

**ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF DRIED GARLIC AND  
ONION AGAINST COMMON BACTERIAL PATHOGENS**

**BY**

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Master of Science in Ecological Public Health at the University of Zambia**

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## **DECLARATION**

I **Constance Kalyati** do hereby declare that the dissertation titled “Assessment of antibacterial properties of garlic and onion against common bacterial pathogens from fresh food” has been carried out by me and was conducted at The University of Zambia, School of Veterinary Medicine in the department of Para-clinical Studies. Information derived from literature has been duly acknowledged in the text and a list of references provided. This dissertation has not been previously presented for another degree at this or any other institution.

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Signature

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Date

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## **DEDICATION**

This work is dedicated to my beloved father Jervas and my beautiful mother Astridah who have always had my back and held me strong in the stormy days. Their sacrifice for my education is unimagined and I remain forever grateful.

## ABSTRACT

According to the World Health Organization, the extensive and sometimes indiscriminate use of antibiotics for treatment and as growth promoters in the animal production industry has resulted in the widespread development of antibiotic resistance. As such, antimicrobial resistance cannot be overemphasized as the discovery trend of resistant strains has continued. Thus, the pursuit of new natural plant-based antibiotics is a call to action for scientists to save the world. Garlic and onions are traditional herbs that have been used since ancient times in the preparation of not only food but medicines as well.

In this study, the aim was to assess if dried garlic, red, and yellow onions possess antibacterial properties and the effect evaporation procedures and temperatures used have on their activity. Cured *Allium sativum* (garlic) and *Allium cepa* (onion) were bought from the local market. The plants were dried, coarsely crushed, and macerated using solvents: ethyl acetate, ethanol, and water. To obtain the crude extracts, two evaporation procedures were used: water bath and oven evaporation; water bath evaporation was at a temperature of 95°C, whereas oven evaporation was at 35°C. To test for antibacterial activity, agar dilution method was used against standard American Type Culture Colony strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* all of which are of public health significance. The activity was then graded as susceptible, intermediate, or resistant.

Among the extracting solvents used, water extracts had the highest yield potential, which was followed by ethanol and finally ethyl acetate in order of decreasing polarity. Results showed that extracts from the oven evaporating procedure were more efficacious compared to those of water bath evaporation procedure indicating that the active ingredient is heat labile to high temperatures and in this case 95°C. The best antibacterial extracting solvent from the oven evaporating procedure was the water extracting solvent, which was effective against all four test organisms. The activity was followed by ethanol, with ethyl acetate being the least effective. Based on the water bath evaporating procedure, extracts obtained from ethanol were more efficacious compared to the two extracting solvents, which showed a higher percentage of bacteria resistance. Following the individual antibacterial assessment of garlic, red onion, and yellow onion with respect to the two evaporating procedures used, observations were made that the percentage of susceptible bacteria to the plant extracts subjected to oven evaporation was higher than the percentage susceptibility of the bacteria to the plant extracts subjected to water bath evaporation. Therefore, based on the findings, it can be said that dried garlic and onions do have antibacterial properties against bacterial pathogens under study and that the plants have a higher potency when exposed to a low evaporation temperature like 35°C as opposed to a high temperature of 95°C. However, evaporation at temperature 35°C and below is recommended so as to minimize the exposure of these plants to heat, thereby raising their antibacterial activity.

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## LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
CLSI	Clinical Laboratory Standards Institute
CNS	Central Nervous System
DMSO	Dimethyl Sulfoxide
DNA	Deoxy Ribonucleic Acid
EPS	Extracellular Polymeric Substances
Hrs	Hours
MH	Muller Hinton
MHA	Muller Hinton Agar
M <sub>i</sub>	Initial Molarity
MIC	Minimum Inhibitory Concentration
MLC	Minimum Lethal Concentration
NKJV	New King James Version
OMZ	Oxygen Minimum Zone
Se	Selenium
SeMCYS	Se-methylselenocysteine
VISA	Vancomycin Intermediate <i>Staphylococcus aureus</i>
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
XLD	Xylosine Lysine Deoxycholate

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background

Nowadays, antibiotics have broad applications in agriculture and animal husbandry and are being used to help increase life expectancy by lowering the deaths due to bacterial infections, especially in developing countries. This has contributed to antibiotic pollution and increased resistance (Kumar et al., 2019). Additionally, common bacteria that produce biofilm, such as *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella spp.*, *Staphylococcus aureus*, and *Escherichia coli*, are a threat to the health of humans. As biofilm-producing bacteria attach to solid surfaces in an aqueous environment, they form a network of extracellular polymeric substances (EPS) and begin living a "multicellular lifestyle," giving biofilm an unexpected degree of complexity and three-dimensional structure. This structure has significance in the survival of bacteria against antimicrobials (Abebe, 2020). The EPS include proteins, polysaccharides, lipids, and Deoxyribo Nucleic Acid (DNA), which are just a few of the compounds that build a protective matrix surrounding bacteria to ensure their survival and integrity (Preda and Săndulescu, 2019). These compounds form a multilayer and may contribute to bacterial resistance to antibiotics due to poor antibiotic penetration and stress adaptation (Stewart, 2002). Over the recent years, clinical infections resulting from biofilms formed by pathogenic microbes in food have culminated in drug resistance (Abebe, 2020). The wide spread antibiotic resistance that has been observed is now posing a serious public health concern, with medical scholars warning of a return to the pre-antibiotic era (Davies and Davies, 2010); be it community- or hospital-acquired infections due to Vancomycin-resistant *Staphylococcus aureus* (VISA) or Vancomycin-resistant Enterococci (VRE).

Studies have shown that crude extracts of medicinal plants could serve as an alternative source of resistance-modifying agents owing to the large variety of secondary metabolites that could act as potential antimicrobials and resistance modifiers by inhibiting the efflux pumps, disrupting the cytoplasm, thereby affecting membrane permeability, and also enhancing the B and T cells-mediated response (Pooja and Tannaz, 2017).

*Allium sativum* (garlic) and *Allium cepa* (onion) are two plants believed to be natives of central Asia and are now available all over the world (Keusgen et al., 2006). They have not only been widely used as flavoring ingredients in cooking but also as medicine through ancient times in India, Egypt, Rome, and Greece (Petrovska and Cekovska, 2010). Garlic and onion both belong to the genus *Allium*. The mature whole alliums are composed mainly of cysteine sulfoxides, and when tissues are crushed, the enzyme allinase is released, converting the cysteine sulfoxides into thiosulfinate (Krest et al., 2000). These compounds are reactive, volatile, odor-producing, and lachrymatory and have a broad range of activity against gram-positive and gram-negative bacteria (Krest et al., 2000).

In Zambia, garlic and onions are not available year-round because of our growing cycles. The short-day onions planted require 10 to 12 hours of daylight to form bulbs and are planted in March, when there is a reduction in rains and more sunlight, avoiding months like January and February that have heavy rains that can wash away the seeds or damage the seed beds. The majority of the short-day onions planted in Zambia take 90–135 days to mature, and there is a shortage of good quality of these plants from April to June. After maturity, these plants can either be sold fresh or preserved by curing and drying, which takes between 2 and 4 weeks (Starkeyayres, 2022). Garlic, on the other hand, is best grown from February to April, and the type grown in Zambia is the soft neck. Garlic adapts to growing conditions, though it takes years for it to adapt. It is grown in soil with a pH between 6.0 and 7.5, requiring more nitrogen during its initial stages. For proper growth, this plant needs fairly even soil moisture during its growing season, with no additional moisture during the last few weeks. The majority of garlic sold on the market is cured and dried because maturation takes nine months, making it unavailable most of the year (Agriculture in Zambia, 2018). Therefore, the assessment of antibacterial properties in plants preserved by drying is necessary, and in this case, those are the garlic and onions that are routinely used for food. These will serve as natural antibiotics year-round, curbing the ever-increasing antibiotic resistance.

Fresh foods harbor and encourage the growth of microbes due to the nutrients and sugars that supplement the growth of bacteria, leading to food spoilage. Food spoilage renders food undesirable or unacceptable for human consumption, and in order to address the issues of microbial

food spoilage and poisoning in fresh foods, an evaluation of materials used as food ingredients such as garlic and onion (red and yellow) was performed, and the efficacy of each was determined.

## **1.2 Problem Statement**

Food contamination with pathogenic microbes is a severe public health problem around the world, causing food-borne diseases every year (Alina and Alexandru, 2018). These food-borne diseases require treatment that may involve the use of antimicrobials, which may or may not be taken according to prescription. As a result, the overuse of antimicrobials has led to antimicrobial resistance (AMR). AMR threatens historic achievements in modern medicine and public health as well as the broader development of antibiotics. Antimicrobial agents like antibiotics are essential to treating some human and animal diseases. Microbes can develop resistance to antimicrobials, meaning that a drug such as an antibiotic is no longer effective in treating the infection. Overuse and misuse of antimicrobials in food-producing animals has contributed to an increasing challenge of antimicrobial-resistant pathogens being passed on to people through the food chain and the environment (Van et al., 2020). Vulnerable populations such as the young, cancer patients, people over the age of 60, and those living with HIV/AIDS suffer the most from antimicrobial resistance. Residues of antimicrobial agents in foods of animal origin are another health concern, as they can cause allergies, alter the intestinal flora, and increase bacterial resistance in humans (WHO/EURO, 2020). Studies have suggested that naturally occurring plants have antimicrobial compounds that serve as defense mechanisms for the plant and have the potential to serve as natural antimicrobial compounds against human pathogens (Hintz et al., 2015). Nevertheless, a few studies have been conducted in Zambia with regards to the ethnomedical properties of plants (Chinsembu, 2016). If nothing is done to investigate alternative plant-based antimicrobials, we shall remain dependent on commercialized antibiotics, which, if misused, will further add to the growing challenge of antimicrobial resistance.

### **1.3 Study Justification**

Foodborne illnesses can have a social and economic impact, place a strain on the health-care system, and impede tourism and trade. These can be caused by a variety of environmental contaminants, such as pollution in water, soil, or air, as well as hazardous food storage and processing. These microbial diseases significantly contribute to the global disease burden and death rate. Antibiotics are given to treat diseases, but if they are abused or overused, they can induce resistance, like in the case of penicillin, which was discovered by Sir Alexander Fleming in 1928 from the fungus *Penicillium notatum* (Sengupta et al., 2013). Penicillin was shown to be effective in preventing bacterial infections among World War II soldiers. Yet, bacterial resistance to penicillin arose almost soon after its introduction, posing a danger to many of the preceding decade's innovations (Ventola, 2015). As a result of unregulated antibiotic usage, by 2050, there will be a significant AMR that will create a larger pandemic than COVID-19, killing 10 million people annually (Singhal, 2022). With this in mind, commonly consumed food products such as onions and garlic should be investigated for potential antibiotic capabilities under various heat exposure settings in order to reduce the costs associated with treating antimicrobial resistance, thus the study. This research is also significant because it will give an inexpensive, readily accessible, and safe antimicrobial extract that may be used to extend the shelf life of fresh foods, rendering them safe for consumption. Moreover, small-scale local market traders have been faced with the challenge of food spoilage and contamination, and because they do not have enough resources to buy food preservatives, they have experienced losses that have affected them greatly.

### **1.5 Objectives**

#### **1.5.1 General Objective**

To assess the antibacterial properties of dried *Allium sativum* and *Allium cepa* against common bacterial pathogens.

#### **1.5.2 Specific Objectives**

1.5.2.1 To determine the effect of water bath and oven evaporating procedure on the antibacterial properties of dried garlic and onion against common bacterial pathogens.

1.5.2.2 To determine which extracting solvent provides better antibacterial activity of dried garlic and onion.

1.5.2.3 To determine the antibacterial properties of dried garlic, red and yellow onion against common bacterial pathogens

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Herb utilization in human Existence

Shrestha and Dhillion (2003) highlighted that throughout history, herbs and spices have been added to meals as medicinal, flavoring, and preservation agents. Medicines were mostly administered orally in the form of juice that was obtained from either the soaked leaves or roots, as they were found to be effective in the treatment of coughs and colds, fever, headaches, skin infections, and digestive problems. Despite being inexpensive goods, herbs and spices are now regarded as valuable as gold or jewels and have been for several millennia (El-Sayed and Youssef, 2019).

The ancient Egyptians employed herbs and spices in food, which have been in use in India and China for centuries. Currently, spices and herbs are used to improve and increase the acceptability of foods by enhancing flavor, aroma, and health. Fritts (2018) discovered that spiced vegetables were preferred over plain varieties by rural high school students, demonstrating how adding spices can improve food palatability.

The majority of current medicine used worldwide comes from indigenous traditional knowledge that has been obtained through trial and error in several different cultures, practiced over a very long period of time, and transferred from generation to generation. Some currently used drugs derived from plants include: Atropine, an anticholinergic derived from *Atropa belladonna*; Cocaine, used for local anesthesia, derived from *Erythroxylum*; Caffeine, a CNS stimulant from *Camellia sinensis*; and Quinine, an antimalarial drug obtained from *Cinchona ledgeriana* (Maridass and De Britto, 2008). Furthermore, 60% of the world's population lives in developing countries that rely directly on plants and herbs for their therapeutic properties. This dependency could be due to a lack of modernized medication, poverty, and others, believe that folk medicine is most effective (Shrestha and Dhillion, 2003).

Herbs and spices have been utilized as food additives all over the world, not only to enhance the flavor, texture, and color of food but also to increase their shelf life by decreasing or eliminating the foodborne pathogens (Lai and Roy, 2004). The antioxidant, antimicrobial, and anticancer properties present in herbs and foods enable them to enhance the health and medical status of human beings. An example is the consumption of tomatoes, which helps prevent the progression of prostate cancer due to its high levels of lycopene (Xu et al., 2016).

Some herbs, such as cinnamon, have been used in dairy products by food investigators to test the consumer's preferences for novelty in the products (El-Sahid and Youssef, 2019). Behrad et al. (2009) concluded that adding cinnamon did not modify the yoghurt fermentation but allowed for the growth of *Lactobacillus* spp. during refrigerated storage. Cinnamon-flavored yogurt containing probiotic bacteria prevented the growth of *Helicobacter pylori* in vitro, though the efficiency of these herbal yogurts to stop the growth of *H. pylori* needs to be investigated further under the extremely acidic environment of the stomach. The health benefits of yoghurt are well known, and several yoghurt-based products are consumed by people all over the world (Fernandez and Marette, 2017). Other herbs include onions, garlic, saffron, turmeric, ginger, black pepper, and cloves.

## **2.2 Use of Onions and Garlic in Foods and ethnomedicine**

Garlic and onions have been and continue to be used as ingredients in cooking because they act as aromatics, adding a savory aroma and flavor to a variety of cuisines. We see the flavors also being incorporated in snacks like the Yoyo Chipsy, Lays, Pringles, and the mini-Cheddars by Bakers (cheese and onion-flavored snacks). In Egypt, 'garlic and onions' were fed to the working class involved in heavy labor, as in the building of pyramids; this presumably was done to maintain and increase their strength (Moyers, 1996). This has further been supported by the Bible record that reads, "We remember the fish that we ate freely in Egypt, the cucumbers, the melons, the leeks, the onions, and the garlic" (Numbers 11: 5, NKJV), presumably referring to the time the Israelites were slaves involved in heavy labor in Egypt.

Garlic has long and widely been used as a medicinal plant, dating back to the ancient times of the Egyptian medical text, the Codex Ebers (1500 BC), which is one of the earliest sources indicating

prescription of garlic for treatment of abnormal growths and also for circulatory ailments, general malaise, and infestation with insects and parasites (Bergner 1996; Lawson 1998).

In ancient Chinese medicine, garlic was prescribed to aid respiration and digestion (Woodward, 1996); this is certainly being practiced even in developing African countries like Zambia, where garlic, onions, lemons, tumeric, and ginger are mixed, boiled, and given to the patient suffering from a cold, and even more so when the world was battling with the COVID-19 pandemic (Orisakwe et al., 2020). Sometimes slices of red onion are kept around the room at night for a patient suffering from the same. This could impact the air the patient is breathing in, causing relief.

### **2.3 Antimicrobial Potential of Garlic and Onion**

Garlic extracts have a wide range of antibacterial activity against both gram-negative and gram-positive bacteria. Reports show that garlic extracts are also effective against antibiotic-resistant bacteria and their toxic products (Wolde et al., 2018; Tsewang et al., 2021). This effect is due to the presence of bioactive compounds, which include flavonoids, tannins, terpenes, saponins, alkaloids, and allicin. In particular, allicin inhibits the growth of bacteria by inhibiting their DNA and protein synthesis partially and also inhibiting RNA synthesis as a primary target (Feldberg et al., 1998).

Onions have the main antimicrobial agents' quercetin and allicin (thio-2-propane-1-sulfinic acid-5-allyl-esters). Quercetin binds to bacteria's DNA gyrase (an enzyme that belongs to the family of topoisomerase II that relieves the supercoil, enabling the process of transcription) (Feldberg et al., 1998). Thus, replication will not take place, thereby inhibiting the growth of bacteria, while allicin inhibits certain thio-containing enzymes in the microorganisms by the rapid reaction of thiosulfates. These enzymes are biological catalysts, and as such, once inhibited, they cannot fully perform, reducing the survival chances of an organism (Feldberg et al., 1998). Red and yellow onions have been reported to be active against some resistant bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* that were isolated from cooking utensils (Anyaeibunam et al., 2019). These showed susceptibility to white onions, whereas in the case of red onions, all were susceptible except *Staphylococcus aureus* (Anyaeibunam et al., 2019).

From these observations, it could be inferred that white onions target both gram-positive and gram-negative bacteria, whereas red onions target gram-negative bacteria.

### **2.3.1 Onion and medicinal uses**

Tsewang et al., (2021) stated that different phytochemical compounds in onion, such as flavenols, sulphur, and seleno compounds, have been identified. Onions have the ability to accumulate selenium (Se) from the soil, and these Se-enriched plants like onions show greater protection against carcinogenesis than the other plants (Tsewang et al., 2021). It is said to reduce the incidence of stomach cancer, cancer of the intestines and lungs, as well as other types of cancer. On the basis of epidemiological studies, the correlation between the amounts of selenium present in cancer patients has been established to be inversely proportional, i.e., patients with cancer have low amounts of selenium in their blood or plasma (Whanger, 2004). *Allium* spp., namely garlic and onions, have shown promising signs of possessing chemopreventive activity against cancers because of the compound se-methylselenocysteine (SeMCYS), which is highly present in plants exposed to high levels of Se (Whanger, 2004). This is achieved as Se regulates the instructions for making p53, a protein that suppresses tumors by containing the proliferation and division of cells (Smith et al., 2004). Some of the potential health benefits of onions include improved heart health, lowering blood glucose, preventing allergies, improving the immune system, and preventing blood clots from forming in blood vessels. It has been recommended to treat colds, coughs, bronchitis, whooping cough, asthma, and other respiratory problems. Onion has been traditionally used for its remedial characteristics in the management of various ailments (Teshika et al., 2018). The essence of *A. cepa* proliferated into ancient Greece, where it was used as a blood purifier for athletes and is taken raw for treating infectious diseases, digestive problems, skin diseases, metabolic diseases, and insect bites, where positive results have been recorded (Teshika et al., 2018).

### **2.3.2 Garlic and Medicinal uses**

Garlic bulbs are the most frequently used in many medicines. In India, they are prepared in several ways, including by extracting the juice or making paste. This has been taken to relieve problems such as cough and fever or applied externally to prevent the graying of hair and improve skin conditions such as eczema and scabies. It has even been applied to the noses of hysterical patients

to calm them down. Garlic also features in traditional medicine in other parts of the world, like Europe, Asia, and South and North America, as folk medicine (Divya et al., 2017).

#### **2.4 Antimicrobial Properties of Garlic and Onion**

Garlic and onions have not only been accepted as ethnopharmaceutical drugs but have also been proved to have therapeutic effects by several scientific research studies (Yetgin et al., 2018; Bhatwalkar et al., 2021). These studies have documented the antibacterial, antiprotozoal, anticancer, antifungal, and antiviral activity of garlic. There have been a number of related researches done on the antimicrobial properties of garlic and onion worldwide, such as the research done in Nigeria, where extracts of garlic and onion were tested on some clinical bacterial isolates using the agar-well diffusion method. Antibacterial effects of aqueous extracts of onions and garlic were observed to be concentration-dependent (Omotola, 2018).

Bhatwalkar et al. (2021) elucidated that garlic possesses antibacterial properties such as antibiofilm, antitoxin, and bactericidal characteristics due to the presence of organosulfur compounds contained in it. Furthermore, antifungal, antiviral, anti-inflammatory, and anticancer benefits have been associated with garlic (Yetgin et al., 2018). In the findings by Bhatwalkar et al. (2021), ethanol extract activity against *Helicobacter pylori* decreased with the decrease in concentration of allicin.

Tests conducted by Kim (1997) on the antibacterial action of onions showed that onion extracts were bactericidal effective against all the tested oral pathogenic bacterial strains, which included *Streptococcus mutans* JC-2, *S. sobrinus* OMZ176, *P. gingivalis* ATCC 332777, and *P. intermedia* ATCC 25611.

#### **2.5 Agar Diffusion Method as a Microbial Susceptibility Test Method**

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and agar diffusion method. The efficacy of antibiotics can be assessed by their ability to suppress bacterial growth, described by the Minimum Inhibitory Concentration (MIC), or by their

ability to kill bacteria, characterized by the Minimal Lethal Concentration (MLC) (Bonev et al., 2015). MIC is usually derived by means of tests in solid media, whereas both MIC and MLC can be determined in broth dilution assays, which involve the application of antibiotic solutions of different concentrations to cups, wells, or paper discs, placed on the surface, or punched into agar plates seeded with the test bacterial strain (Bonev et al., 2015). Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth and no lawns are formed on the area of inoculation. Bonev et al. (2015) further alludes that the value of the MIC is determined as the zero intercept of a linear regression of the squared size of these inhibition zones,  $x$ , plotted against the natural logarithm of the antibiotic concentration,  $c$ .

$$\ln \text{MIC} = \ln c - x^2/4Dt$$

Here,  $D$  is the diffusion coefficient, presumed to be independent of concentration and the time of antibiotic diffusion.

## **2.6 Significance of using Garlic and Onion on microbes**

Microorganisms have been part of human and animal existence. While some microorganisms are probiotics (good for restoring the natural balance of bacteria in the gut), others are bad and have the potential to cause diseases like diarrhea and vomiting. It is such conditions that have led us to try and understand the medicinal value of garlic and onions.

### **2.6.1 Organic materials, rare toxicity from overdose**

Traditional medicines are organic materials that are generally regarded as safe once used appropriately as medicine, dietary supplements, or food supplements. These plants are considered safe as they have a long history of use in treating diseases based on the accumulated knowledge over several centuries. Documented hospitalizations or fatalities due to the toxicity of herbs are not common; not even the United States National Poison Control Centers have a category in their database for adverse reactions to herbs (Nasri and Shirzad, 2013). For instance, the traditional medicine used as an antimalarial medicine in Ghana has not had any reports of adverse reactions or cytotoxicity in normal human cells (Komlaga et al., 2016; Komlaga et al., 2015).

Nevertheless, the toxicity of medicinal plants in the formal herbal industry could be attributable to insufficient quality assurance, non-compliance with the standards of good manufacturing practice, inadequate access to the information required for the effective use of herbal medicines, and inappropriate approaches to their use (Mensah et al., 2019). These toxicity problems could be enhanced by the adulteration of herbal remedies, i.e., the addition of synthetic drugs, leading to drug-drug interactions. Other potentially toxic compounds include botanicals, pathogenic microorganisms, toxins, pesticides and fumigants, agrochemical residues, and heavy metals (WHO, 2004; Palombo, 2006; Balammal, 2009).

### **2.6.2 High level of Antimicrobial Resistance**

Sir Alexander Fleming discovered the antibiotic penicillin from a fungus, *Penicillium notatum*, in 1928, which launched the modern era of antibiotics (Sengupta et al., 2013). Since then, antibiotics have revolutionized contemporary medicine and saved millions of lives. Ventola (2015) goes on to say that when antibiotics were initially recommended in the 1940s to treat severe infections, penicillin was effective at preventing bacterial infections in individuals who fought in World War II. Shortly thereafter, penicillin resistance developed into a serious clinical issue, resulting in a threat to many of the innovations from the previous decade (Ventola, 2015).

New beta-lactam antibiotics were created in response to penicillin resistance. Nevertheless, the first instance of methicillin-resistant *Staphylococcus aureus* emerged after the beta-lactam antibiotics were discovered, developed, and deployed in 1962 in the United Kingdom and in the United States in 1968. Unfortunately, it has eventually been observed that there is resistance to practically every antibiotic that has been developed to date (Ventola, 2015). Also, in 1972, the clinical use of vancomycin was established with a motive to treat methicillin resistance in both *S. aureus* and the *staphylococci* that don't produce coagulase (Loomba, 2010). Figure 2.1 shows the trend of when a specific antibiotic was introduced and when the resistance to the drug was identified. Since then, antibiotics have revolutionized contemporary medicine and saved millions of lives. Ventola (2015) goes on to say that when antibiotics were initially recommended in the 1940s to treat severe infections.



Figure 2. 1: Development of Antibiotic Resistance: Key Events Timeline (Ventola, 2015).

The abbreviations are as follows: PDR (pan-drug-resistant), R (resistant), and XDR (extensively drug-resistant). The dates above follow early literature reports of resistance, whereas those of pan-drug-resistant *Acinetobacter* and *Pseudomonas* are based on health care transmission or outbreaks.

AMR has stimulated interest in medicinal plants since they are potential antimicrobials with a variety of secondary metabolites and compounds that can modify resistance. There are currently about 25–50% of pharmaceuticals that have been derived from plants (Pooja, 2017); this is discussed further in Chapter 2. As such, there is a need for this research. It will also stimulate interest in further scientific inquiry on the isolation of the active ingredient and even come up with a new highly efficacious antimicrobial drug from the *Allium spp.*

### **2.6.3 Combating Antimicrobial Resistance**

Gupta and Birdi (2017) highlight that because crude extracts of medicinal plants have a wide variety of secondary metabolites, they could serve as an alternative source of resistance-modifying agents. These secondary metabolites (alkaloids, tannins, and polyphenols) have the potential to be antimicrobials and resistance modifiers as they can bind to protein domains. This enables the herbalists to also present themselves as effective modulators of host-related cellular processes via immune response, mitosis, apoptosis, and signal transduction. Thus, they may exert their activity not only by killing the microorganism but also by affecting key events in the pathogenic processes, which include exposure, adhesion, invasion, and infection; thereby, the bacteria, fungi, and viruses may have a reduced ability to develop resistance to botanicals (Gupta and Birdi, 2017).

### **2.6.4 Prophylaxis in food**

Prophylaxis is a proactive intervention that aims at preventing disease before it occurs. The *Allium spp.* (garlic and onions) contain a bioactive antibiotic called Allicin that can help kill bacteria before infections occur. Optimal host nutritional status plays a vital role in immune system function and is essential in limiting the impact of emerging viral infections. A strong immune system is acquired through the maintenance of a balanced diet supplemented with vitamins C and B6, manganese, zinc, selenium, and fiber obtained from garlic and onions, all of which play a role in enhancing the immune system (Chakraborty et al., 2022).

Because herbs do have antimicrobial properties, they can be used as preservatives to replace synthetic preservatives, which have been and continue to be used in foods. Nevertheless, these synthetic preservatives may have negative implications for health and significant drawbacks, such as handling hazards, concerns about residues on food, and threats to the human environment.

Spices and herbs (garlic, mustard, cumin, clove, cumin, cumin, thyme, basil, pepper, ginger, and rosemary) are more eco-friendly as they are natural with little to no side effects, effective, and non-toxic compounds with more than just one health benefit. (Abdel-Aziz, Aeron, and Kahil, 2016)

### **2.6.5 Cheap and readily available**

Zambia and the majority of African countries are considered developing countries, and this entails that a large percentage of our population does not have the capacity to buy synthetic products, so herbs such as garlic and onions are an alternative that is cheap and readily available and can even be accessed in our local markets. Unlike drugs, garlic and onions are not regulated for purity and potency as they are considered food integrators and readily available in the market without prescription.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study design

An experimental study design was used to assess the efficacy of yellow onion, garlic, and red onion against selected bacterial strains: *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. typhi*.

#### 3.2 Extraction procedure

The garlic, red, and yellow onion plants were purchased from local markets. 5 bulbs of each plant were rinsed thoroughly using distilled water and then dried for 21 days in an oven set at 35°C situated in a well-ventilated environment. The plants were observed from time to time, monitoring their drying progress until crumbly dry. The onion layers were peeled in preparation for drying, as shown in figures 3.1 and 3.2.



Figure 3. 1: Red and yellow onion preparation for drying



Figure 3. 2: Garlic preparation for drying

After drying, the plants were crushed separately using a sterilized mortar and pestle. 5g of the crushed garlic and onions were weighed and macerated in 100 ml of solvents (ethyl acetate, ethanol, and water).



Figure 3. 3: Showing apparatus to be used

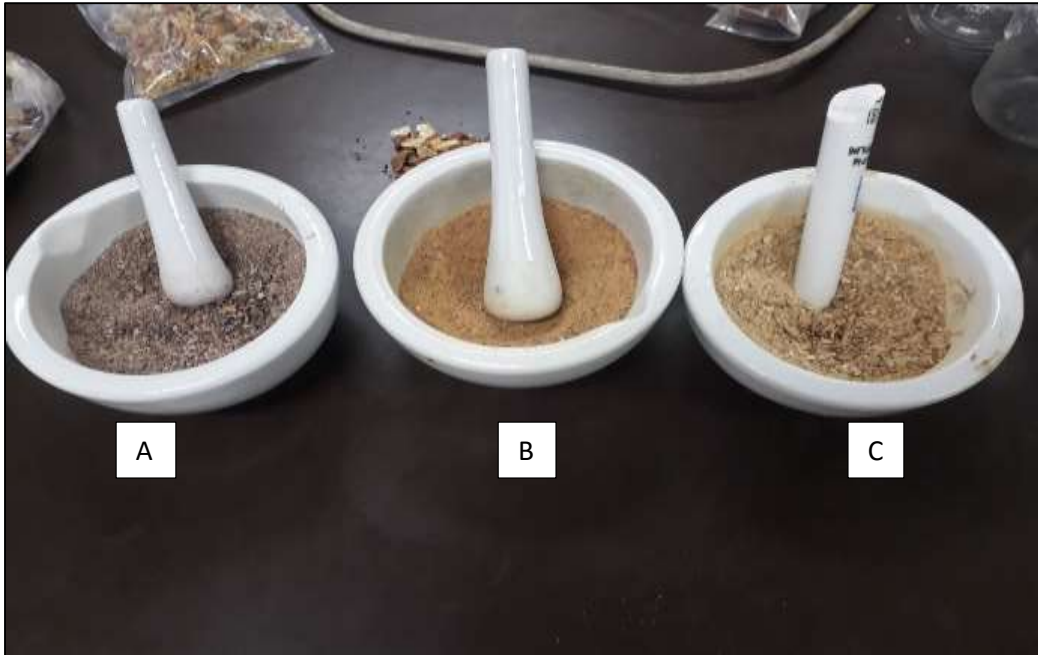


Figure 3. 4: Crushed A-Red Onion, B-Garlic and C-Yellow Onion.

The flasks containing the coarsely powdered plants in the solvents were left on a shaker for 6 hours and then allowed to stand for 18 hours to obtain a crude extract, as shown in figure 3.5. The extract was then sterilized by filtration using a 45 $\mu$ m pore-size filter paper (Figure 3.6).



Figure 3. 5: Extracts on a shaker



Figure 3. 6: Filtered extracts following extraction from onions and garlic

After filtering, the extracts were evaporated using the following methods:

- a) Drying by vaporization in a boiling water bath; this took 6 hours, following which the resulting substrate was chilled in a desiccator.
- b) Drying the filtrate by gently heating it in a dry oven at 40°C for twenty days and then chilling it in a desiccator.

The cooled extract was then kept at 4°C until it was used. The yield from the method was calculated using the following formula:

$$\text{Product yield} = (\text{amount after extraction in grams}) / (\text{quantity before extraction in grams}) 100$$

### 3.3 Determination of antimicrobial activity

The antibacterial activity of garlic, red, and yellow onion extracts was tested using the agar dilution method. Beginning with each representative extractive solvent, the weighed extracts were totally dissolved in 1.5 ml of Dimethyl Sulfoxide (DMSO) to attain an initial concentration ( $M_i$ ). The water extracts had a high yield and were too thick to be dissolved in 1.5 mL; therefore, 3 mL of DMSO were used. This concentration was recorded as  $M_i$ . The extracts were then transferred into sterile Eppendorf tubes. Mueller-Hinton (MH) agar media was used for the growth of bacterial cultures as well as assessing the antibacterial properties of garlic and onion, and Mueller-Hinton broth (Oxoid, UK) was used for MIC determination. For the assessment of antibacterial activity, the agar dilution method was employed; 9.5g of Muller-Hinton agar (Oxoid, UK) was dissolved into 250 ml of distilled water in a conical flask. The solution was brought to a boil so as to dissolve the mixture completely; thereafter, 19 ml of the dissolved Muller-Hinton agar was transferred into eleven 50-ml Falcon tubes to ensure accuracy and precision. With the lids loosely tightened, the falcon tubes (containing the dissolved MH agar) were autoclaved at 121 °C for 15 minutes.

Whilst the agar was molten at about 50 °C, 1 ml of the extract was pipetted into the 19 ml MH agar to make up to 20 ml as the final volume ( $V_f$ ). This was mixed and then poured into the petri dish. For the second run, however, the 1 ml of extract was pipetted into 14 ml of the MH agar (this was done in an endeavor to increase the concentration of the extracts in the agar) to make up to 15 ml as the final volume ( $V_f$ ). The resultant outcome shows: E-garlic extract from ethanol and G-red onion extract from water diluted in MH agar.

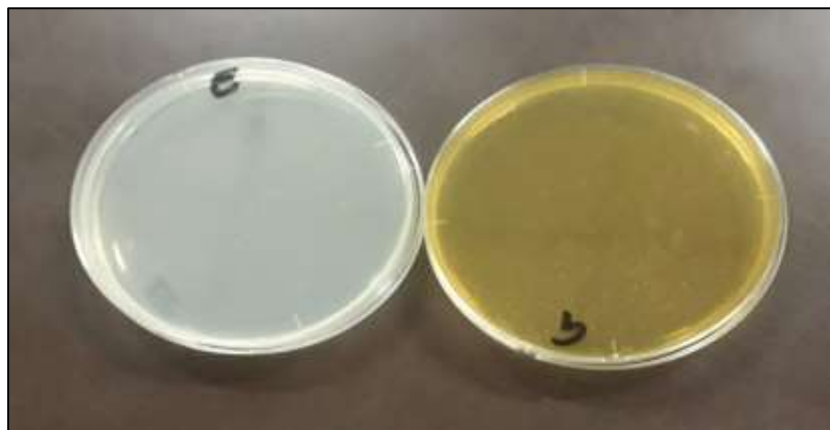


Figure 3. 7: Petri-dishes with extracts diluted in Muller Hinton Agar.

### **3.3.1 Culture of Bacteria isolates for antimicrobial activity tests**

Different media were used for the growth of the bacterial isolates under study. MacConkey (Oxoid, UK), a selective and differential media that allows for the growth of gram-negative bacteria, was used to grow *E. coli*, which formed a pure culture of pink colonies. Nutrient agar was used for the growth of *P. aeruginosa*, which, after 18 hours of incubation, showed a green coloration. *S. aureus* was grown on Blood Agar (Oxoid, UK) and appeared grayish, showing beta hemolysis around the colonies. Xylose Lysine Deoxycholate (XLD, Oxoid, UK), a selective media for enteric pathogens, was used for the growth of *S. typhi*, and colonies appeared red with black centers. For antimicrobial activity, the bacterial isolates were suspended in normal saline, where spot inoculation was done onto the MHA at the CLSI recommended inoculum rate of 0.5 McFarland, and labeled for bacterial test strain identification. All the test organisms were spot inoculated using sterile swabs on all the solvent extracts incorporated into MHA and labeled from A-I with two controls: one with DMSO incorporated into MHA, one without any extract, and the other with only MHA. Thereafter, the plates were incubated at 37 °C for 24 hours. A result comparison was done based on the sensitivity analysis and graded as susceptible, intermediate, or resistant. Susceptible meant no single colony was observed; intermediate implied partial growth (reduced growth compared to that of the control); and resistant implied full growth just as the control, i.e., the control had no extract incorporated in it.

### **3.3.2 Efficacy testing of the extracts**

Sterile inoculating loops were used to pick 3-5 colonies of well-isolated colonies on the cultured plates and emulsified in 3 ml of physiological normal saline. The turbidity of the suspension of the test organisms obtained was adjusted to match the 0.5 McFarland standard (Cheesebrough, 2000).

The MIC was defined as the lowest concentration that completely inhibited the growth of microorganisms for 24 hours. The yellow onion water extract was used for the MIC (the reason for choosing yellow onion was because, despite being subjected to a 95°C evaporation temperature, *S. typhi* remained susceptible). A serial dilution in Mueller-Hinton broth was prepared, beginning with 10% and decreasing to the lowest final dilution concentration of 1.56%. *Salmonella typhi* was used for the MIC determination because it was the most susceptible organism to both water bath evaporation extracts and oven evaporation extracts. A control was also added to each dilution,

vortexed, and incubated for 24 hrs. Inoculation was performed on Nutrient agar, and observations and data were collected.

## CHAPTER FOUR

### RESULTS

#### 4.1 Percentage Yield Obtained

The percentage yield is the ratio of the quantity obtained to the initial quantity multiplied by a hundred. This was calculated in Excel and the results are shown in the tables. Tables 4.1 and 4.2 show the percentage yields obtained from the boiling water bath evaporating procedure (95°C) and oven evaporating procedure (40°C), respectively.

Table 4. 1: Yields obtained from water bath evaporating procedure

<b>Solvent Type</b>	<b>Herb</b>	<b>Extracted Mass(mg)</b>	<b>Initial Mass (mg)</b>	<b>Extracted Yield (%)</b>
Ethyl acetate	Red Onion	13	5000	0.26
	Garlic	10	5000	0.2
	Yellow Onion	50	5000	1
Ethanol	Red Onion	615	5000	12.3
	Garlic	583	5000	11.7
	Yellow Onion	332	5000	6.64
Water	Red Onion	1293	5000	25.9
	Garlic	4424	5000	88.5
	Yellow Onion	2920	5000	58.4

According to the table above, the extract with the highest yield is the one with water as the extracting solvent. The percentage yields of red onion, garlic, and yellow onion water extracts were 25.9%, 88.5%, and 58.4%, respectively. The second-best yield comes from the ethanol-extracting solvent, with red onion yielding 12.3%, garlic yielding 11.7%, and yellow onion yielding 6.64%. The lowest yields were achieved from ethyl acetate, which had the maximum yield from yellow onions (1%), followed by red onions (0.26%), and garlic (0.2%).

The next table also highlights the yield obtained the oven evaporation. Results are as shown in Table 4.2.

Table 4. 2: Yields obtained from Oven evaporation procedure

<b>Solvent Type</b>	<b>Herb</b>	<b>Extracted Mass(mg)</b>	<b>Initial Mass (mg)</b>	<b>Extracted Yield (%)</b>
Ethyl acetate	Red Onion	41	5000	0.82
	Garlic	0	5000	0
	Yellow Onion	236	5000	4.72
Ethanol	Red Onion	2668	5000	53.4
	Garlic	20	5000	0.4
	Yellow Onion	348	5000	6.96
Water	Red Onion	1897	5000	37.9
	Garlic	3390	5000	67.8
	Yellow Onion	1883	5000	37.7

In the case of the oven evaporation technique, the highest yield was obtained from the water extract, with garlic having the highest yield of 67.8%, followed by red onion with 37.9% and yellow onion with 37.7%. For the ethanol extracting solvent, red onions gave the highest yield with 53.4%, followed by yellow onions with 6.96%, and garlic with 0.4%. Ethyl acetate recorded low percentage yields, with the highest yield of 4.72% from yellow onions, followed by red onions with 0.82%, and the least from garlic with 0% or no extraction observed. Figure 4.1 is a conical flask showing the quality of the extract obtained after the evaporation of ethanol from red onion extract.



Figure 4. 1: Quality of ethanol red onion extract upon evaporation

#### 4.1.2 Comparative yield values from water bath and oven evaporation methods

The two evaporating procedures are compared in the scatter plot diagram performed using Excel, with the x-axis having extracting solvents labeled from 1-9, beginning with ethyl acetate (red onion) all the way to water (yellow onion), and the y-axis percentages from 0-100%. In all the two evaporation methods used, water extraction gave the highest yields, with the least yields from ethyl acetate.

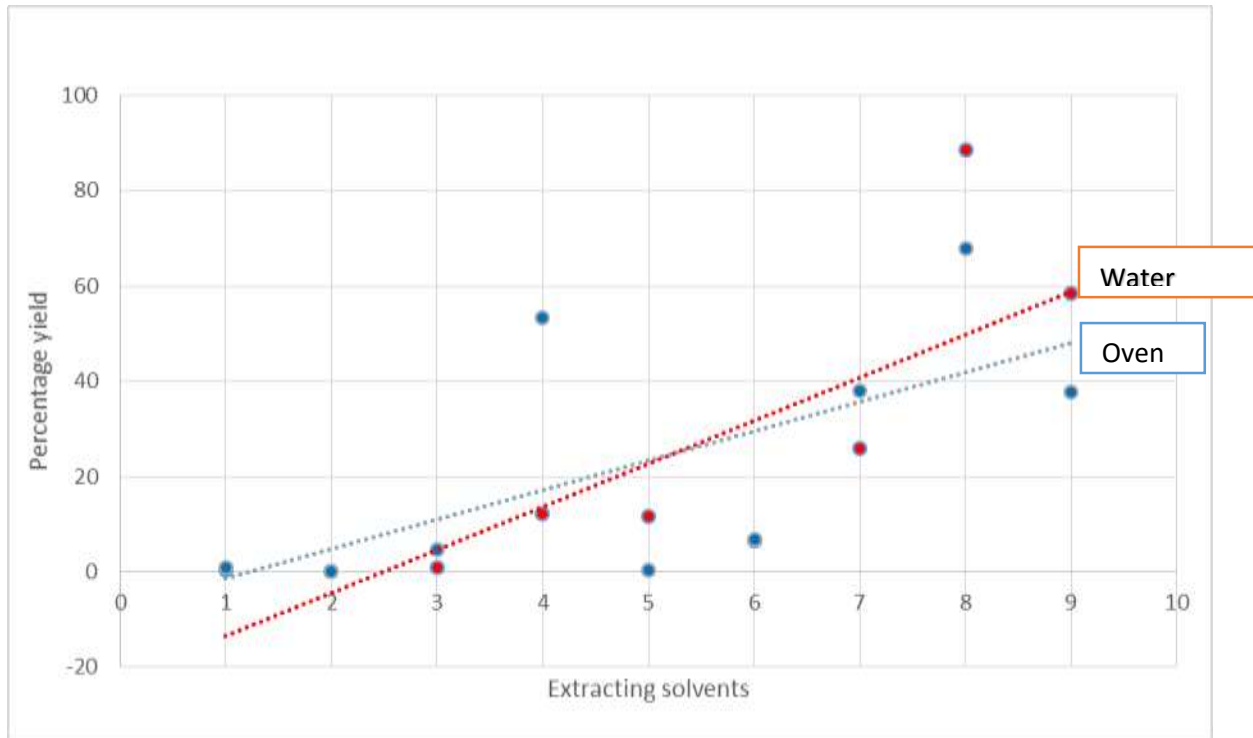


Figure 4. 2: Percentage yield obtained from the two evaporating procedures

## 4.2 Efficacy of the Extracts

### 4.2.1 Water bath extracted extracts

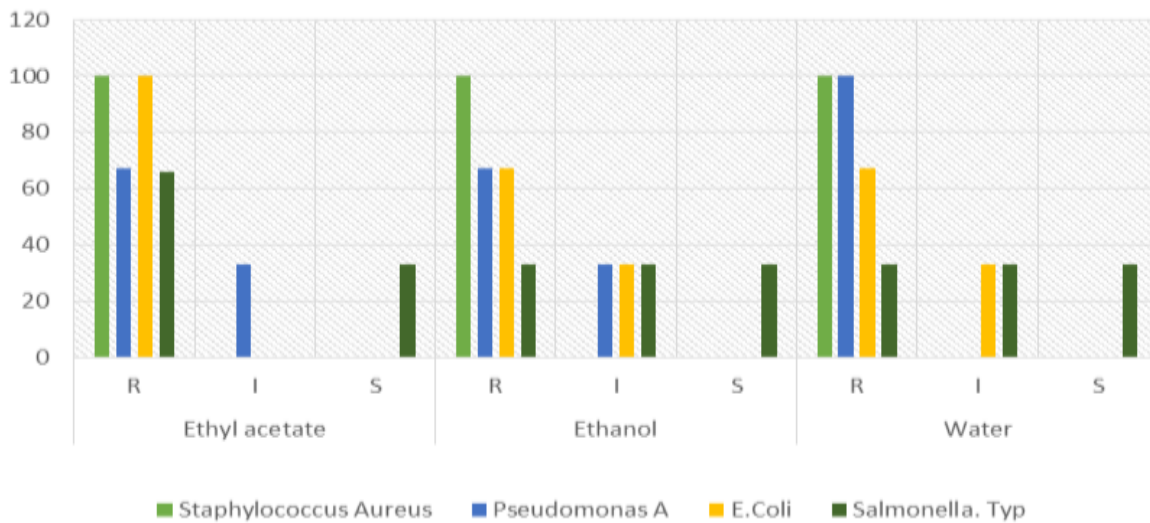
The efficacy of the different extracts obtained using the water evaporating procedure is indicated in Table 4.3. Only *Salmonella typhi* showed some sensitivity to ethyl acetate extracts, while the other bacteria showed resistance. *Pseudomonas aeruginosa* showed some intermediate sensitivity for ethyl acetate and ethanol-extracted extracts, with *Escherichia coli* also exhibiting the same phenomenon with ethanol and water extracts.

Table 4. 3: Efficacy for water bath extracts

Percentage Activity for Different Solvents against the four Bacteria					
Solvents	Microbial Activity	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Ethyl Acetate	R	100	67	100	67
	I	0	33	0	0
	S	0	0	0	33
Ethanol	R	100	67	67	33
	I	0	33	33	33
	S	0	0	0	33
Water	R	100	100	67	33
	I	0	0	33	33
	S	0	0	0	33

Abbreviations are as indicated: R- Resistant, I-Intermediate and S-Susceptible

The data was plotted in excel using a bar graph of percentage activity against solvents.



Where: R- Resistant, I-Intermediate and S-Susceptible

Figure 4. 3: Bar plot for percentage activity against solvent extracts

From figure 4.3, above: both *S. aureus* and *E. coli* showed a 100% resistance to all the three plant extracts extracted using ethyl acetate. *P. aeruginosa* and *S. typhi* recorded a resistance of 67% implying that two of the three treatments were not able to clear the bacteria. A, 33% intermediate for *P. aeruginosa* and a 33% susceptibility for *S. typhi* was recorded.

For the ethanol extract, only *S. aureus* recorded a 100% resistance from all the three plants extracted using ethanol, *P. aeruginosa* and *E. coli* recorded a 67% resistance and *S. typhi* a 33% resistance. *P. aeruginosa*, *E. coli* and *S. typhi* showed a 33% intermediate with the other 33% susceptibility being *S. typhi*.

From the extracts extracted using water *S. aureus*, *P. aeruginosa*, *E. coli* and *S. typhi* showed 100%, 100%, 67% and 33% resistance respectively. *E. coli* and *S. typhi* were both 33% intermediate and another 33% susceptible recorded for *S. typhi*.

#### **4.2.2 Oven extracted extracts**

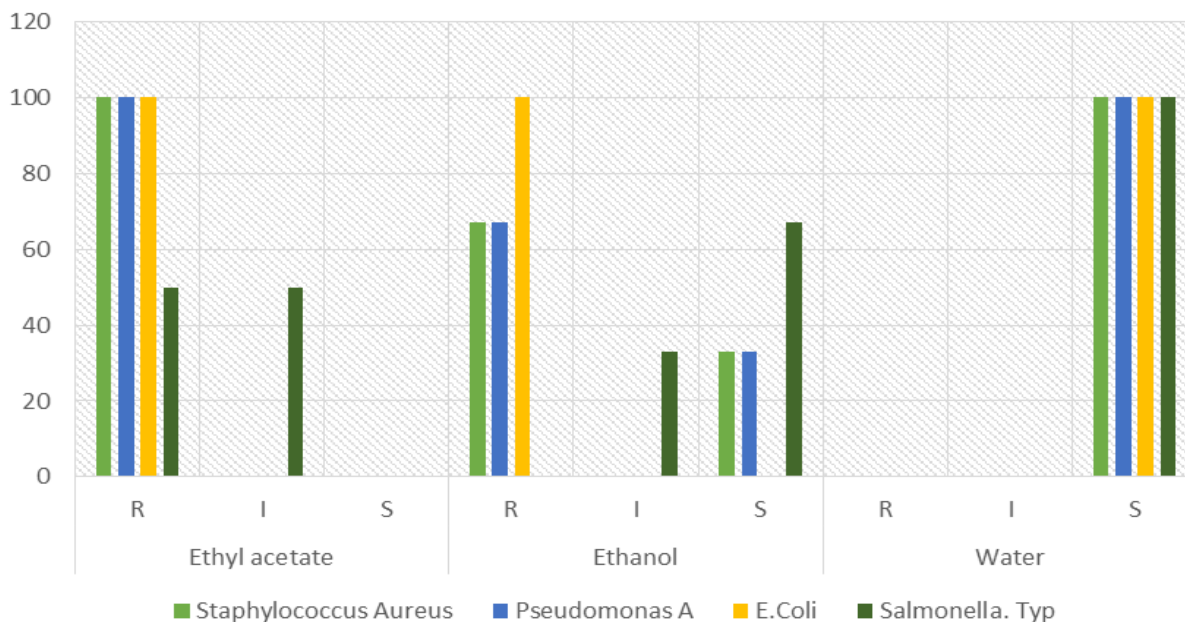
The efficacy of the different extracts obtained using the oven evaporating procedure is indicated in Table 4.4. All four test organisms showed sensitivity to the water extracts. *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed resistance to extracts obtained using ethyl acetate, while for ethanol, the two showed some resistance and some sensitivity. *Salmonella typhi* recorded some intermediate and sensitivity to the extracts. *Escherichia coli* was dominantly resistant to both ethyl acetate and ethanol extracts.

Table 4. 3: Efficacy for oven extracts

<b>Percentage Activity for Different Solvents against the four Bacteria</b>					
<b>Solvents</b>	<b>Microbial Activity</b>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
Ethyl Acetate	R	100	100	100	50
	I	0	0	0	50
	S	0	0	0	0
Ethanol	R	67	67	100	0
	I	0	0	0	33
	S	33	33	0	67
Water	R	0	0	0	0
	I	0	0	0	0
	S	100	100	100	100

Abbreviations are as indicated: R- Resistant, I-Intermediate and S-Susceptible

The data was plotted as indicated in Figure 4.4 showing percentage activity against solvents.



Where: R- Resistant, I-Intermediate and S-Susceptible

Figure 4. 4: Percentage bacterial activity against solvent extracts from garlic, red and yellow onions

The oven evaporated extracts were also reacted with *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhi*. For the ethyl acetate extracting solvent, *S. aureus*, *P. aeruginosa*, *E. coli* and *S. typhi* showed resistance of 100%, 100%, 100% and 50% respectively. Ethanol extracts demonstrated varying activity against *S. aureus*, *P. aeruginosa* and *E. coli*, as a resistance of 67%, 67% and 100% was recorded respectively with *S. typhi* being the only intermediate with a record of 33%. Three organisms were susceptible to the ethanol extracts and these were *S. aureus*, *P. aeruginosa*, and *S. typhi* giving percentages of 33, 33 and 67 respectively.

*S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhi* were all 100% susceptible to the water extracts, thus neither resistance nor intermediate results were observed. The figures 4.5 and 4.6 show the susceptibility of all test organisms to the water extract and two controls respectively. Control-1 was purely MH agar whereas control-2 had DMSO in place of extract incorporated in the media.



Figure 4. 5: Susceptibility of all four organisms to water extract



Figure 4. 6: Showing controls of MH agar plates

### 4.3 Individual Assessment of Plants

The extracts from red onion, garlic and yellow onion were assessed individually based on susceptibility, intermediate, and resistance to the four bacteria namely: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The activity performance was based on all the three extracting solvents used for the water bath evaporating procedure and results are as shown in the figure 4. 6 where 50% of the organisms were resistant, 17% susceptible, and 33% intermediate to red onion. Figure 4. 7 shows the response of the organisms to garlic, while figure 4. 8 shows that of yellow onion.

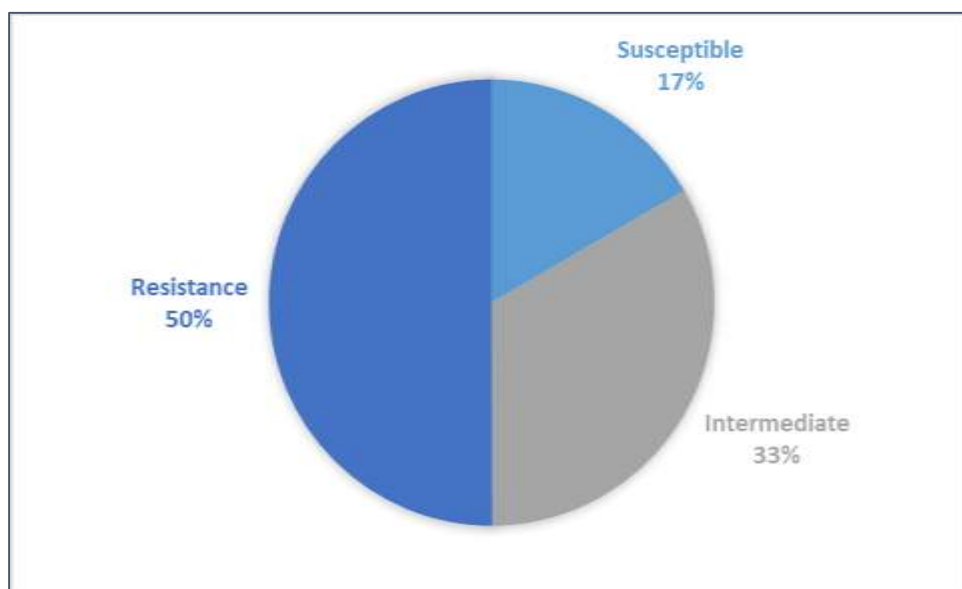


Figure 4. 7: Antibacterial activity of red onion (Water bath evaporation)

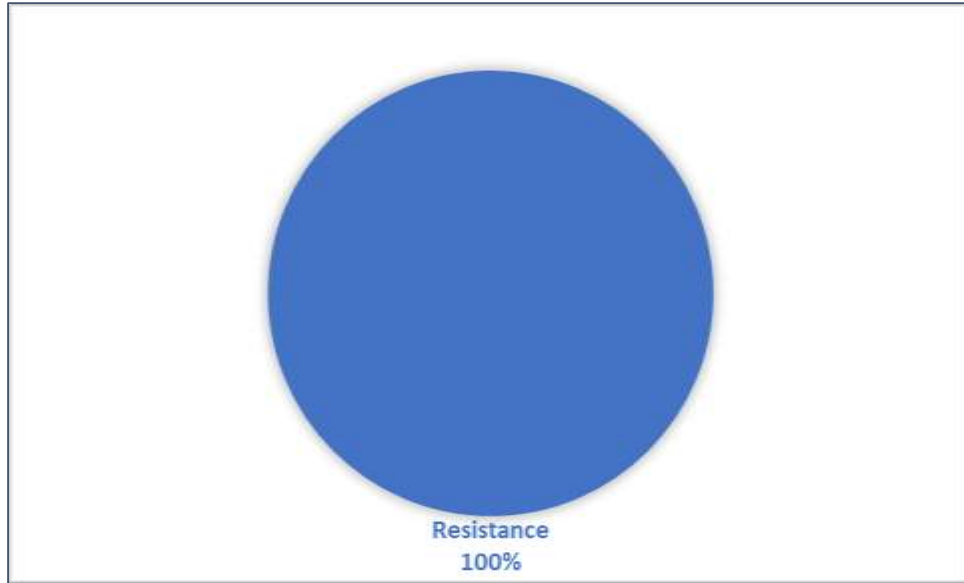


Figure 4. 8: Antibacterial activity of garlic (Water bath evaporation)

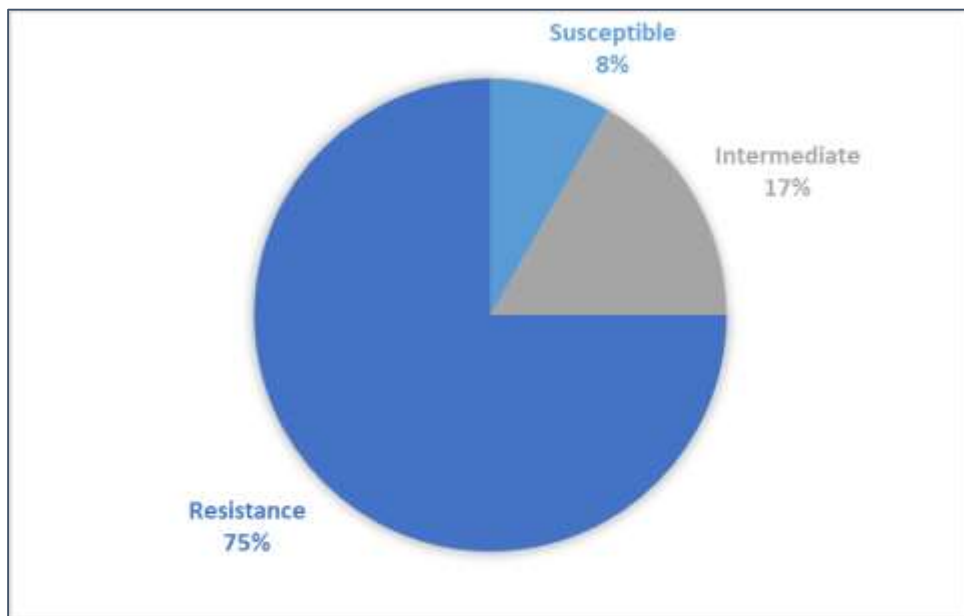


Figure 4. 9: Antibacterial activity of yellow onion (Water bath evaporation)

Figures 4. 9, 4. 10 and 4. 11 illustrates the individual plants' activity performance in all the three extracting solvents used which were oven evaporated. In all the extracts, resistance was above 50%, while susceptibility was lower and varied depending on the extract source as either garlic, red or yellow onion. Garlic had the highest susceptibility at 75% while red onion had the lowest

susceptibility with 33%. The highest susceptibility was observed in *Salmonella typhi*, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Escherichia coli* was the least sensitive.

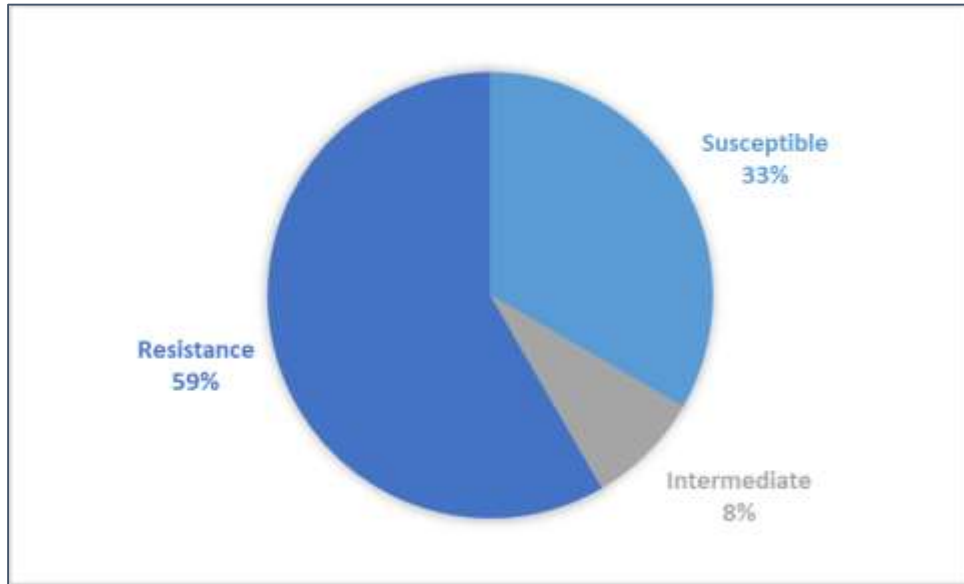


Figure 4. 10: Antibacterial activity for red onion (Oven evaporation)

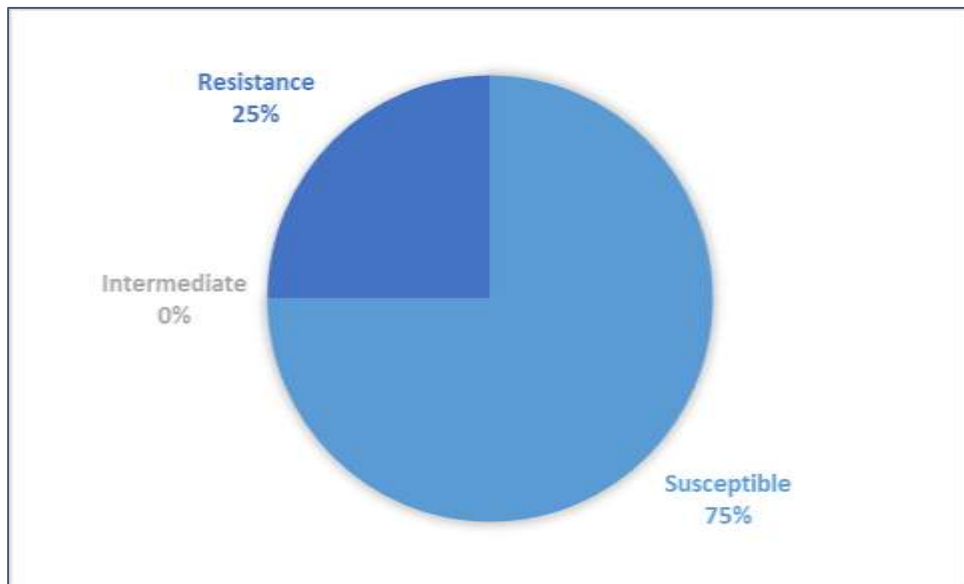


Figure 4. 11: Antibacterial activity of garlic (Oven evaporation)

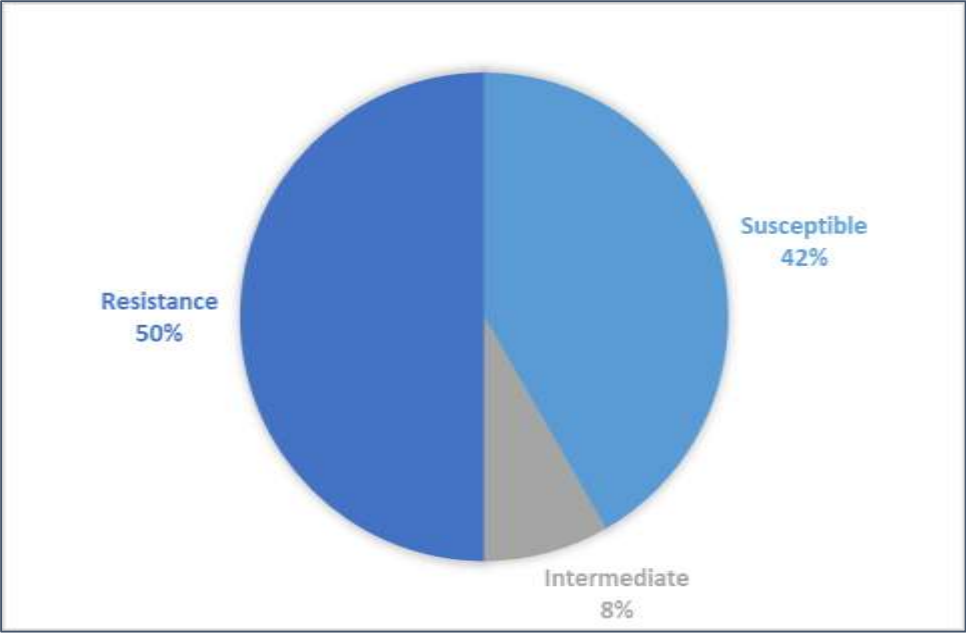


Figure 4. 12: Antibacterial activity of yellow onion (Oven evaporation)

## CHAPTER FIVE

### DISCUSSION

The importance of garlic and onions cannot be overemphasized, especially with AMR becoming a public health threat (Gupta and Birdi, 2017). The findings of the antimicrobial effect of the plants (garlic and onions) are discussed in this chapter.

The medicinal properties of garlic and onions have been previously investigated (Tsewang et al., 2021). In order to ascertain this observation with the Zambian-grown onion and garlic, which are usually cured and dried, an antibacterial assessment against common bacteria was done. The possible active agents were extracted using water, ethyl acetate, and alcohol. The ability of solvents to extract large amounts of bioactive compound from the plant is what is referred to as "yield potential," and it follows the principle that like dissolves like. Therefore, a non-polar solvent will dissolve a non-polar compound, whereas a polar solvent will dissolve a polar compound, and as such, the amount of extract obtained is dependent on the extracting solvent (Wolde et al., 2018). Different extracting solvents used were water (H<sub>2</sub>O), ethanol (CH<sub>3</sub>CH<sub>2</sub>OH), and ethyl acetate (CH<sub>3</sub>COOCH<sub>2</sub>CH<sub>3</sub>) in the order of decreasing polarity. Generally, from the findings, water extract gave the highest yield, followed by ethanol, and finally ethyl acetate. This can arguably be said that the three plants, red onion, garlic, and yellow onion, contain a high amount of polar compounds, which were extracted mostly by the water solvent with the highest polarity. These results are of great benefit considering that water is the most abundant and utilized solvent used in cooking and the preparation of onions and garlic.

From the results obtained via the water bath extraction procedure, *S. aureus* was 100% predominantly resistant across all three extracting solvents, *S. typhi* was susceptible with 33% across solvents, and *P. aeruginosa* and *E. coli* had a varying response with the best performance record of an intermediate. In comparison to the extracts evaporated using oven evaporation, susceptibility to water extracts was 100% for all bacteria tested, indicating that water was able to not only dissolve but also preserve the active ingredient in red onion, garlic, and yellow onion. However, this was not the case for water bath evaporation extracts, and thus the temperature (95°C)

could have minimized the amount of active ingredient present. This is because onions and garlic contain volatile compounds that contribute to activity, and a lot was lost during heat evaporation. Reyes et al. (2018) highlight the major active ingredient in garlic as allicin (diallylthiosulfinate), which is an organosulfur containing compound responsible for the pungent garlic smell and is only present when the garlic is crushed, allowing the allinase enzyme to act on allin (S-allylcysteine sulfoxides). Allinase is an enzyme, and enzymes are proteins in nature, thus by applying heat the morphology and configuration of the protein is distorted, leading to denaturation and ineffectiveness of the enzyme. This can be further argued following the results obtained from the individual assessment of the plants shown in figures 4.6 to 4:11. The susceptibility of the four microorganisms to red onion from the water bath evaporation extracts was 17%, which increased to 33% for the oven evaporation extracts. For garlic, the water bath evaporation extracts showed 100% resistance to all four bacteria and later showed 75% susceptibility to the oven evaporation extracts and 25% resistance of the bacteria to the oven evaporation extracts. The case for yellow onions was not any different, as susceptibility moved from 8% for the extracts obtained using a water bath to 42% for the extracts obtained using oven evaporation, and as such, it can be deduced that heat definitely has an impact on the antibacterial activity of the *Allium* sps. Although there was some susceptibility and intermediate activity observed for red and yellow onions, they could have come from other secondary metabolites contained in the plants and quercetin, which also possess antibacterial properties. A study conducted by Hossain et al. (2013) on the effect of temperature and the extraction process on antioxidant activity also shows that an increase in the extraction temperature leads to a reduction and breakdown in the antioxidant content. Concentration is another factor that could have had an impact on the efficacy of the plants, as the initial weight for all plant materials was 5 g, but depending on the type of solvent used, different yields were obtained. Plant extracts that were extracted using water had a higher concentration after solubilization with DMSO and thus showed a higher potency against the bacteria under study than extracts from the two solvents obtained by oven evaporation.

Lambert (2002) describes *P. aeruginosa* as a notorious organism to control with antibiotics. Resistance in bacteria occurs via plasmids, in which both mutations on the chromosomes and the transfer of resistant genes via transformation occur. When bacteria produce enzymes that chemically modify, degrade, and even inactivate antibiotics, this is known as antibiotic

inactivation. Modification of the target site is done by altering the sites that interact with antibiotics. Gupta and Birdi (2017) report that *P. aeruginosa* can develop resistance to imipenem by mutational loss of porin proteins, thereby modifying the outer membrane permeability. Nevertheless, water extracts of the plants from oven-extracted extracts were clearly able to eliminate *P. aeruginosa*, and a study by Anayaegbunam (2019) shows also the activity of onions against *P. aeruginosa*. The other school of thought on the activity of these extracts could be on the make-up nature of the bacteria as Gram positive and Gram negative. Gram positive bacteria have a thick cell wall, whereas Gram negative bacteria have a relatively thin cell wall; this results in the cells having different properties in response to antibiotics (Mai-Prochnow, 2016). In this study, *Staphylococcus aureus*, a Gram-positive bacterium, was sensitive to all oven-evaporated plant water extracts. This is as observed by other workers (Deresse, 2010; Nejad et al., 2014).

The best performing extract was yellow onion with the most susceptible being *S. typhi* and as such MIC was performed which should inhibition between 6% and 3% of the extract concentration. *Salmonella typhi* is a gram-negative bacterium that is of public health significance as it is responsible for typhoid fever, with developing countries being affected (Ashurst, 2022). Zambia being a developing nation has not been spared with the most recent outbreak in 2022. With the results obtained home remedies, or rather use of onions, and garlic should be encouraged to minimize the burden of bacterial diseases.

These results were followed up for about seven days, and the observation for the water evaporated extracts changed with the progression of time, with those that were susceptible changing to intermediate and those that were intermediate to resistance. The ones that recorded resistance-maintained overtime thus shows the instability of the active component upon exposure to a water bath evaporation of 95°C. On the other hand, oven evaporated extracts maintained their results over time thereby maintaining the effectiveness of the extracts.

The use of garlic and onions is ecologically friendly in fighting bacteria pathogens, as they do not leave any residues as in the case of antibiotics which eventually may exceed some tolerable levels and lead to toxicity and antimicrobial resistance. Like any other herbs, garlic and onions are a natural resource that neither produce harmful toxins nor have any negative impact on the

ecosystem (Nasri and Shirzad, 2013). With reference to the results obtained in this study, eating raw garlic and onions or applying them as a powder to our foods should be normalized other than boiling the plants, as this reduces their potency.

## **CHAPTER SIX**

### **CONCLUSION**

#### **6.1 CONCLUSION**

1. Garlic, Red and yellow onion do possess antibacterial properties and can be used to minimize the disease burden.
2. The activity of these plants is negatively affected by the high temperatures they are exposed to and water provides a better solvent for garlic and onion active compound.

#### **6.2 RECOMMENDATIONS AND FUTURE ENDEAVOURS**

1. Further research should be done to purify the active ingredients present in dried garlic and onions.
2. Temperature of 35°C and below should be used to evaporate the plant extracts so as to increase their efficacy.
3. Local food processors can make use of the available plants' garlic and onion' to reduce food spoilage pathogens and food poisoning caused by microbial contamination thereby promoting public health.
4. Farmers should attempt supplementing feed with garlic and onion to boost the health of animals rather than administering antibiotics to promote animal health.
5. Garlic and onion use should be encouraged in our cooking and uncooked foods like salads.
6. Local traders should be enlightened on how they can use garlic and onion in food preservation and shelf-life extension.

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## APPENDIX 1: APPROVAL OF RESEARCH PROPOSAL



**THE UNIVERSITY OF ZAMBIA  
SCHOOL OF VETERINARY MEDICINE  
OFFICE OF THE ASSISTANT DEAN (POSTGRADUATE)**

Telephone: 293727  
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Lusaka, Zambia

Your Ref:

Our Ref:

24<sup>th</sup> September, 2021

Constance Kalyati  
Department of Disease Control  
School of Veterinary Medicine  
University of Zambia  
P.O. Box 32379  
**LUSAKA**

Dear Constance Kalyati,

**RE: APPROVAL OF RESEARCH PROPOSAL**

At the meeting of the School Board of Graduate Studies held on 24<sup>th</sup> September, 2021, your research proposal entitled '**Assessment of garlic and onion's antimicrobial properties against common fungal and bacterial pathogens in food environments: An in vitro study**' was tabled and discussed. I am therefore pleased to inform you that the research proposal was subsequently approved by the Board.


On behalf of the Board, I wish you success as you apply for ethical approval and carry on with your research activities.

Yours sincerely

Dr Chisoni Mumba  
**ACTING ASSISTANT DEAN (PG), SCHOOL OF VETERINARY MEDICINE**

Cc     Director, DRGS  
       Dean, School of Veterinary Medicine  
       Head, Disease Control  
       File

## APPENDIX 2: RESEARCH ETHICAL APPROVAL



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16<sup>th</sup> February, 2022.

**Ref. No. 2021-Nov -021**

The Principal Investigator  
Ms. Constance Kalyati  
Department of Paraclinical studies  
School of Veterinary medicine  
University of Zambia  
Lusaka, Zambia

Dear Ms Kalyati

**RE: ASSESSMENT OF GARLIC AND ONIONS'S ANTIMICROBIAL PROPERTIES AGAINST COMMON FUNGI AND BACTERIAL PATHOGENS FOUND IN FOOD ENVIRONMENTS SUCH AS E. COLI, SALMONELLA AND STAPHYLOCOCCUS AUREUS: AN IN-VITRO STUDY.**

Reference is made to your protocol submission. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Ordinary	Approval No. <b>2021-Nov-021</b>
Approval and Expiry Date	Approval Date: 16 <sup>th</sup> February 2022	Expiry Date: 15 <sup>th</sup> February, 2023
Protocol Version and Date	Version - Nil.	15 <sup>th</sup> February, 2023
Information Sheet, Consent Forms and Dates	• English.	15 <sup>th</sup> February, 2023
Consent form ID and Date	Version - Nil	15 <sup>th</sup> February, 2023
Recruitment Materials	Nil	15 <sup>th</sup> February, 2023
Other Study Documents	Data Collection Sheet, Focus Group Discussion.	15 <sup>th</sup> February, 2023
Number of participants approved for study	-	15 <sup>th</sup> February, 2023

Where Research Ethics and Science Converge

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

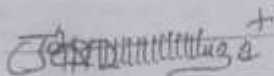
#### **Conditions of Approval**

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,  
**ERES CONVERGE IRB**



Dr. Jason Mwanza  
Dip. Clin. Med. Sc., BA., M.Sc., PhD  
**CHAIRPERSON**