
S. aureus	7.00 ± 0.00	9.50 ± 0.71	0.00 ± 0.00	8.50 ± 2.12	6.00 ± 0.00	44.00 ± 1.41
P. aureginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.50 ± 0.70
E. coli	9.00 ± 1.41	10.50 ± 0.71	0.00 ± 0.00	8.00 ± 1.41	8.50± 0.71	46.00 ± 1.41
P. syringae	6.00 ± 0.00	6.50 ± 0.71	0.00 ± 0.00	6.00 ± 0.00	6.50± 0.71	35.00 ± 0.00
B. subtilis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	47.50 ± 3.53
P. mirabillis	6.50 ± 0.71	6.50 ± 0.71	7.00 ± 4.14	8.00 ± 1.41	7.00 ± 0.00	39.00 ± 1.41

KEY

a - Chloramphenical, K06 –Eldoret indigenous (Ziwa), K07 – kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret

4.3.2 Antibacterial activity of the aqueous phase sample

After decanting the solid phase sample from the aqueous phase samples, the liquid samples were also screened for activity against the bacteria. This was general screening to determine any antibacterial activity but the sample concentration was not determined at this level. The undiluted aqueous phase samples showed a lot of activity (Table 5). All the five tested samples showed some inhibition to all the tested bacteria. K03L showed the highest activity of 20.00 mm when tested against *S. aureus*. The least activity was an inhibition zone of 6.00 mm which was given by sample K05L against *Shigella ssp* and sample K03 against *S. typhi, B. subtilis* and *P. syringe* (Table 4.5).

Table 4. 5: inhibition zones of undiluted aqueous phase samples of *S. nigrum* complex

Bacteria species	Samples (undiluted)					[a] STD
	K01L	**K02L**	**K03L**	**K04L**	**K05L**	
Shigella ssp	12.50±0.70	9.00±1.41	8.00±1.41	0.00±0.00	6.00±0.00	9.50±0.70
S. aureus	9.00±0.00	8.00±0.00	20.00±5.65	15.00±7.07	9.00±0.00	44.00±1.41
E. coli	12.00±1.41	6.00±0.00	8.00±0.00	9.50±0.70	10.00±0.00	46.00±1.41
P. aureginosa	7.50±2.12	11.00±1.41	10.00±0.00	9.00±1.41	7.50±0.70	6.50±0.70
P. mirabillis	11.50±2.12	7.00±1.41	8.00±1.41	7.50±2.12	10.00±0.00	39.00±1.41
S. typhi	9.50±0.70	11.00±1.41	6.00±0.00	7.00±1.41	9.00±0.00	22.50±0.70
B. subtilis	9.50±0.70	8.50±0.70	6.0 ± 0.00	8.00±2.82	10.50±0.70	47.50±3.53
P. syringae	8.00±0.00	8.00 ± 1.41	6.00 ± 0.00	7.50±0.70	9.00±0.00	35.00±0.00

Key: a = Chloramphenical, KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture

After drying the aqueous phase samples to a constant weight, the samples were diluted to known concentrations and then screened for antimicrobial activity (Table 4.6). The samples showed activity against all the bacteria species except *P. aureginosa* which showed resistance to samples K01L, K02L, K03L and K05L. Sample K01L was also not active against *Shigella ssp.* The rest of the samples were active against all the other tested bacterial species. The highest activity was seen for sample K04L against *S. aureus* with an activity index of 29.00 mm. The smallest activity was shown by samples K06L aginst *Shigella ssp* which had an activity index of 6mm. Samples K06L, K07L and K08L also had a small inhibition of 6 mm against *E. coli*. The rest of the samples had activity index of between 6.00 mm and 29.00 mm (Table 4.7).

Table 4. 6: Dilutions prepared for the aqueous phase samples used during screening

Samples	K01L	K02L	K03L	K04L	K05L	K06L	K07L	K08L	K09L	K10L
Concentration of sample prepared (g/ml)	0.745	1.015	0.815	1.020	0.733	0.091	1.200	0.223	0.306	1.320

Key: KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 –Eldoret indigenous (Ziwa), K07 – kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 – Eldoret A

Table 4. 7: Inhibition zones of screening for the antibacterial activity of the liquid samples

Bacteria	Inhibition zones (mm)										
	Samples										[a] STD
	K01L	K02L	K03L	K04L	K05L	K06L	K07L	K08L	K09L	K10L	
Shigella spp	0.00±0.00	10.00±0.00	10.00±0.00	14.00±0.00	10.00±0.00	6.00±0.00	13.50±0.71	8.00±1.41	13.5±0.71	14.50±0.71	9.50±0.70
S. typhi	11.00±1.41	15.00±1.41	20.00±0.00	12.00±0.00	15.00±1.41	7.00±0.00	8.50±0.71	9.00±1.41	19.00±1.41	24.50±0.71	22.50±0.70
S. aureus	14.00±0.00	21.00±1.41	21.00±1.41	29.00±1.41	22.00±0.00	6.50±0.71	10.00±1.41	8.50±0.71	9.00±1.41	10.00±0.00	44.00±1.41
P. aureginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21.00±1.41	0.00±0.00	7.00±1.41	8.50±0.71	9.00±0.00	19.00±1.41	20.00±0.00	6.50±0.70
E. coli	10.00±0.00	13.00±1.41	16.00±0.00	10.00±0.00	13.00±1.41	6.00±0.00	6.00±0.00	6.00±0.00	9.50±0.71	10.00±0.00	46.00±1.41
P. syringae	20.50±0.71	12.00±0.00	10.00±0.00	14.00±0.00	16.00±0.00	8.50±0.71	8.00±1.41	7.50±0.71	11.00±1.41	20.00±0.00	35.00±0.00
B. subtilis	14.00±0.00	13.00±1.41	11.00±1.41	10.00±0.00	15.00±1.41	9.00±1.41	21.00±1.41	10.50±0.71	17.00±1.41	21.00±1.41	47.50±3.53
P. mirabilis	20.00±0.00	10.00±0.00	14.00±0.00	19.00±1.41	20.00±0.00	8.00±0.00	8.00±0.00	7.50±0.71	15.00±1.41	21.00±1.41	39.00±1.41

Key: a – Chloramphenical, KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 –Eldoret indigenous (Ziwa), K07 – kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret A

Table 4. 8: MIC values of the liquid samples against bacteria

Bacterial	MIC values (g/ml)									
	Samples									
	K01L	**K02L**	**K03L**	**K04L**	**K05L**	**K06L**	**K07L**	**K08L**	**K09L**	**K10L**
E. coli	0.49	0.60	0.40	0.70	0.42	0.09	1.20	0.22	0.30	0.60
S. aureus	0.35	0.20	0.16	0.10	0.14	0.09	0.70	0.22	0.16	0.60
P. syringae	0.21	0.70	0.56	0.60	0.35	0.09	1.20	0.22	0.14	0.30
B. subtilis	0.49	0.70	0.64	0.60	0.35	0.09	0.20	0.22	0.04	0.20
P. mirabilis	0.14	0.80	0.48	0.20	0.14	0.09	1.20	0.16	0.30	0.20
S. typhi	0.49	0.50	0.16	0.70	0.35	0.09	1.20	0.22	0.12	0.80
Shigella spp	0.75	0.70	0.56	0.60	0.49	0.09	0.60	0.22	0.14	0.30
P. aureginosa	0.75	1.01	0.82	1.02	0.73	0.09	1.20	0.22	0.12	1.32
Mean	**0.46**	**0.65**	**0.47**	**0.57**	**0.37**	**0.09**	**0.94**	**0.21**	**0.17**	**0.54**

Key: KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 –Eldoret indigenous (Ziwa), K07 – kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret A

4.4 MIC values of *S. nigrum* complex samples against bacteria

The Minimum inhibition concentration value was determined against all the test bacterial species. Sample K01L had an MIC value of 0.49 g/ml when tested against *E. coli*, *B. subtilis* and *S. typhi*. This was the highest MIC value for the sample K01L. The rest of the MIC values were 0.35 g/ml, 0.21 g/ml and 0.14 g/ml for the bacteria *S. aureus*, *P. syringae* and *P. mirabilis* respectively (Table 4.9).

Table 4. 9: Inhibition zones of bacteria at different concentrations of sample K01L

Bacteria	**K01L sample concentration(g/ml)**							^{b}**STD**	**MIC(g/ml**
	0.70g/ml	0.63 g/ml	0.49 g/ml	0.35 g/ml	0.21 g/ml	0.14 g/ml	0.07 g/ml		
E. coli	9.67±0.58	8.00±1.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.49**
S. aureus	13.67±0.58	12.00±0.00	7.67±0.58	6.33±0.58	0.00	0.00	0.00	0.00	**0.35**
P. syringae	19.00±1.00	17.67±0.58	14.67±0.58	9.33±0.58	6.33±0.58	0.00	0.00	0.00	**0.21**
B. subtilis	10.67±0.58	9.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.49**
P. mirabillis	18.67±0.58	17.33±0.58	14.00±0.00	10.00±0.00	7.67±0.58	6.00±0.00	0.00	0.00	**0.14**
S. typhi	9.33±0.58	7.67±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	**0.49**

KEY = b –Methanol, K01L – Kisumu Ahero Aqueous phase sample

Sample K02L had the MIC values ranging from 0.80 g/ml to 0.20 g/ml. The highest MIC value was seen against *P. mirabilis* while the least MIC value was seen against *S. aureus.* When tested against *P. syringae, Shigella spp* and *B. subtilis*, the sample gave an MIC value of 0.7 g/ml, followed by *E. coli* and *S. typhi* which gave MIC values of 0.6 g/ml and 0.5 g/ml respectively (Table4.10).

Table 4. 10: Inhibition zones of bacteria at different concentrations of sample K02L

Bacteria species	**K02 sample concentrations (g/ml)**							**^{b}STD**	**MIC(g/ml**
	1.00	**0.80**	**0.70**	**0.60**	**0.50**	**0.20**	**0.10**		
Shigella spp	10.00±0.00	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.70**
E. coli	12.00±0.00	9.33±0.58	8.00±0.00	6.33±0.58	0.00	0.00	0.00	0.00	**0.60**
S. aureus	18.67±0.58	15.67±0.58	13.67±0.58	12.33±0.58	9.67±0.58	6.00±0.00	0.00	0.00	**0.20**
P. syringae	9.67±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.70**
B. subtilis	12.33±0.58	8.00±1.00	6.33±0.58	0.00	0.00	0.00	0.00	0.00	**0.70**
P. mirabilis	9.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.80**
S. typhi	13.67±0.58	9. 33±0.58	7. 67±0.58	7.00±0.00	6.33±0.58	0.00	0.00	0.00	**0.50**

KEY

b- Methanol, K02L – Bungoma bitter

Sample K03L had the highest MIC value of 0.64 g/ml which occurred when the sample was tested against *B. subtillis*. This was followed by MIC value of 0.56 g/ml which occurred when the plant sample was tested against *Shigella spp* and *P. syringae*. *E. coli* and *P. mirabilis* both gave MIC values of 0.40 g/ml and 0.48 g/ml respectively. When tested against both *S. typhi* and *S. aureus*, the sample gave the least MIC value of 0.16 g/ml (Table 4.11).

Table 4. 11: Inhibition zones of bacteria at different concentrations of sample K03L

Bacteria species	**K03L sample concentrations (g/ml)**									**[a]STD**	**MIC (g/ml)**
	0.80	**0.72**	**0.56**	**0.48**	**0.40**	**0.32**	**0.24**	**0.16**	**0.08**		
Shigella	9.67±0.58	8.00±1.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.56**
E. coli	14.00±0.00	11.33±1.57	8.00±1.00	7.33±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	**0.40**
S. aureus	18.67±0.58	17.33±0.58	14.00±0.00	12.33±0.58	10.00±0.00	8.67±0.58	7.67±0.58	6.00±0.00	0.00	0.00	**0.16**
P. syringae	9.33±0.58	7.67±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.56**
B. subtilis	9.67±0.58	8.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.64**
P. mirabilis	12.00±0.00	9.33±0.58	7.00±0.00	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	**0.48**
S. typhi	18.67±0.58	17.00±0.00	13.67±0.58	12.33±0.58	9.67±0.58	8.33±0.58	6.67±0.58	6.00±0.00	0.00	0.00	**0.16**

Key: a - Chloramphenicol, K03L – Kakamega Butsotso liquid sample

For sample K04L, the MIC values had a narrow range with the highest MIC value being 0.7 g/ml when the sample was tested against *E. coli* and *S. typhi*. This was followed by an MIC value of 0.60 g/ml when tested against *Shigella spp*, *P. syringae* and *B. subtilis*. *P. mirabilis* and *S. aureus* had MIC values of 0.20 g/ml and 0.10 g/ml respectively (Table 4.12).

Table 4. 12: Inhibition zones of bacterial species at different concentrations of sample K04L

Bacterial species	**K04L sample concentrations (g/ml)**									**[b]STD**	**MIC**
	1.00	**0.90**	**0.70**	**0.60**	**0.50**	**0.40**	**0.30**	**0.20**	**0.10**		
Shigella.spp	12.67±0.58	10.67±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.60**
E. coli	9.00±0.00	7.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.70**
S. aureus	24.67±0.58	19.67±0.58	16.67±0.58	16.00±0.00	14.33±0.58	9.33±0.58	8.00±0.00	7.33±0.58	6.00±0.00	0.00	**0.10**
P. syringae	10.33±0.58	9.00±0.00	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.60**
B. subtilis	9.33±0.58	7.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.60**
P. mirabilis	18.67±0.58	17.00±0.00	13.67±0.58	12.33±0.58	9.67±0.58	8.33±0.58	6.67±0.58	6.00±0.00	0.00	0.00	**0.20**
S. typhi	9.33±0.58	7.00±1.00	2.00±3.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.70**

KEY: b – methanol, K04L – Bungoma less bitter sample

The sample K05L had the highest MIC value of 0.49 g/ml when tested against *Shigella spp. E. coli* also gave a high MIC value of 0.42 g/ml for the same sample. This was followed by an MIC value of 0.35 g/ml which occurred when the sample was tested against *P. syringae, B. subtilis* and *S. typhi.* The least MIC value for the sample K05L was 0.14 g/ml which occurred when the sample was tested against *S. aureus* and *P. Mirabilis* (Table 4.13).

Table 4. 13: Inhibition zones of bacteria at different concentrations of sample K05L

Species	**K05L sample concentrations (g/ml)**									**[b]STD**	**MIC**
	0.70	**0.63**	**0.49**	**0.42**	**0.35**	**0.28**	**0.21**	**0.14**	**0.07**		
Shigella.spp	11.00±0.00	8.00±1.00	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.49**
E. coli	12.67±0.58	10.67±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.42**
S. aureus	18.67±0.58	17.00±0.00	13.67±0.58	12.33±0.58	9.67±0.58	8.33±0.58	6.67±0.58	6.00±0.00	0.00	0.00	**0.14**
P. syringae	13.33±0..58	10.67±0.58	7.33±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.35**
B. subtilis	12.33±0.58	10.67±0.58	8.33±0.58	7.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.35**
P. mirabillis	18.67±0.58	17.33±0.58	14.00±0.00	12.00±0.00	10.00±0.00	8.67±0.58	7.67±0.58	6.00±0.00	0.00	0.00	**0.14**
S. typhi	14.67±0.58	11.67±1.53	7.00±1.00	6.33±0.58	2.00±3.46	0.00	0.00	0.00	0.00	0.00	**0.35**

KEY: b = methanol, K05L – Bungoma agriculture liquid sample

Sample K07L only had three microbes with inhibition zones of at least 10 mm which therefore proceeded to MIC assay. These microbes are *Shigella spp, S. aureus* and *B. subtilis* having MIC values of 0.60 g/ml, 0.70 g/ml and 0.20 g/ml respectively (Table 4.14).

Table 4. 14: Inhibition zones of bacterial species at varying concentrations of sample K07L

Species	**Concentrations of sample K07L(g/ml)**									**[b]STD**	**MIC**
	1.00	0.90	0.80	0.70	0.60	0.50	0.40	0.20	0.10		
Shigella spp	10.00±0.00	9.00±0.00	7.66±0.58	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.60**
S. aureus	10.00±0.00	8.33±0.58	7.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.70**
B. subtilis	18.67±0.58	17.33±0.58	15.33±0.58	14.00±0.00	12.00±0.00	10.00±0.00	8.67±0.58	6.00±0.00	0.00	0.00	**0.20**

Key: b = methanol, K07L – Kisumu Agriculture Liquid sample.

Sample K10L had a high MIC value of 0.8 g/ml when tested against *S. typhi*. This was followed by an MIC value of 0.6 g/ml when tested against *S. aureus* and *E. coli*. The most common MIC value was 0.3 g/ml which occurred when the sample was tested against *Shigella spp*,

P. aureginosa and *P. syringae*. The smallest MIC value was 0.2 g/ml which occurred when the sample was tested against *P. mirabilis* and *B. subtillis* (Table 4.15).

Table 4. 15: Inhibition zones of bacteria at varying concentrations of sample K10L

Species	K10L sample concentrations (g/ml)									bSTD	MIC(g/ml
	1.00	**0.80**	**0.70**	**0.60**	**0.50**	**0.40**	**0.30**	**0.20**	**0.10**		
P. mirabilis	16.67±0,58	13.00±1.00	11.33±0.8	10.67±0.58	9.33±0.58	8.33±0.58	7.33±0.58	6.00±0.00	0.00	0.00	**0.20**
S. typhi	14.33±0.58	6.66±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.80**
S. aureus	14.67±0.58	10.33±0.58	8.00±1.00	6.67±0.58	0.00	0.00	0.00	0.00	0.00	0.00	**0.60**
Shigella spp	13.67±0.58	11.00±0.00	10.33±0.58	8.333±0.58	8.00±0.00	7.67±0.58	6.00±0.00	0.00	0.00	0.00	**0.30**
P .aureginosa	14.67±0.58	12.67±0.58	11.33±0.58	10.00±0.00	8.67±0.58	7.67±0.58	6.00±0.00	0.00	0.00	0.00	**0.30**
P .syringea	16.67±0.58	12.67±0.58	11.67±0.58	10.67±0.58	9.67±0.58	7.67±0.58	6.00±0.00	0.00	0.00	0.00	**0.30**
E. coli	10.67±0.58	8.33±0.58	7.33±0.58	6.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00	0.00	**0.60**
B. subtilis	19.00±1.00	16.00±0.00	14.67±0.58	11.67±1.53	9.33±0.58	7.00±1.00	6.33±0.58	6.00±0.00	0.00	0.00	**0.20**

Key: b – Methanol, K10L – Eldoret AMP

Sample K09L had the MIC concentration values ranging between 0.16 g/ml and 0.04 g/ml. *B. subtilis* gave the smallest MIC value of 0.04 g/ml. This was followed by *S. typhi* and *P. aureginosa* both giving MIC values of 0.12 g/ml. *Shigella spp* followed by giving an MIC value

of 0.14 g/ml. The highest MIC value for sample K09L was 0.16 g/ml which occurred when the sample was analyzed against *S. aureus* (Table 4.16).

Table 4. 16: Inhibition zones of bacteria at different concentrations of sample K09L

Species	K09L sample concentrations (g/ml)									^{b}STD	MIC(g/ml
	0.20	**0.16**	**0.14**	**0.12**	**0.10**	**0.08**	**0.06**	**0.04**	**0.01**		
Shigella	10.33±0.58	7.66±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.14**
S. typhi	17.33±0.58	10.00±0.00	7.33±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	**0.12**
P.syringae	12.33±0.58	8.67±0.58	6.33±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.14**
P. aureginosa	12.00±0.00	8.67±0.58	7.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.12**
B. subtilis	18.67±0.58	15.67±0.58	14.00±0.00	12.33±0.58	10.00±0.00	8.67±0.58	7.67±0.58	6.00±0.00	0.00	0.00	**0.04**
S. aureus	9.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.16**

Key: b- Methanol, K09L – Kakamega indigenous liquid sample

Sample K08L was only tested for MIC against *B. subtillis* and the MIC value was 0.16 g/ml. the rest of the bacteria had inhibition zones which were smaller than 10 mm and therefore did not proceed to MIC assay (Table 4.17).

Table 4. 17: Inhibition zones of bacteria at different concentrations of sample K08L

Species	K08L sample concentrations (g/ml)									^{b}STD	MIC(g/ml)
	0.20	**0.16**	**0.14**	**0.12**	**0.10**	**0.08**	**0.06**	**0.04**	**0.01**		
B. subtilis	9.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16

Key

b- Methanol, K08L – Edoret ziwa A1

The MIC assay was also performed against the solid samples which had activity index of at least 10mm. These were K07S, K05S and K04S. Sample K07S was only tested against *E. coli* and it gave an MIC value of 0.12 g/ml. Sample K05S was tested against *P. aureginosa* and *S. typhi* where the MIC values were 0.0175 g/ml and 0.020 g/ml respectively. K04S was tested against *P. aureginosa* and the MIC value was 0.0175 g/ml (Tables 4.18a – 4.18c).

Table 4.18 a: Inhibition zones of bacteria of different concentrations of sampleK04S (Bungoma less bitter)

Species	K04S sample concentrations (g/ml) *10^{-3}							[b]STD	MIC(g/ml
	25.0	**0.00**	**20.0**	**17.5**	**15.0**	**12.5**	**10.0**		
P. aureginosa	13.67 ± 0.58	0.00	7.83 ± 0.76	6.16 ± 0.29	0.00	0.00	0.00	0.00	**0.0175**

Key: b- Methanol, K04S – Bungoma less bitter solid phase sample

Table 4.18 b: Inhibition zones of bacteria at different concentrations of sample K05S

Bacterial species	K05S sample concentrations (g/ml) *10^{3}							[b]STD	MIC
	25.0	**22.5**	**20.0**	**17.5**	**15.0**	**12.5**	**10.0**	**0.00**	
P. aureginosa	12.33 ±0.57	9.00 ±1.00	6.67 ±0.57	2.00 ±3.46	0.00	0.00	0.00	0.00	**0.0175**
S. typhi	11.67 ± 0.58	9.00 ± 1.00	6.66 ± 1.58	0.00	0.00	0.00	0.00	0.00	**0.020**

Key: b- Methanol, K05S – Bungoma agriculture solid phase sample

Table 4.18 c: Inhibition zones of bacteria at different concentrations of sample K07S (Kisumu Agriculture)

Species	K07S sample concentrations (g/ml)								[b]STD	MIC(g/ml)
	0.20	**0.18**	**0.16**	**0.14**	**0.12**	**0.10**	**0.08**	**0.01**		
E. coli	10.00±0.00	9.00±0.00	7.66±0.58	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	**0.12**

Key: K07S – Kisumu Agriculture solid sample

4.5 Antifungal activity of *S. nigrum* complex samples

The antifungal activity for the *S. nigrum* complex samples was determined against *F. culmorum, F. avenaceum* and *F. moniliforme*. These are fungi which are notorious in causing plant diseases. They are also known to be major cause of food spoilage and food poisoning in serials and grains where they cause food intoxication that lead to death or illness of humans and animals upon consumption of food contaminated with these fungi.

4.5.1 Antifungal property of the solid samples

Fusarium culmorum was only inhibited by samples K01S, K04S, and K05S. *F. moniliforme* was resistant to all the tested samples except the reference standard nystatin where there was an activity index of 14.00 mm. *F. avenaceum* was inhibited by samples K02L, K03S and K04S (Table 4.19a).

The three fungi were (*F. culmorum, F. moniliforme* and *F. Avenaceum*) were all completely resistant to samples K06S K07S, K08S, K09S and K10S (Table 4.19b).

Table 4.19 a: Screening for the antifungal activity of solid samples at 25 mg/ml

Fungi	**Samples**					**Nystatin**
	K01S	**K02S**	**K03S**	**K04S**	**K05S**	
F. culmorum	12.00 ± 0.00	0.00±0.00	0.00±0.00	12.50±2.12	7.50±0.71	16.50±2.12
F. moniliforme	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	14.00±1.41
F. avenaceum	0.00±0.00	8.00 ± 0.00	6.00 ± 0.00	7.00 ± 1.41	0.00±0.00	24.00±1.41

Key: KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture

Table 4.19 b: Screening for the antifungal activity of solid samples at 100 mg/ml (KO6S – K10S)

Fungi	**Samples**					**Nystatin**
	K06S	K07S	K08S	K09S	K10S	
F.culmorum	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	16.50±2.12
F.moniliforme	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	14.00±1.41
F. avenaceum	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	24.00 ± 1.41

Key: K06 –Eldoret indigenous (ziwa), K07 – Kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret AMP

4.5.2 Antifungal property of the aqueous phase samples

The antifungal activity of the aqueous phase samples was also determined and they exhibited a considerable amount of antifungal activity. *F. culmorum* was inhibited by all the tested samples except sample K06L, K08L and K09L. *F. moniliforme* was inhibited by most of the tested samples except sample K03L, K06L, K08L and K09L. The fungi *F. avenaceum* was inhibited by all the tested samples with very high activity indexes recorded. Sample K05L had an activity index of 26.00mm, which is higher than that of nystatin, a standard antifungal drug (Tables 4.20a and 4.20b).

Table 4.20 a: Screening for the antifungal activity of liquid samples (K01L – K05L)

Fungi	Samples					Nystatin
	K01L	K02L	K03L	K04L	K05L	
F. culmorum	21.00±1.41	19.00±1.41	24.00±0.00	18.00±2.82	16.00±0.00	16.50±2.12
F. moniliform	9.00±1.41	10.00±0.00	0.00±0.00	12.00±2.82	6.00±0.00	14.00±1.41
F. avenaceum	14.00±0.00	15.00±1.41	24.00±0.00	23.00±1.41	26.00±0.00	24.00±1.41

Key: KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture

Table 4.20 b: Screening for the antifungal activity of liquid samples (K06L – K10L)

Fungi	Samples					Nystatin
	K06L	**K07L**	**K08L**	**K09L**	**K10L**	
F. culmorum	0.00±0.00	6.50±0.71	0.00±0.00	0.00±0.00	7.50±0.71	16.50±2.12
F. moniliforme	0.00±0.00	8.50±0.71	0.00±0.00	0.00±0.00	6.50±0.71	14.00±1.41
F. avenaceum	6.50±0.71	7.50±0.71	6.00±0.00	6.50±0.71	8.50±0.71	24.00±1.41

Key:

K06 –Eldoret indigenous (Ziwa), K07 – Kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret AMP

4.6 Determination of the MIC of the antifungal properties of *S. nigrum* complex samples

4.6.1 Determination of MIC of solid samples against fungi

The MIC was also determined for the samples which gave an activity index higher than 10 mm. For all the solid samples, only *F. culmorum* proceeded to the MIC assay when tested against samples K04S and K01S with the samples having MIC values of 20 mg/ml and 17.5 mg/ml respectively (Table 4.21).

Table 4.21: Inhibition zones of *F. Culmorum* at different concentrations of samples K04S and K01S

Samples	**Concentration of samples(mg/ml)**							**[b] STD**	**MIC**
	25	22.5	20	17.5	15	12.5	10		
KO4S	14.00±1.00	10.67±0.58	7.00±0.00	0.00	0.00	0.00	0.00	0.00	**20**
KO1S	12.00±0.00	9.67±0.57	7.00±1.00	6.00±0.00	0.00	0.00	0.00	0.00	**17.5**

Key: b – methanol, K04S – Bungoma less bitter solid sample, K01S – Kisumu Ahero solid sample

4.6.2 MIC of the liquid samples against fungi

Sample K01L was tested against *F. culmorum* and *F. avenaceum* giving MIC values of 0.35 g/ml for both fungi (Table 22). Sample K02L was analyzed for MIC against *F. culmorum, F. moniliforme* and *F. avenaceum* with the MIC values being 0.2 g/ml, 0.6 g/ml and 0.7 g/ml for the three fungi respectively (Table 4.23).

Sample K03L was analyzed for MIC against *F. culmorum* and *F. avenaceum.* With the two fungi having MIC values of 0.16 g/ml and 0.24 g/ml respectively (Table 4.24). K04L was tested for MIC value against the three fungal species. The MIC values were 0.7 g/ml, 0.9 g/ml and 0.3 g/ml for the fungi *F. culmorum, F. moniliforme* and *F. avenaceum* respectively (Table 4.25). Sample K05L was analyzed for MIC against the fungi *F. culmorum* and *F. avenaceum* with MIC values of 0.28 g/ml and0.14 g/ml respectively (Table 4.26).

Table 4.22: Inhibition zones of fungi at varying concentrations of sample K01L

Fungi	Concentrations of sample K01L (g/ml)							^{b}STD	MIC(g/ml
	0.70	**0.63**	**0.49**	**0.35**	**0.21**	**0.14**	**0.07**		
F. culmorum	16.00±0.00	14.00±1.00	10.67±0.58	7.00±0.00	0.00	0.00	0.00	0.00	**0.35**
F. avenaceum	1100±0.00	9.67±0.57	7.00±1.00	6.00±0.00	0.00	0.00	0.00	0.00	**0.35**

KEY- b= Methanol, K01L = Kisumu Ahero liquid sample

Table 4.23: Inhibition zones of fungi at varying concentrations of sample K02L

Species	Concentrations of sample K02L(g/ml)							^{b}STD	MIC(g/ml)
	1.00	**0.80**	**0.70**	**0.60**	**0.50**	**0.20**	**0.10**		
F. culmorum	18.00±0.00	15.33±0.58	12.00±0.00	10.00±0.00	8.67±0.58	6.00±0.00	0.00	0.00	**0.20**
F. moniliforme	10.67±0.58	8.00±0.00	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	**0.60**
F. avenaceum	14.00±1.00	10.67±0.58	7.00±0.00	0.00	0.00	0.00	0.00	0.00	**0.70**

KEY = b- Methanol K02L – Bungoma bitter liquid sample

Table 4.24: Inhibition zones of fungi at varying concentrations of sample K03L

Species	**Sample concentration**									**[b] STD**	**MIC**
	0.80	**0.72**	**0.56**	**0.48**	**0.40**	**0.32**	**0.24**	**0.16**	**0.08**		
F. culmorum	24.67±0.58	19.67±0.58	16.67±0.58	16.00±0.00	14.33±0.58	9.33±0.58	8.00±0.00	6.00±0.00	0.00	0.00	**0.16**
F. avenaceum	23.67±0.58	19.67±0.58	15.00±0.00	14.33±0.58	9.33±0.58	8.00±0.00	6.00±0.00	0.00	0.00	0.00	**0.24**

KEY: b- Methanol K03L – Kakamega Butsotso liquid sample

Table 4.25: Inhibition zones of fungi at different concentrations of sample K04L

Fungi	**Concentrations of sample K04L (g/ml)**									**[b]STD**	**MIC(g/ml)**
	1.00	**0.90**	**0.80**	**0.70**	**0.60**	**0.40**	**0.30**	**0.20**	**0.10**		
F. culmorum	16.00±0.00	14.00±1.00	10.67±0.58	7.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.70**
F.moniliforme	10.67±0.58	7.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.90**
F.avenaceum	17.00±0.00	15.67±0.58	13.67±0.58	12.33±0.58	9.67±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	**0.30**

KEY: b – Methanol K04L – Bungoma less bitter

Table 4.26: Inhibition zones of fungi at different concentrations of sample K05L

Fungi species	**Concentrations of sample K05L (g/ml)**									**^{b}STD**	**MIC(g/ml)**
	0.70	**0.63**	**0.49**	**0.42**	**0.35**	**0.28**	**0.21**	**0.14**	**0.07**		
F. culmorum	15.67±0.58	13.67±0.58	9.67±0.58	8.33±0.58	6.67±0.58	6.00±0.00	0.00	0.00	0.00	0.00	**0.28**
F. avenaceum	24.67±0.58	19.67±0.58	16.67±0.58	16.00±0.00	14.33±0.58	9.33±0.58	8.00±0.00	6.00±0.00	0.00	0.00	**0.14**

Key: b- Methanol, K05L – Bungoma Agriculture Liquid sample

4.7 Antimicrobial activity for the isolated crystal

The crystals isolated from sample K06L were analyzed for antibacterial and antifungal activity against the eight test bacteria and three fungi. There was a considerable amount of activity exhibited by the crystals. The highest activity was 14 mm which was seen when the crystal was analyzed against *S. typhi.* This was followed by the activity against *P. syringae* and *P. mirabilis* both having activity index of 10 mm. The rest of the microbes were inhibited with activity index values less than 10 mm except for *F. culmorum* where there was completely no inhibition (Table 4.27).

Table 4.27: Screening for the antimicrobial activity of the crystals

Microbe	Inhibition zones (mm)	Standard
S. typhi	14.00±1.41	22.50±0.70 [a]
P. syringae	10.00±0.00	35.00±0.00 [a]
E. coli	7.50±0.71	46.00±1.14 [a]
S. aureus	6.50±0.71	44.00±1.41 [a]
B. subtilis	7.00±0.00	47.50±3.53 [a]
Shigella spp	8.00±0.00	9.50±0.70 [a]
P. mirabilis	10.00±0.00	39.00±1.41 [a]
P. aureginosa	6.00±0.00	6.50±0.70 [a]
F. culmorum	0.00±0.00	16.50±2.12[b]
F. avenaceum	8.00±0.00	24.00±1.41[b]
F. moniliforme	6.50±0.71	14.00±1.41[b]

Key: a –Chloramphenical, b -Nystatin

4.8 MIC values of the crystals

The MIC values of the crystal were determined against the microbes which showed activity index values higher of at least 10mm. These were the bacteria *S. typhi*, *P. syringae* and *P. mirabilis. S. typhi* had an MIC of 0.12 g/ml while *P. syringae* and *P. mirabilis* both had an MIC value of 0.14 g/ml (Table 4.28).

Table 4.28: Inhibition zones of the bacteria at different concentrations of the crystals

Bacteria species	**Concentrations of the crystal (g/ml)**							**[a]STD**	**MIC(g/ml)**
	0.2	**0.18**	**0.16**	**0.14**	**0.12**	**0.10**	**0.08**		
S. typhi	13.67±0.58	11.67±0.58	9.33±0.58	7.33±0.58	6.00±0.00	0.00	0.00	0.00	**0.12**
P. syringae	9.67±0.58	8.00±0.00	7.33±0.58	6.00±0.00	0.00	0.00	0.00	0.00	**0.14**
P. mirabilis	10.00±0.00	8.33±0.58	7.00±0.00	6.00±0.00	0.00	0.00	0.00	0.00	**0.14**

Key: a – chloramphenicol

4.9 Phytochemical composition of *S. nigrum* complex extracts

Saponins and cardiac glycosides were found to be present in all the ten samples. Tannins were present in samples K01, K02, K03 K04 and K05 but absent in all the other samples. Sterols were found to be present on samples K01, K02, K03, K04, K05 and K06. Flavonoids were present in all the samples except sample K09 and K10. Plobatanins were present in samples K04L, K06, K07, K08, K09, and K10, but absent in samples K01, K02, K03 K04S and K05. Terpenoids were found to be present in all the tested samples except sample K03, K07, K09 and K01. Phenolic compounds were found to be present in all the samples except sample K02. Anthraquinons were seen to be present in the fast five samples but absent in samples K06, K07, K08 K09 and K01 (Table 4.29 and 4.30).

Table 4.29: Phytochemical composition of solid samples of *S. nigrum* complex extracts

Compound	K01S	K02S	K03S	K04S	K05S	K06S	K07S	K08S	K09S	K10S
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	-	-	-	-	-
Sterols	+	+	+	+	+	+	-	-	-	-
Cardiac glycosides	-	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	-	-
Phlabotanins	-	-	-	-	-	+	+	+	+	+
Terpenoides	+	+	-	+	+	+	-	+	-	-
Alkaloids	-	-	+	-	+	+	-	+	-	-
Phenolic compounds	+	-	+	+	+	+	+	+	+	+

Key

(+) - represent presence of the tested compound in the sample, (-) – represent absence of the tested compound in the sample, S- solid samples

KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 – Eldoret indigenous (Ziwa), K07 – Kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret AMP

Table 4.30: Phytochemical composition of liquid samples of *S. nigrum* complex

Compound	K01L	K02L	K03L	K04L	K05L	K06L	K07L	K08L	K09L	K10L
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	-	-	-	-	-
Sterols	+	+	+	+	+	+	-	-	-	-
Cardiac glycosides	-	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	-	-
Phlabotanins	-	-	-	+	-	+	+	+	+	+
Terpenoides	+	+	-	+	+	+	-	+	-	-
Alkaloids	-	-	+	-	+	+	-	+	-	-
Phenolic compounds	+	-	+	+	+	+	+	+	+	+

Key

(+) - represent presence of the tested compound in the sample, (-) – represent absence of the tested compound in the sample, L – liquid samples,

KO1 – Kisumu Ahero, KO2 – Bungoma bitter, KO3 – Kakamega Butsotso, KO4 – Bungoma less bitter, KO5 – Bungoma Agriculture, K06 – Eldoret indigenous (Ziwa), K07 – Kisumu Agriculture, K08 – Eldoret Ziwa (A1), K09 – Kakamega indigenous, K10 –Eldoret AMP

CHAPTER 5

DISCUSSION

5.1 Percentage yields

During evaporation of the plant samples, some samples demonstrated high level of frothing thereby producing soap-like foam which is an indication of high concentration of saponins (Ravi *et al.,* 2009).

The test samples showed a high variation in the percentage yields, based on the fact that the same solvent and extraction time was used for all the samples, the variation can be attributed to the difference in the amount and type of the soluble compounds in the various dry samples (Edeoga *et al.,* 2005).

The aqueous phase samples showed a variation in the texture with samples K10L, K07L and K04L remaining in the liquid state even after drying the samples to a constant weight, most of the other samples remained semi-solid while a few formed solids, this is associated to the variation in the amount of oily compounds present in the samples. The samples which were in liquid state after drying had very high concentration of oily compounds. This was also confirmed by a positive grease test on the liquid samples. A research done by Ravi *et al.,* (2009) on *S. nigrum* samples also confirmed presence of fixed oils and fats in high concentration.

During sample preparation, samples K04L and K06L formed crystals after being left undisturbed for a long time to allow for drying. This was an indication that the samples had a pure compound which was crystallisable. The similarity in the appearance of the crystals from both samples was an indication of presence of the same compound in these two samples.

5.2 Antibacterial activity of *S. nigrum* complex samples

The samples were screened for antimicrobial activity. Against each test sample, a plain disc containing methanol was also placed and allowed to dry for the same duration as the sample. Absence of any inhibition zones around the methanol discs indicated that the activity observed was entirely due to the samples and not due to any residual methanol in the samples. This is the similar procedure that was used by Souza *et al.,* (2005) in order to eliminate a possibility of antimicrobial activity resulting from the solvent.

5.2.1 Antibacterial activity of solid phase samples

The solid samples were tested for antibacterial activity at two different sample concentrations. Samples K01S to K05S were tested at a concentration of 25 mg/ml while samples K06S to K10S were tested at a sample concentration of 100 mg/ml. On average, there was more antibacterial activity seen for the samples at higher concentration than for the samples at lower concentration. This was statistically proven by the T-test results whereby the T value of the paired means which was 24.52 was out of the expected range of 5.925 – 6.959 at 95% confidence interval (Appendix 2). This implies that the two means of the 25 mg/ml concentration and 100 mg/ml sample concentration are significantly different. This can only mean that the antibacterial activity of the tested samples is dose dependent, and the higher the dose, the higher the antibacterial activity of the solid samples. These results agreed with the results of Sheen (2009).

Samples K01S to K05S were all active against *S. typhi*, *P. aureginosa* and *E. coli.* These bacteria are notorious in causing gastro-intestinal infections, urinary tract infections, dermatitis, bacteremia, (Kenneth 2012), traveler's diarrhea, typhoid fever,

paratyphoid fever, salmonellosis and other nosocomial infections (Tarun*,* 2011). Mostly these bacteria affect immune-compromised patients and also are associated with neonatal meningitis (Tarun*,* 2011). Samples K01 to K05 are therefore capable of treating these infections. This explains the successful use of the extracts from *Solanum* species in traditional treatment of stomach infections (Edmonds and Chweya, 1997)

These samples showed no activity against *Shigella,* similar results to the results observed by Sheen (2009). However, the activity of chloramphenical; the standard antibiotic used, was very small when tested against *Shigella spp.* This can only mean that *Shigella* species used is highly resistant to antibiotics. Other bacteria which showed total resistance against the five samples are *S. aureus***,** *P. syringae, B. subtilis* and *P. mirabilis.* These results can be associated to the low concentration of the samples used because they disagree with results observed by other scientists in the previous studies on the same plants (Sheen, 2009; Aliero and Afolayan, 2005).

Solid samples K06S to K10S which are *S. nigrum Mill* and *S. scabrum* respectively were not active against *P. aureginosa* and *B. subtilis* and showed total resistance. Sample K08S which is a sample of the *S. nigrum* species showed no inhibition against *P. aureginosa, E. coli, P. Syringae and B. Subtilis.* The rest of the samples showed activity against all the other tested bacteria with sample K07S from the *S. scabrum* showing the highest activity of 10.50mm against E. coli. Sample K06S was not active against *Shigella ssp*. This variation in activity can be associated to variation in the presence of many phytochemical compounds in the samples and also the occurrence of the compounds in different concentrations in the different samples (Gugulothu *et al.,* 2011).

5.2.2 Antibacterial activity of the aqueous phase samples

The samples K01L to K05L were initially screened for antibacterial activity against the undiluted liquid samples and they all showed a considerable antibacterial activity. The samples showed activity against all the tested bacterial species. When tested against *Shigella spp* and *P. aureginosa*, the samples even had higher activity index than chloramphenicol, the standard antibiotic. This result can be used to suggest that the extracts from solanum species can be considered as better antibiotics than the existing standard drugs, this is more so because the extracts used in this study are crude extracts whose active compounds form just a fraction of the whole sample as compared to the antibiotic which is a pure compound (Edeago *et al*, 2005).

After drying the samples and diluting them at known concentrations, the liquid samples were then screened for antibacterial activity. The aqueous phase samples have a higher efficacy to bacteria than the solid phase samples. This was proved by comparing the means of the inhibition zones of the two samples using T- test. The calculated T value was 15.57 which fall outside the expected tabulated range of 7.297 – 9.412 at 95% confidence interval. This implies that there is a significant difference between the means of the samples at the two different phases. The samples showed activity against all the bacteria species except *P. aureginosa* which showed resistance to samples K01L, K02L, K03L and K05L. During the initial screening of the undiluted samples, the samples had some activity against *P. aureginosa* as discussed above, but after diluting the samples, there was no activity of these four samples against *P. aureginosa*. This means that there is need for a high active ingredient concentration when dealing with *P. aureginosa* because it is known to be highly resistant to antibiotics and the resistance increases further after exposure of the bacteria to antibiotic substances (Selina, 2012). The fact that other samples were able to inhibit this bacterium can be associated to difference in

the type and quantity of phytochemical compounds in the samples. Sample K01L was also not active against *Shigella ssp.* This can be associated to absence of cardiac glycosides in the sample because it is the only compound which tested present in all samples except K01. Cardiac glycosides are known to have ability to inhibit microbial growth in plants and thereby inhibiting decay of plants (Clifford *et al.,* 1973) and the same antibacterial activity is effective in preventing microbial infections on humans upon consumption (Kessler *et al.,* 2003). The rest of the samples were active against all the other tested bacterial species. The highest activity was seen for sample K04L against *S. aureus* with an activity index of 29.00mm while the smallest activity was shown by samples K06L against *Shigella ssp* which had an activity index of 6mm. The rest of the samples had activity index of between 6.00mm and 29.00mm (Table 8). This variation in activity can be associated to the different combinations of phytochemical compounds in the ten samples and not by some specific compounds (Bai, 1990)

Statistical analysis was done in order to compare the efficacy of the samples against Gram negative bacteria and Gram positive bacteria using T. Test. The calculated T value was -3.15 and it falls in the expected tabulated range of -6.852 to -1.498 at 95% confidence interval (Appendix 4). This implies that there was no significant difference in the antibacterial activity of the samples against Gram positive and Gram negative bacteria.

5.3 MIC of *S. nigrum* complex samples against bacteria

The samples showed a variation in the MIC values against the different bacteria. This variation can be associated to the variation in type and amount of phytochemical compounds like saponins and cardiac glycosides, which were found to be present in all the samples and have been proven to have antibacterial activity (Harbone, 1973;

Foerster, 2006; Al-Bayati and Al-mola, (2008). Other compounds like tannins, sterols, Flavonoids, plabotanins and terpenoids were present in some samples and absent on others in no particular order. This qualitative variation and the possible quantitative variation in presence of the phytochemical compounds are responsible for the variation in MIC values of the samples against the different bacterial species. Since the samples showed decreasing inhibition zones as the concentration was reduced, it shows that the antibacterial activity of the samples was dose dependent. Higher MIC values show that the bacteria are resistant to the test sample (Sheen, 2009). This can be confirmed by complete lack of inhibition when the sample is tested against very highly resistant bacterial species like *S. dysentaria* and *P. aureginosa*.

5.4 Antifungal activity of *S. nigrum* complex samples

5.4.1 Antifungal property of the solid phase samples

The ten samples were screened against *F. culmorum, F. avenaceum and F. moniloforme*. These are *Fusarium* species of fungi known to cause plant infections, food poisoning and various mycoses. *F. culmorum* was only inhibited by samples K01S, K04S, and K05S while *F. avenaceum* was inhibited by samples K02S, K03S and K04S. Therefore these samples can be considered as potential treatments root rot of cabbages, ear rot, among other diseases caused by these two fungi. The three fungi were completely resistant to samples K06S K07S, K08S, K09S and K10S. This can be associated to lack of tannins in these samples. According to Victor *et al.,* (2005), tannins have considerable antifungal activity.

Fusarium culmorum is plant pathogenic and causes seedling blight, foot rot, ear blight, common root rot and other diseases of cereals, grasses, and a wide variety of monocots and dicots (Rodriguez *et al.,* 2008). It is also capable of producing Mycotoxins in plants

especially cereals. The most common mycotoxins of *F. culmorum* in wheat are deoxynivalenol (DON) and 3-acetyldeoxynivalelon (3-ADON) which are harmful to human and animal health. (Marasas, 1984) This shows a potential application of these plant samples in control and treatment of plant diseases as well as preventing food poisoning by Mycotoxins in cereals and grains.

Fusarium avenaceum is a globally distributed fungus commonly isolated from soil and a wide range of plants. Severe outbreaks of crown and stem rot of the flowering ornamental, lisianthus (Eustoma grandiflorum), have been attributed to *F. avenaceum*. This fungus has been reported to be an occasional pathogen of stored cabbage (Geeson, 1983).

Fusarium moniliforme proved to be the most resistant to all the tested samples and cannot be eliminated by any of the tested samples. However, the standard antibiotic used was active against the fungi.

5.4.2 Antifungal property of the aqueous phase samples

The aqueous phase samples showed antifungal activity against the tested fungal pathogens. *F. culmorum* was inhibited by all the tested samples except sample K06L, K08L and K09L. *F. moniliforme* was inhibited by most of the tested samples except sample K03L, K06L, K08L and K09L. The fungi *F. avenaceum* was inhibited by all the tested samples with very high activity indexes recorded. Sample K05L had an activity index of 26.00mm against *F. avenaceum*, which is higher than that of nystatin. This shows that extracts from the *S. scabrum* can be considered as potential substitutes for the standard antibiotics used in maintenance if fungal infections in plants. The sample K05 was found to have eight of the tested phytochemical compounds; these are tannins, saponins, sterols, cardiac glycosides, flavonoids, terpenoids, alkaloids, and phenolic

compounds. Presence of these compounds in the proportions present in the sample K05L made the sample to have the highest activity index against the *F. avenaceum* (Gugulothu *et al.,* 2011).

Since the phytochemical composition of the liquid samples was found to be similar to that of the solid phase samples, then the difference in activity can be associated to the difference in concentrations of the samples used for screening; and also the difference in percentage of the phytochemical compounds between the two phases. A research done by Anokwuru *et al.,* (2011) on methanol extracts *S. scabrum* where sample K05 belongs as a species proved a high concentration of flavonoids and phenolic compounds which are known to have antifungal activity (Scalbert, 1991; Pashin *et al.,* 1986).

5.5 MIC of the antifungal properties of *S. nigrum* complex samples

5.5.1 MIC of solid samples against fungi

Fusarium culmorum was analysed for MIC assay when tested against samples K04S and K01S thereby having MIC values of 20 mg/ml and 17.5 mg/ml for the two samples respectively. There is a very small difference between the two MIC values. This can be associated to similarity in presence of most of the phytochemical compounds in these two samples because both samples had tannins, cardiac glycosides, sterols, Saponins flavonoids, and terpenoids, all of which have been shown to poses antifungal activity (Ogwenwenmo *et al.,* 2007; Victor *et al.,* 2005; Scalbert, 1991).

The samples showed a decrease in activity as the concentration of the sample decreased. This shows that the antifungal activity of the sample was dose dependant.

5.5.2 MIC of the aqueous phase samples against fungi

The MIC value of the aqueous phase samples was variable ranging between 0.35 g/ml and 0.2 g/ml. This variation can be associated to the difference in the type and amount of phytochemical compounds in the samples. This variation in quality and quantity of the phytochemical compounds can be proved by the fact that these samples were different in their physical state due to difference in the percentage composition of oily substances in the samples thereby making some of the aqueous phase samples to be in liquid state after drying to a constant weight; an observation that agrees with the findings of Ravi *et al.,* (2009).

The gradual decrease in antifungal activity as the concentration of the samples decreased showed that the antifungal activity of these samples is dose dependent.

5.6 Antimicrobial activity for the isolated crystal

The crystal showed a high activity index when tested against the susceptible microbes and only one fungus; *F. culmorum*); was completely resistant to the crystal. This is because it is a pure compound with only one possible active compound which happens not to inhibit growth of these fungi.

A crystal is a pure compound that is formed through aggregation of similar molecules in a process of crystallization. *S. typhi* had an MIC of 0.12 g/ml while *P. syringae* and *P. mirabilis* both had an MIC value of 0.14 g/ml. These are relatively low MIC values which mean that the crystal is highly active against these bacterial species.

5.7 Phytochemical composition of *S. nigrum* complex samples

Extracts of *S. nigrum* were found to poses a wide range of different phytochemical compounds. These compounds are tannins, Saponins, terpenes, terpenoids, cardiac

glycosides, phenolic compounds, anthraquinones, flavonoids and phlobatanins All these compounds are known to be biologically active and hence the observed antimicrobial activity (Kessler *at el*., 2003).

This study agrees with the results of a research done by Ravi *et al.,* (2009) on the phytochemical composition of *S. nigrum* which revealed presence of carbohydrates glycosides, saponins, tannins, alkaloids, flavonoids, fats and oils, phytosterols and coumarines. His study revealed anti-inflammatory and anti-convulsion activity in the samples which was associated the activity to presence of flavanoids.

Thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* should be subjected *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans (Das *et al.,* 2009).

All the plant extracts contained tannins; they can therefore be recommended for treatment of diarrhoea (Yu *et al.,* 2000; Gertrudes (2006), inhibit multiplication of retroviruses because of their ability to inhibit reverse transcriptase (Nonak *et al.,* 1990), treatment of asthma and other respiratory diseases (Burkil, 1994). These tannins can be extracted and used to prepare insecticide for spraying on plants (Buttler, 1998). Tannins have been reported to be toxic to filamentous fungi, yeasts and bacteria (Scalbert, 1991). Their antimicrobial action is made possible by their capacity for protein complexation through hydrogen and covalent bonding and inactivation of microbial adhesions, enzymes and cell envelope transport proteins (Gertrudes, 2006). The consumption of tannins as green teas (Gertrudes, 2006) and wines prevents different illnesses (Serafini *et al.,* 1994) and inhibits viral reverse transcriptase.

Flavonoids were present in most of the extracts. Their health promoting effects include anti-inflammatory, anti-viral, anti-cancer, anti-oxidant, and anti-allergic effects (Balch

and Balch, 2000) which make *S. nigrum* complex samples useful in all these medical applications.

Saponins found in the samples are known to reduce the level of low density cholesterol and are therefore useful in human diet for controlling cholesterol levels (Assiak *et al.,* 2001). They also control cancer by interfering with cholesterol rich membranes of cancer cells (Dong *et al.,* 2005). They also have antioedema and immunoregulatory effects (Victor *et al.,* 2005), antibacterial, antifungal, anti-inflammatory, antiviral (Al-bayati and Al-mola, 2008), and anti-protozoan (Cheeke, 1998) activities which add to the beneficial application of *S. nigrum* complex.

Another compound which was found in all the extracts was steroids which have important application in the pharmaceutical companies in the production of sex hormones used to bring hormonal balance in expectant and lactating women.(Victor *et al.,* 2005). This may be the reason why the leaves of *S. nigrum* are recomended as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance. This is because steroidal structure could serve as potent starting material in synthesis of these hormones (Okwu, 2001). It has been proved that steroids also have antimicrobial effects against bacteria causing stomach infections. It has also been shown to have analgesic and anti-inflammatory effects, and also used in decreasing serum cholesterol levels (William, 2008)

Cardiac glycosides were present in all the samples except sample K01. Therefore all the studied species of *S. nigrum* complex can be used in treatment of cardiac disorders except K01 (Clifford *et al.,* 1973; Leverin and McMatron, 1999).

Phlobatanins and alkaloids were not common in most samples, but they were found to be present in some of the samples. Research shows that these compounds have

antimicrobial (Ogwenwenmo *et al.,* 2007), anticancer (Sneden, 2005), anti-inflammatory (Sofowora, 1993) effects and also act as immune boosters (Jeffery and Harborne, 2000).

Alkaloids have their antibacterial affect based on the fact that they help the white blood cells to dispose harmful microorganisms, (Jeffery and harbone, 2000). Glycosides are responsible for the characteristic bitter taste of the black night shade. They are in high concentration in unripe berries and they prevent insects and birds from feeding on the immature fruits and seeds. They also prevent decay of damaged plant tissues (Ogwenwenmo *et al.,* 2007).

Phenolic compounds, especially the hydroxylated phenols have been found to be toxic to microrganisms with their relative activity increasing with increasing level of oxidation (Scalbert, 1991), number of hydroxyl groups attached to the main structure and their specific sites (Pashin *et al.,* 1986). Phenols and flavonoids have antibacterial activity associated to their ability to complex with nucleophilic amino acids in proteins and the bacterial cell wall leading to destruction of the protein structure and subsequent enzyme inactivation and loss of function (Mason and Wasserman, 1987).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, it was concluded that Species of S. nigrum complex found in western Kenya are *S. nigrum, S. villosum* and *S. scabrum w*ith different variants occurring in the same species. Phenotypic variations observed among samples of the same species were associated to genetic variation and also the different environmental conditions of growth.

The test samples have antibacterial and antifungal activity against Gram positive and Gram negative bacterial species and also against plant pathogenic fungi of the genus *Fusarium.*

A wide array of phytochemical compounds is present in the samples and they include saponins, cardiac glycosides, phlobatannins, tannins, flavanoids, phenolic compounds, terpenes and sterols. These compounds are responsible for the observed antibacterial and antifungal activity of the samples.

6.2 Recommendations

These plants are edible, safe and effective in management of various ailments; therefore the following recommendations were made:

- Research should be carried out on determination of the relative percentage of the phytochemical compounds in the samples.

- Consumption of the plants from the *S. nigrum* complex by immune-compromised patients is recommended in order to fight nosocomial infections and opportunistic infections.

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APPENDICES

Appendix I: Photographs of the samples collected

(Source: Author, 2013)

Solanum nigrum Mill

Solanum villosum

Solanum scabrum

Appendix II: T- test for comparing the antimicrobial activity of samples at concentrations of 100mg/ml and 25mg/ml

***** **Two-sample T-test (paired)** *****
Calculated using one-sample t-test with the null hypothesis that the mean of C1 - C2 is equal to 0

One-sample t-test

Variate: **100mg** **(**C1), **25mg** **(**C2) **[1].**

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
C1-C2	328	6.442	22.64	4.759	0.2628

95% confidence interval for mean: (5.925, 6.959)

Test of null hypothesis that mean of C1-C2 is equal to 0

Test statistic t = 24.52 on 327 d.f.

Probability < 0.001

Appendix III: T. test for comparing the activity of the solid phase and the aqueous phase samples

*****Two-sample T-test (paired) *****
Calculated using one-sample t-test with the null hypothesis that the mean of C1 - C2 is equal to 0

1. One-sample t-test

Variate: Liquid & SOLID PHASES [1].

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
C1-C2	220	8.355	63.32	7.957	0.5365

95% confidence interval for mean: (7.297, 9.412)

Test of null hypothesis that mean of C1-C2 is equal to 0

Test statistic t = 15.57 on 219 d.f.

Probability < 0.001

Appendix IV: T-test for comparing the efficacy of the samples against Gram positive and Gram negative bacterial species

***** **Two-sample T-test (paired)** *****
Calculated using one-sample t-test with the null hypothesis that the mean of C1 - C2 is equal to 0

One-sample t-test

Variates: gram negative (C1), gram positive (C2).

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
C1-C2	40	-4.175	70.05	8.369	1.323

95% confidence interval for mean: (-6.852, -1.498)

Test of null hypothesis that mean of C1-C2 is equal to 0

Test statistic t = -3.15 on 39 d.f.

Probability = 0.003