# PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *Carica papaya* L. Var. WAINAMALO EXTRACTS AGAINST SELECTED MICROORGANISMS IN MASENO, KENYA.

**BY**

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**SCHOOL OF PHYSICAL AND BIOLOGICAL SCIENCES**

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## DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university or institution. All sources of information have been duly acknowledged in the reference unless inadvertent omission.

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## DEDICATION

I dedicate this piece of work to my lovely and caring parents Mr. Joseph Arum and Mrs. Eve Arum and to my siblings who make it all worthwhile.

## ABSTRACT

*Carica papaya* L. Variety Wainamalo has been used by the Luo community in Lake Victoria region as medicine for a long time and this constitute the reason for choice of this plant for this study. The number of emerging multi-drug resistant microbial strains is continuously increasing and has become a serious threat to successful treatment of infectious and opportunistic diseases of Human Immunodeficiency Virus-Acquired Immune Deficiency Syndrome (HIV-AIDS) victims. Plant derived antimicrobials have received considerable attention in recent years. It affects not only the economy, but the general well-being of people with more serious impacts in developing countries. Many plants have been used because of their antimicrobial traits such as compounds synthesized in the secondary metabolism. Little is known about the phytochemical and antimicrobial activities of *C. papaya* Variety Wainamalo extracts on pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus* and *Candida albicans,* which are known to cause many opportunistic infections among Human Immunodeficiency Virus-Acquired Immune Deficiency Syndrome(HIV-AIDS) infected patients in Lake Victoria region. This study was set to determine the phytochemical and antimicrobial activity of extracts of *Carica papaya* L. Var. Wainamalo and to determine the minimum inhibitory concentration of seed, leaf and bark extracts on *Escherichia coli, Staphylococcus aureus* and *Candida albicans.* *Carica papaya* plant materials were collected from a farm in Kiboswa, Kisumu County and were taken to Maseno university botany laboratory for processing. Fruits, bark and leaf materials were washed with tap water, rinsed in sterile distilled water, and dried under room temperature for 30 days. Fruits were cut open to extract the seeds. The materials were then cut into small pieces and ground into powder separately. One hundred grams powder was transferred into five hundred millimeter of water, 95% ethanol and 95% acetone in conical flasks. The powder was added, stirred and mixture was allowed to stand for 24 hours. Then the mixture was filtered through a Whatman filter paper No 1 after decantation. Phytochemical compounds of the leaf, seed and bark were then extracted using soxhlet apparatus using water, 95% ethanol and 95% acetone respectively. The filtrates were concentrated with a rotary evaporator at $45^0$C. The three test organisms were subjected to five *C. papaya* extract concentrations of 0% (control), 25%, 50%, 75% and 100% using disc diffusion method and Mueller Hinton agar replicated three times. Plant extracts were isolated and MIC was determined by serial dilution. Zone of inhibitions were measured in millimeters. Analysis of variance was carried out using SAS package. Treatment means were separated and compared using Tukey LSD at significance level $P = 0.05$ . The study revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, glycosides, anthoscyanins and tepernoids. Athraquinones were found to be absent in seed and bark extracts. There were significant differences among plant parts extracts, solvents used and microorganisms tested. The acetone extracts of the seed, leaf and bark did not show any activity against *C. albicans*. Ethanol bark and seed extracts demonstrated higher activities against the test microbes with the highest activity (9.82 and 8.87 mm) against *S. aureus.* Ethanol leaf extract had higher inhibition of 8.13mm in *E. coli.* Higher ethanol extracts inhibitions may be attributed to more active components present as a result of high polar solubility properties of ethanol. Minimum Inhibitory Concentration for *E. coli* and *S. aureus* was 0.025mg/ml while for *C. albicans* was 0.05mg/ml. The antimicrobial activity of the extracts on the tested microorganisms may be due to growth inhibition resulting from alteration of the cell biochemical activities and disruption of cell wall integrity. Differences in minimum inhibitory concentration may be due to variable sensitivity of the microbes to the phytochemical substances in *C. papaya* extracts. The results provide evidence that *C. papaya* L. Var. Wainamalo may serve as a potential source of new antimicrobial agents in the treatment of infections caused by the three test organisms. Purification of bioactive compounds can, thus, be further studied for the development of novel antimicrobial therapies.

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## ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| **AMR** | Antimicrobial Resistance |
| **ANOVA** | Analysis of Variance |
| **CA-MRSA** | Community Acquired Methycilin Resistant *Staphylococcus aureus* |
| **CDC** | Centre for Disease Control |
| **ETEC** | Enterotoxigenic *Escherichia coli* |
| **EPEC** | Enteropathogenic *Escherichia coli* |
| **EIEC** | Enteroinvasive *Escherichia coli* |
| **EHEC** | Enterohemorrhage *Escherichia coli* |
| **KEMRI** | Kenya Medical Research Institute |
| **MRSA** | Methycilin Resistant *Staphylococcus aureus* |
| **Ppt** | Precipitate |
| **Soln** | Solution |
| **STEC** | Shiga Toxin producing *Escherichia coli* |
| **VRSA** | Vancomycin Resistant *Staphylococcus aureus* |
| **WHO** | World health Organization |
| **LSD** | Least significance difference |

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Infectious diseases are reported to be the world's major human threat and accounting for almost 50,000 deaths every day (Lohedas *et al.,* 2015). The frequency and diversity of life-threatening infections caused by pathogenic microorganisms has increased steadily; becoming an important cause of morbidity and mortality and continues to be a major problem in many developing countries, especially amongst children (Lohedas *et al.,* 2015). The number of emerging multi-drug resistant microbial strains is continuously increasing and has become a serious threat to successful treatment of infectious and opportunistic diseases of HIV-AIDS victims (Wagate *et al*., 2009). Plant derived antimicrobials have received considerable attention in recent years (Sahle and Okbatinase, 2017). It affects not only the economy, but the general well-being of people with more serious impacts in developing countries. Many plants have been used because of their antimicrobial traits such as compounds synthesized in the secondary metabolism. Little is known about the phytochemical and antimicrobial activities of *C. papaya* Var.Wainamalo extracts on pathogenic microorganisms such as pathogenic *Escherichia coli,* pathogenic *Staphylococcus aureus* and *Candida albicans,* which are known to cause many opportunistic infections among HIV-AIDS infected patients in Lake Victoria region. The Luo community of Kenya relies heavily on ethno-medicine to manage human ailments and have traditionally used plants to treat diseases of microbial origin (Maima *et al.,* 2014). Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli, Vibrio cholerae*, *Aeromonas* spp., *Klabsiella* spp., *Shigella* spp., *Staphylococcus aureus, Pseudomonas* spp. and *Salmonella*

spp. are the most common. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Wagate *et al*., 2009).

With the continuous use of antibiotics, diseases causing microorganisms, especially bacteria (Methicillin Resistant *Staphylococcus aureus*, Multi Drug Resistant *Mycobacterium tuberculosis, Escherichia coli)* and fungi (*Candida* sp, *Aspergillus*) have become resistant to these antibiotics. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppressant and allergic reactions (Namita and Mukesh, 2012). In recent times, the use of herbal medicine in developing countries has increased, owing to the fact that western orthodox medicines are relatively expensive and readily not available (Orchue and Momoh, 2013). Continuous use of antibiotics also lead to excessive growth of other microorganisms which are beneficial in the human body, for example, constant use of antibiotic may lead to increase in the number of *Candida albicans* which at the end lead to candidiasis. This has created an immense clinical problem in the treatment of infectious diseases caused by the named microorganisms. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Furthermore these antibiotics are expensive and are not easily accessed by poor people from rural areas. The *C. papaya* variety used in this study was selected because the communities of people living around the Lake Victoria region use the plant to treat various infections related to HIV-AIDS and this plant has been documented to contain some antimicrobial activities.

According to World Health Organization, WHO (2011), there is lack of scientific evidence to evaluate the safety and efficacy of traditional medicine. Therefore, there is need to screen medicinal plants for better understanding of their properties, safety and efficacy (Doughari *et al*.,

2008) and also to validate their traditional uses and identify the active compounds (Demet *et al*., 2008). Infectious diseases are very common and have adverse effects to the human beings. The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies and academia, because many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). Emergence of resistant strains of pathogenic microorganism has also continued to pose a major health concern about the efficacy of several drugs; most importantly antibiotics in current use (Lohedas *et al.*, 2015). This explains the increasing research on various plants, and the upsurge in mass-media advert placement on herbal preparations by countless 'traditional doctors'. Aruljothi *et al.* (2014) defined medicinal plant as a plant in which one or more organs contain substances that can be used for therapeutic purposes or which a precursor for the manufacturing is of drugs useful for disease therapy.

Plants have major advantages mostly for being the most effective and cheaper alternative sources of drugs. The local use of natural products from plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, and Africa (Bibitha *et al*., 2002). It is however noted that medicinal plants are readily available, they have little side effects and there is extensive local knowledge on herbal medicine amongst the communities (Rojas *et al*., 2006; Doughari *et al*., 2008). Pawpaw (*Carica papaya* L.) is commonly known for its food and nutritional values throughout the world. The medicinal properties of pawpaw fruit and other parts of the plant are also well known in traditional systems of medicine. Each part of pawpaw tree possesses economic value when it is grown on a commercial scale (Krishna *et al*., 2008). Even though the active compounds are normally extracted from all parts of the plant, the concentration of these compounds varies from structure to structure (Aruljothi *et al*., 2014). However, parts known to contain the highest concentration

of the principles are preferred for therapeutic purposes and it can either be the leaves, stem, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits, and the seeds (Kafaru, 1994). Various parts of the pawpaw plant, which include the leaves, fruit, seed, latex, and root, are known to contain bioactive compounds (Anibijuwon and Udeze, 2009). The plant parts are found to possess some properties like analgesic, amoebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral and vermifuge (Anibijuwon and Udeze, 2009).

The presence of phytochemical constituents in medicinal plants make them useful for healing as well as curing of human diseases (Yahaya *et al*., 2017). Phytochemicals are naturally occurring compounds in the medicinal plants (Wadood *et al*., 2013). Large populations of the world, especially in developing countries depend on the traditional system of medicine to treat variety of diseases (Yahaya *et al*., 2017).

Minimum inhibitory concentration (MIC) is the lowest concentration able to completely inhibit any visible growth after overnight incubation with media (Prescott *et al*., 1999; Yahaya *et al*., 2017). MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials.

Chymenopapain and papain are the two important bioactive compounds present in *C. papaya*. (Aruljothi *et al*., 2014). Antimalarial and antiplasmodial activity has been noted in some preparations of the plant (Aruljothi *et al*., 2014). The leaves of the pawpaw plants contain chemical compounds of karpain, a substance which kills microorganisms that often interfere with the digestive function (Udoh *et al*., 2005). Pawpaw leaf-extracts have phenolic compounds,

such as protocatechuic acid, p-coumaric acid, 5, 7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid (Romasi *et al*., 2011; Jyotsna *et al*., 2014).

During the last few decades, considerable progress has been achieved regarding the therapeutic properties of pawpaw. The use of *C. papaya* L. in traditional medicine relies on papain, the active principle which exerts an ulcer protective effect. Studies from other parts of the world have indicated that *C. papaya* possesses antimicrobial, antioxidant and anti-inflammatory activities (Aruljothi *et al*., 2014) but similar studies are lacking in Kenya and yet this plant is used by local communities in the Lake Victoria region to treat infections related to HIV-AIDS. The phytochemical compounds and antimicrobial properties of the *C. papaya* L. varieties growing in the Lake Victoria region have not been studied yet the pharmaceutical value and concentration of active ingredients in each plant vary depending on climatic and edaphic factors (Musyimi *et al*., 2007; Rajakaruna *et al*., 2002). Several factors such as phenological age of plant, percent humidity of the harvested material and the method of extraction have been identified as possible sources of variation for the chemical variation, toxicity and bioactivity of the extracts (Musyimi *et al*., 2007; Rajakaruna *et al*., 2002). The efficacy of treatment with *C. papaya* is dependent on the quantity of the different compounds in the preparations. In Indonesia, pawpaw leaves are used as feed for animals after parturition-2 leaves boiled in water fed every 2 days for 1week (Shivananda *et al*., 2007).

In Nigeria, it is used for treatment of upper respiratory tract ailment and cancer of the uterus. In Ivory Coast, it is used for treating mental instability (Anibijuwon *et al.,* 2009). In Trinidad, it is used for treating scorpion bites and hypertension (Anibijuwon *et al.,* 2009). In Cote d'Ivoire and Samoa, it is used for toothache and in Mexico to treat tuberculosis (Anibijuwon *et al.,* 2009). In Honduras and Turkey, it is used for alleviating liver ailments, constipation and as a laxative. In

Philippines, India, Madagascar and Malaya, it is used for treating arthritis and rheumatism. In Honduras, Japan, Panama and West Africa, it is used for the treatment of diarrhea and dysentery (Anibijuwon *et al.,* 2009).

Research findings also support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds (Sahle and Okbatinsae, 2017). Approximately two thirds of HIV/AIDS patients in many developing countries seek symptomatic relief and manage opportunistic infections through the use of traditional medicine (WHO, 2011). Therefore, it is sensible to study plants from the local flora which are used for treatment of such infections (Okemo *et al*., 2011). It is estimated that more than 70% of the pathogenic bacteria are resistant to at least one of the antibiotics commonly used to treat them (Okemo *et al*., 2011). This calls for urgent and continued need to find new antimicrobial compounds with varied chemical structures and new mechanisms of action (Parekh and Chanda, 2007) and has necessitated the need to search for new antimicrobial substances from other sources including plants (Doughari, 2006; Duraipandiyan *et al*., 2006).

The microorganisms (pathogenic *Staphylococcus aureus,* pathogenic *Escherichia coli* and *Candida albicans*) used in this study were chosen because they are known to cause infections such as boils, impetigo, diarrhea, thrush and candidiasis among the communities within the Lake Victoria region and especially among the HIV/AIDS patients. Pawpaw plants especially leaf, bark and seed extracts have been documented to contain antimicrobial activities by previous studies (Anibijuwon *et al.,* 2009). Little information on the phytochemical and antimicrobial activity of pawpaw extracts generally on pathogenic *Staphylococcus aureus,* pathogenic *Escherichia coli* and *Candida albicans* is available in Kenya, despite the fact that

this plant is used locally to manage infections caused by some of these pathogenic microorganisms. The people from the Lake Victoria region also have a strong cultural belief in traditional medicine. This study aimed at investigating the phytochemical, antimicrobial activity and minimum inhibitory concentration of *Carica papaya* L. Var. Wainamalo seed, leaf and bark extracts on pathogenic *Staphylococcus aureus*, pathogenic *Escherichia coli* and *Candida albicans*.

**1.2 Problem Statement**

According to WHO (2011), there is lack of scientific evidence to evaluate the safety and efficacy of traditional medicine. Therefore, there is need to screen medicinal plants for better understanding of their properties, safety and efficacy (Doughari *et al*., 2008) and also to validate their traditional uses and identify the active compounds (Demet *et al*., 2008). Infectious diseases are very common and have adverse effects to the human beings. These diseases cause death and lower productivity of people infected and affected. Emergence of resistant strains of pathogenic microorganisms has continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use. Antibiotic resistance has been observed in *E. coli*, *S. aureus* and *C. albicans* to the synthetic drugs which is becoming a serious public health issue, with high treatment costs. Despite these scientific facts on the antimicrobial potentials of other *C. papaya* varieties, little information exits showing a comparison on the potencies of extracts from different parts in varied extraction solvents. Before this study little was known about the antimicrobial activity of *C. papaya* Var. Wainamalo bark, seed and leaf extracts. Hence, this study was initiated to fill gaps of the unknown pertaining to presence of phytochemical compounds occurring in the leaf, seed and bark extracts of *C. papaya*,

determine the effect of its seed, leaf and bark extracts on growth and its Minimum Inhibitory Concentration (MIC) on *Staphylococcus aureus, Escherichia coli* and *Candida albicans*.

Several studies have addressed phytochemicals in seed, leaf and bark of *C. papaya* and that they vary depending on the ecological zone and variety of the plant yet there is little information of phytochemicals in leaf, seed and bark of *Carica papaya* varieties in Kenya especially the Lake Victoria region. There has also been research work carried out in other parts of the world especially Asian and West African countries but little work has been carried out on the antimicrobial effect of *C. papaya* leaf, seed and bark extracts in Kenya especially around the Lake Victoria region. This scenario stands out in many countries, with negative impact on human health. One of the methods to obtain new substances against drug-resistant bacteria is to prospect for bioactive compounds in plants. Synthetic antibiotics are becoming expensive especially to the poor communities in Kenya, hence there is need to search for alternative sources of antimicrobial agents. In studies done by Aruljothi *et al*. (2014), only leaves were used but in this research, leaves, barks and seeds of *C. papaya* L. Var. Wainamalo were used. In addition to that the acetone and ethanol solvent was used for extraction in this study unlike Aruljothi *et al*. (2014) who used methanol and water for extraction. However in the current study, paper disc method was used for antimicrobial test unlike the well diffusion method used by Aruljothi *et al*. (2014).

### 1.3 Justification of the Study

According to Rukangira, 2001, traditional medicine is one of the ways to achieve total health care coverage of the world`s population. Medicinal plants are used for primary healthcare by about 70 % - 90 % of populations in developing countries (WHO, 2011). Medicinal plants can even cure deadly diseases such as cancer and AIDS that have resisted conventional drugs (Jamil *et al*., 2007). Claims by the traditional healers about the efficacy of herbal preparations have

little validation and documentation yet they treat many people. The Luo community of Kenya relies mostly on ethno-medicine to manage human ailments and have traditionally used plants to treat diseases of microbial origin (Maima *et al.,* 2014).

It is therefore imperative that phytochemical and bioassay analysis is done to find out the efficacy of the medicinal plants. It is vital therefore that the efficacy of phytochemical activity of *C. papaya* L. Var. Wainamalo is validated and documented. This supports the main objective of this study to investigate the phytochemical and antimicrobial activity of the crude extracts of the selected *C. papaya* L. Var. Wainamalo plant using in vitro method to validate and document scientific antifungal and antibacterial activities of this variety. Medicinal plants have long been the subject of human curiosity and need. Plant derived products are present in 14 of 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medical practitioners and they form an important part of the health-care system.

The data from this research work will be useful to pharmaceutical industries to have an alternative to using synthetic drugs to ones derived from the *Carica papaya* parts; it will also open opportunities for other researchers to improve their knowledge and understanding of the antimicrobial effect of *Carica papaya* seed, leaf and bark extracts on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. It will also help offer an alternative to synthetic antibiotics which are very expensive and at times not affordable to the poor.

## 1.4 Objectives

### 1.4.1 General Objective

To determine the phytochemical compounds and antimicrobial activity of *Carica papaya* L. Var. Wainamalo seed, leaf and bark extracts on pathogenic *Staphylococcus aureus,* pathogenic *Escherichia coli* and *Candida albicans.*

### 1.4.2 Specific Objectives

1. To assess the presence of phytochemical compounds in the leaf, seed and bark extracts of *Carica papaya* L. Var. Wainamalo.
2. To determine the effect of Carica papaya L. Var. Wainamalo seed, leaf and bark extracts on growth of pathogenic *S. aureus,* pathogenic *E. coli* and *C. albicans.*
3. To determine the Minimum Inhibitory Concentration (MIC) of leaf, seed and bark extracts of *C. papaya* L. Var. Wainamalo on pathogenic *S. aureus,* pathogenic *E. coli* and *C. albicans.*

## 1.5 Hypotheses

1. *Carica papaya* L. Var. Wainamalo does not possess many phytochemical compounds in its bark, roots and leaves.
2. *Carica papaya* L. Var. Wainamalo seed leaf and bark extracts have no effect on growth of pathogenic *Staphylococcus aureus,* pathogenic *Escherichia coli* and *Candida albicans.*
3. Minimum inhibitory concentration of *Carica papaya* L. Var. Wainamalo leaf, seed and bark extract on pathogenic *Staphylococcus aureus,* pathogenic *Escherichia coli* and *Candida albicans* does not vary.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1Traditional uses of medicinal materials

The growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries (Aruljothi *et al*., 2014). The use and history of herbs dates back to the time of early man, who had the crudest tools as his implements (Aruljothi *et al*., 2014). They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known also as the first art of treatment available to man (Kafaru, 1994).

Investigations into the antimicrobial activities of local medicinal plants and plant products have exposed plants as potential sources of therapeutic agents. Much research work has been done on medicinal plants which are employed in the treatment of various diseases caused by bacteria and fungi among others (Aruljothi *et al*., 2014). According to World Health Organization/WHO (2011), there is lack of scientific evidence to evaluate the safety and efficacy of traditional medicine. Therefore, there is need to screen medicinal plants for better understanding of their properties, safety and efficacy (Doughari *et al*., 2008) and also to validate their traditional uses and identify the active compounds (Demet *et al*., 2008). Infectious diseases are very common and have adverse effects to human beings. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases (Aruljothi *et al*., 2014). Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive (Ahmad and Beg, 2001). However, these complementary components give the plant as a whole a safety and

efficiency much superior to that of its isolated and pure active components (Ahmad and Beg, 2001).

The active components are normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. The parts known to contain the highest concentration of the principles are preferred for therapeutic purposes. They include leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds (Kafaru,1994). Some of the active principles singly or in combination inhibit greatly the life processes of microbes, especially the disease causing ones. This may be by binding their protein molecules, acting as chelating agents, altering their biochemical systems, preventing utilization of available interests to the microorganisms, or causing inflammation of microbial cells (Menza *et al.,* 2013).

The bitter taste, pungent and repulsive smell in some plants have been found to have repressive ability over the metabolic activities of a wide range of microorganisms There has been increased sustained interest in the production of plant based drugs for treatment of many diseases (Aruljothi *et al.*, 2014). Moreover, people are increasingly using herbal medicine to overcome mild or serious illnesses (Aruljothi *et al.,* 2014).

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternative because of their antimicrobial properties (Akujobi *et al.,* 2011). In recent years many people take antibiotics for the treatments of diseases whose microorganisms have become resistant to (Akujobi *et al.,* 2011). These synthetic antibiotics are however expensive especially to people from poor communities. The emergence and persistence of antimicrobial resistance is driven by varied factors including the

indiscriminate use of antibiotics and variable drug efficacy and presents a major threat to the control of infectious diseases (Omulo *et al.,* 2015).

For instance it has been reported that 68% of pregnant women aged between 23 –35 years have vaginal candidiasis (Omulo *et al.,* 2015). *Staphylococcus aureus* has also been reported to be resistant to antibiotics especially methicillin (Omulo *et al.*, 2015).

### 2.2 *Carica papaya*

Pawpaw is a giant herbaceous plant, resembling a tree but not woody. It belongs to the family Caricaceae, plant division; Tracheobionta, sub division; spermatophyte, class; magnoliopsida, order; brassicales, family; caricaceae, genus; *Carica* and Species; *papaya.* Several species have been used as remedy against a variety of diseases (Alabi *et al*., 2012). It belongs to the fruits and vegetable class; a native to the tropics of America, perhaps from Southern Mexico and neighboring Central America. It was first cultivated in Mexico several centuries before the emergence of Mesoamerican classical civilizations (Alabi *et al*., 2012).

Pawpaw grows to a height of 5-10 meters, with spirally arranged leaves confined to the top of the trunk. Lower trunk is conspicuously scarred where leaves and fruits are borne. Leaves are large, 50-70cm in diameter, deeply palmately lobed, with 7 lobes (Akujobi *et al.,* 2011).

Pawpaw plants are generally dioecius, with short stalked female (pistillate) flowers, which are 5- petalled, waxy and white, borne on separate plants from the male (staminate) flowers, which are borne on long panicles (up to 1.8 m). Plants may also bear hermaphrodite or perfect flowers, which have both pistil and stamens, or they may be monoecious, bearing separate male and female flowers on same plant. The fruit that develops varies in shape depending on the flower type. Fruits from female flowers are usually oval to round and smaller than the fruits that

develop perfect flower, which are cylindrical or club-shaped, up to 50 cm long and 20cm wide. The fruits, can weigh up to 9kg although common commercial cultivars generally, produce fruits that weigh 0.5 to 2.25 kg, and have a thin but tough waxy skin. Green fruits contain latex, which disappears as the fruit ripens to light or dark yellow. The flesh of the fruit varies from yellow to orange to red and is thick and juicy, with a central cavity filled with many small black seeds.

The plant grows best on deep, well drained soils with high organic matter. Most soil types are suitable but they don't grow well in heavy clay and water logged soils. Being a tropical plant, it grows best in warm- hot climate and an altitude below 2100m above the sea level with annual rainfall of about 1000mm which is well distributed. They are short live perennial trees whose economic life is about 4 years and they have a lifespan of up to 10 years, (Remberia and Wamoho, 2014).

The plant is of different varieties which include;

i. Mountain; grows at high altitudes with small fruits only suitable for jam and preservative.
ii. Honey dew; an Indian variety with medium height and produces oval juicy medium size fruits.
iii. Kiru; produces large fruits and high yield of papain.
iv. Solo; Hawaiian variety that produces round sweet fruits with uniform size shape.
v. Solo sunrise; produces smooth pear shaped fruits of high quality, weighing 400-500gms. It has a deep red flesh and very sweet. It is also resistant to ring spot disease.

vi. Sunset; a Hawaiian variety which is dwarf and high yielding. It produces a red flesh and very sweet. It has same characteristics as the solo variety.

vii. Solo sunset; produces red/ pink fruits and very sweet.

viii. Mexican red; rose fleshed pawpaw that's lighter in flavor.

ix. Kapoho; solo type with yellow- orange flesh color. The fruits are smaller than that of sunrise.

x. Waimanalo; Hawaiian variety that produces smooth, shiny fruits with short neck. The fruits have a yellow flesh which is thick, sweet and firm.

The plant is majorly grown in the Coastal, Lake and Eastern regions of Kenya. The whole plant has its own medicinal value. It is a rich source of three antioxidants; vitamin C, vitamin A and vitamin E, the minerals; Magnesium and potassium; the B vitamin pantothenic acid and folate and fiber (Aravind *et al*., 2013). It also contains flavonoids and carotenes. They are often used fresh in fruit salads and desserts, as well as prepared in juices and jams or dried. Some South East Asian dishes call for the unripe fruits to be cooked and used as vegetable according to Al Nayem *et al.* (2013).

The fruit is valued for its proteolytic enzymes including papain, is used like bromelain, a similar enzyme found in pineapple to treat sport injuries, other cases of trauma and allergies (Annie *et al*., 2004). Papain also aid in digestion and is used as a meat tenderizer. Papain has been used in medicine to treat ulcers and reduce skin adhesions following surgery and studies have also shown that it has antimicrobial properties (Aravind *et al.*, 2013). Papain is also used to clarify beer, prepare wool and silk for dyeing and to remove hair from hides' before tanning (Orchue *et al.*, 2013).

Carpaine, an alkaloid present in pawpaw, can be used as a heart depressant, amoebicide and diuretic. It has lycopene which is a cancer fighting enzyme (Orchue *et al.,* 2013). The leaves are made into tea as treatment for malaria (Orchue *et al.,* 2013).In some parts of Asia, the young leaves of the pawpaw are steamed and eaten like spinach (Orchue *et al.,* 2013). Some other benefits of the leaf include:

(i) Dengue fever- pawpaw leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver (Orchue *et al.,* 2013).

(ii) Cancer cell growth inhibition- Recent research on pawpaw leaf tea extract has demonstrated cancer cell growth inhibition. It appears to boost the production of key signaling molecules called Th1-type cytokines, which help regulate the immune system (Orchue *et al.,* 2013).

(iii) Antimalarial and antiplasmodial activity- pawpaw leaves are made into tea as a treatment for malaria (Orchue *et al.,* 2013).

(iv) Facilitate digestion- The leaves of the pawpaw plants contain chemical compounds of carpain, a substance which kills microorganisms that often interfere with the digestive function. Additional benefits of pawpaw leaves: As an acne medicine, increase appetite, ease menstrual pain and relieve nausea (Orchue *et al.,* 2013).

The medicinal folk use the leaves poultice onto nervous pains and elephantoid growths. The leaf is smoked for asthma relief in various remote areas (Al Nayem *et al.,* 2013). Japanese believe that eating pawpaw prevent rheumatism. Dietary pawpaw does reduce urine acidity in humans while the flowers have been used for jaundice. The young leaves and to lesser degree other parts contain carpain, an active bitter alkaloid which has a depressing action on the heart. The plant is strong amoebicide (Al Nayem *et al.,* 2013).

**Table 2.1. Phytoconstituents present in pawpaw plant parts**

| **Phytoconstituents** | *Carica papaya* **part** |
|---|---|
| Enzyme<br>Papain, chymopapain | Unripe fruit |
| Carotenoids<br>B carotene, crytoxanthin | Fruits |
| Carposide | Roots |
| Glucosinolates<br>Benzyl isothiocynate, pawpaw oil | Seeds |
| Minerals<br>Ca, K, Mg,Zn,Mn,Fe | Shoots, leaves |
| Monoterpernoids<br>Linalool,4-terpinol | Fruits |
| Flavonoids<br>Myricetin, kaemferol | Shoots |
| Alkaloids<br>Carpinine, carpaine, vitamin C and E | Leaves |

Source: Nwofia *et al.,* 2012: Vijay *et al.,* 2014.

### 2.3 *Escherichia coli*

*Escherichia coli* is a gram-negative, facultatively anaerobic, rod shaped bacterium that is common but not the most abundant inhabitant of the human intestine (Chima *et al*., 2016). It also lives in the intestine of many other animals, wild as well as domestic. They are part of the normal flora of the gut (Chima *et al*., 2016). Normally, *E. coli* does not cause disease although some strains frequently cause diarrhea, especially the drug resistant *E. coli*, and is the most common cause of urinary tract infections (Chima *et al*., 2016). One strain designated 015:H7 is

particularly virulent and has been responsible for several dangerous diseases outbreaks in people consuming contaminated foods (Chima *et al*., 2016).

The bacterium was first identified from stools of healthy children by T. Escheric (1857-1911) as enteropathogenic strain. Certain strains of *E. coli* which were antigenetically related to each other were linked to severe infantile gastroenteritis (Chima *et al*., 2016). More than 700 serotypes of *E. coli* have been identified (Chima *et al*., 2016); the different *E. coli* serotypes are distinguished by their O and H antigens on their bodies and flagella (Chima *et al*., 2016). *Escherichia coli* serotypes that are responsible for the numerous reports of contaminated foods and beverages are those that produce shiga toxin (stx) (Chima *et al*., 2016); so called since the toxin is virtually identical to that produced by *Shigella dysenteris* type also causing bloody diarrhea and hemolytic uremic syndrome in emerging nations (Chima *et al*., 2016).

*Escherichia coli* 0157:H7 is so dangerous and has had many deaths attributed to it, the bacteria can survive in many ecosystems and often requires as low as <50 to set up a house keeping in a victims intestinal tract and cause infection (Chima *et al*., 2016). *Escherichia coli* 0157:H7 was first recognized as a food borne pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis associated with consumption of contaminated hamburgers (Riley *et al*., 1983). Centre for Disease Control (CDC) has estimated that 85% of *E. coli* 0157:H7 infections are food borne in origin (Mead *et al.*, 1997). In fact, consumption of any food and beverage contaminated by animal especially cattle can result in contracting the disease. Foods that have been sources of contamination include, beef, sausages, unpasteurized milk and cheese, unpasteurized apple fruit and cider (Mead *et al.*, 1997). Other sources of food includes: orange juice, alfalfa and radish sprouts (Mead *et al.*, 1997), Lettuce, spinach and water.

Enteric *E. coli* is classified on the basis of serological characteristics and virulence properties (Wikipedia.com). The virotypes include:

i. Enterotoxigenic *E. coli* (ETEC) is the causative agent of diarrhea without fever in humans, pigs, sheep, goats, cattle, dogs and horses. The virotypes use fimbrial adhesions to bind entocype cells in the small intestines. ETEC; can produce two toxins: LT enterotoxin similar to cholera toxin in structure and function, ST enterotoxin causes cAMP accumulation in target cells and subsequent secretions of fluid and electrolytes into the intestinal lumen (Chima *et al*., 2016.)

ii. Enteropathogenic *E. coli* (EPEC) causes diarrhea in humans, rabbits, dogs, cats and horses. Like ETEC, EPEC also causes diarrhea but the molecular mechanisms of colonization and atiology are different. EPEC lacks fimbriae, ST and LT toxins, but they use intimin to bind to the host (Chima *et al*., 2016.) Adherence to the intestinal mucosa causes are arrangement of actin in the host cells, causing significant deformation (Chima *et al*., 2016).

iii. Enteroinvasive *E. coli* (EIEC) are found only in humans, the patient's shows profuse diarrhea and high fever (Chima *et al*., 2016). They are highly invasive and utilize protein adhesions for bonding to their host cells (Chima *et al*., 2016).

iv. Enterohemorrhagic *E. coli* (EHEC) infects humans, cattle and goats, the sole member is 01157:H7, causing bloody diarrhea and elicits no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment, is moderately invasive and possesses a phage- encoded shiga toxin that can elicit an intense inflammatory response (Chima *et al*., 2016).

v. Enteroaggregative *E. coli* (EAggEC) mainly found in humans (Wikipedia.com), they have fimbriae that aggregate tissue culture cells. It binds to host cells causing watery diarrhea without fever. They are noninvasive.

**2.4 *Staphylococcus aureus***

*Staphylococcus aureus* is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. It is positive for catalase and nitrate reduction. It is estimated that 20% of the human population are long term carriers of *S. aureus* which can be found as part of the normal skin flora and in anterior nerves of nasal passages (Oyedera, 2010). This bacterium appears as grape like clusters when viewed through a microscope and has large, round, golden yellow, colonies often with hemolysis when grown on blood agar plates (Ryan and Ray, 2004).

*Staphylococcus aureus* belongs to the domain; bacterium, kingdom; eubacteria, phylum; firmicutes, class; bacilli, order; bacillales, family; staphylococcaceae, genus; *Staphylococcus* and species; *aureus.* Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning (Oyedera, 2010). Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies (Cole *et al*., 2001). The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine (Cole *et al*., 2001).

*Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. This name was later appended to *Staphylococcus aureus* by Friedrich Julius Rosenbach who was credited by the official system of nomenclature at the time. It is estimated that 20% of the human population are long-term

carriers of *S. aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages (Cole *et al*., 2001).

*Staphylococcus aureus* is the most common species of staphylococcus to cause infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies (Ryan and Ray, 2004). *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections (Ryan and Ray, 2004). These infections and diseases are common among the HIV and AIDS infected people within the Lake Victoria region.

*Staphylococcus aureus* can infect tissues when the skin or mucosal barriers have been breached. *Staphylococcus aureus* can spread through contact with pus from an infected wound , skin – to – skin contact with an infected person by producing hyaluronidase that destroys tissues and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person (Ryan and Ray, 2004). *Staphylococcus aureus* is one of the causative agents of mastitis in dairy cows. Its large polysaccharide capsule protects the organism from recognition by the cow's immune system defenses (Ryan and Ray, 2004).

*Staphylococcus aureus* can be categorized into different types as follows;

**Methicillin Resistant** *Staphylococcus aureus* **(MRSA);** are strains of the *Staphylococcus aureus* that are resistant to the action of methicillin and related beta-lactam antibiotics

(e.g. penicillin, oxacillin, amoxicillin). MRSA have evolved resistance not only to beta-lactam antibiotics, but to several classes of antibiotics. Some MRSA are resistant to all but one or two antibiotics, including vancomycin (Ryan and Ray, 2004).

MRSA are often sub-categorized as Hospital-Associated MRSA (HA-MRSA) or Community-Associated MRSA (CA-MRSA), depending upon the circumstances of acquiring disease. Based on current data, these are distinct strains of the bacterial species.

i. HA-MRSA occurs most frequently among patients who undergo invasive medical procedures or who have weakened immune systems and are being treated in hospitals and healthcare facilities such as nursing homes and dialysis centers. MRSA in healthcare settings commonly causes serious and potentially life threatening infections, such as bloodstream infections, surgical site infections or pneumonia (Ryan and Ray, 2004).

ii. About 75 percent of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively. However, CA-MRSA strains display enhanced virulence, spread more rapidly and cause more severe illness than traditional HA-MRSA infections, and can affect vital organs leading to widespread infection (sepsis), toxic shock syndrome and pneumonia. It is not known why some healthy people develop CA-MRSA skin infections that are treatable whereas others infected with the same strain develop severe, fatal infections (Ryan and Ray, 2004). Studies have shown that rates of CA-MRSA infection are growing fast. In 1999, four children in Minnesota and North Dakota were reported to have died from fulminant CA-MRSA infections (Ryan and Ray, 2004). One study of children in south Texas found that cases of CA-MRSA increased 14-fold between 1999 and 2001. By 2007,

CA-MRSA was the most frequent cause of skin and soft-tissue infections seen in emergency departments in the United States (Ryan and Ray, 2004).

The first documented *S. aureus* strain with complete (≥16µg/ml) vancomycin resistance, (VRSA) was reported by in the United States in 2002, (Ryan and Ray, 2004). CA-MRSA (Community-acquired MRSA) has now emerged as an epidemic that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis (Namita and Mukesh, 2012). CA-MRSA infections now appear to be endemic in many urban regions and cause most CA- *S. aureus* infections (Namita and Mukesh, 2012).

Methicillin Resistance *S. aureus* (MRSA) is usually transmitted from patient to patient on the hands of health care workers by direct contact with a person who has a purulent lesion or is and asymptomatic carrier of the pathogenic strain (Namita and Mukesh, 2012). Colonized heath care workers with dermatitis or paronychia are especially likely to transmit MRSA to patients (Namita and Mukesh, 2012). Transmission by air bone route is much less likely to occur except in burn units where aerosolized MRSA may contaminate environmental surfaces (Namita and Mukesh, 2012). Hospital admission for serious MRSA infection is acceptable medical practice because the patient will usually require intravenous antibiotic therapy with vancomycin (Marthe *et al*., 2014). An acute care setting is needed because vancomycin can have adverse reactions, such as ototoxicity, and in some instances produce nephrotoxicity (Marthe *et al*., 2014).

Several studies have shown that the prevalence of community- associated MRSA varies geographically. Global outbreaks have been reported from the United States to Saudi Arabia to New Zealand (Marthe *et al*., 2014). In a population- based surveillance study of 3 communities

in 2001-2002, researchers from CDC reported 1,647 cases of community- associated MRSA infection from Atlanta, Minnesota and Baltimore.

### 2.5 *Candida albicans*

*Candida albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Ryan and Ray, 2004; Hube, 2007).

Although often referred as "dimorphic", *C. albicans* is polyphenic. When cultured in standard yeast laboratory medium, it grows as ovoid "yeast" cells. However mild environmental changes in temperature and pH can result in morphological shift to pseudo-hyphal growth (Hube, 2007). Pseudo-hyphae share many similarities with yeast cells, but their role during candidiasis remains unknown.

*Candida albicans* belongs to the Kingdom: Fungi, division: Ascomycota class: Saccharomycetes order: Saccharomycetales family: Saccharomycetaceae genus: *Candida* and species: *albicans*. When *Candida albicans* are grown in a medium that mimics the physiological environment of a human host, they grow as "true" hyphae. Its ability to form hyphae has been proposed as virulence factors as these structures are often observed invading tissue and stains that are unable to form hyphae are defective causing infection. *Candida albicans* can also form chlamydospores, the function of which remains unknown (Berman and Sudbery, 2002; Staib and Morschhäuser, 2007). The fungus is normally present on the skin and the mucous membranes such as the vagina, mouth or rectum. The fungus can also travel through the blood stream and affect the throat, intestines, and heart valves.

*Candida albicans* becomes an infection agent when there is alteration of the normal flora of the body allowing its growth. When growth persists it weakens the intestinal walls, penetrating

through into the blood stream and releasing its toxic byproducts throughout the body as they spread; these toxins cause damage to body tissues and organs making them immune susceptible (Lederber, 2004). *Candida albicans* causes thrush, a type of candidiasis that develops in the mouth or throat especially in young children and yeast infection, which develops in the vagina.

Antifungal drug resistance has been studied most extensively with the diploid pathogenic yeast *Candida albicans*. *Candida albicans* is ubiquitous commensal, residing on the mucosal surfaces of the mouth, digestive tract, or genitourinary system of 15%-60% of healthy humans, depending on the sample group (Brij *et al.,* 2015). *Candida albicans* is also a good example of an opportunistic pathogen, causing both superficial infections and invasive fungal disease in immune compromised individuals (Brij *et al.,* 2015).

Species of *Candida* are the fourth most common cause of nosocomical blood stream infections, with *C. albicans* being the most commonly encountered (Brij *et al.,* 2015). *Candida albicans* has been the fungal pathogen of choice for studying drug resistance because it is more easily manipulated and contained than other medically important pathogens, such as *Cryptococcus neoformans, Aspergillus fumigates and Histoplasma capsulatum* (Brij *et al.,* 2015). *Candida albicans* is wet yeast, classified as a relatively low risk to research personnel. In common with other countries in the American Biological Safety Association Health Canada considers *C. albicans* to be a risk group agent, unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment.

As a research system, *C. albicans* also offers a range of molecular-genetic tools (Brij *et al.,* 2015) a complete genome sequence (Brij *et al.,* 2015) and a sufficiently close phylogenetic relationship to the model yeast system, *Saccharomyces cerevisae,* that many genes and

pathways have highly similar counterparts in both yeasts. Unfortunately, *C. albicans* is, as yet, not amenable to conventional genetic analysis. Although *C. albicans* cells rendered homo- or hemizygous for mating-type- like genes are able to mate, meiosis has not been observed (Sarah, 1999). This disadvantage can be overcome with complementary studies using *S. cerevisiae* as a genetic stand- in. it is notable that some of the key studies of drug resistance (Ogunjobi and Ogunjobi, 2011). Dimorphism and virulence (Ogunjobi and Ogunjobi, 2011) have exploited this synergy. It's increasingly becoming resistant to first line and second line antifungal medications especially fluconazole, ampicillin and echinocandins. Approximately 7% of all candida infection isolates tested by CDC are resistant to fluconazole (Ogunjobi and Ogunjobi, 2011).

There has been a lot of research work on *C. papaya* antimicrobial effect on several microorganisms including *E. coli, S. aureus* and *C. albicans* majorly in African countries including Nigeria, Gabon, and Cameroon but there is very little work which has been done on *C. papaya* plant varieties especially growing in Kenya. The World Health Organization underscores the importance of herbal plants as best source of a variety of drugs and promotes further scientific investigations unto determination of properties, safety and efficacy of plant drugs. There is very little documented work on *C. papaya* Var. Wainamalo phytochemicals screening and its antimicrobial effect on *E. coli, S. aureus* and *C. albicans,* which are the most common pathogenic microorganisms infecting many HIV-AIDS victims around the lake Victoria region.

### 2.6 Antimicrobial activity of plant extracts

An antimicrobial agent is defined as chemical substances that kills or inhibit the growth of microorganisms. It may either be a synthetic chemical or a natural product (Oyedera, 2010). Antimicrobial agent may be grouped into two namely cidal or static. A bacteriocidal agent is one

that kills bacteria at all concentrations while a bacteriostatic agent is one that only inhibits the growth and development of bacteria (Oyedera, 2010).

Since the discovery of penicillin in 1928, antibiotics and other antimicrobial therapies have been used to control both old and new emerging pathogens, resulting in global improvements in disease outcomes and increments in life expectancy (Omulo *et al.,* 2015). However, the rapid emergence of antimicrobial resistance (AMR) by microbial pathogens threatens to reverse the public health gains made since widespread use of antibiotics was adopted. AMR is not a recent phenomenon (Omulo *et al.,* 2015) and with decreasing options for and production of newer antibiotics the control of diseases has become a challenge, particularly in low and middle income countries where infectious diseases, poverty and malnutrition are endemic.

The emergence of AMR is a complex process often involving the interplay of human, environmental and pathogen-related factors (Omulo *et al.,* 2015). In sub-Saharan Africa, the endemicity of acute respiratory infections, diarrheal diseases, HIV and AIDS, tuberculosis, malaria and helminthic infections has increased the demand for antimicrobial therapies both for prophylaxis and treatment (Omulo *et al.,* 2015). Further, shortfalls in the healthcare environment ranging from limited diagnostic capacity and resources, unregulated access to antibiotics, constrained access to and poor health facilities (Omulo *et al.,* 2015).

### 2.7 Challenge for antibiotic resistance

Antibiotics are the main basis for therapy provision of bacterial and fungal infections (Khan *et al*., 2009). Since their discovery, it was believed that this would eventually lead to the eradication of infectious diseases. On the contrary, overuse and indiscriminate use of antibiotics has led to the emergence of multi-drug resistant strains of several groups of microorganisms

(Harbottle *et al*., 2006; Khan *et al*., 2009; Wagate *et al*., 2009), and this has become a global concern (Parekh and Chanda, 2007). This emergence of multidrug-resistant pathogens threatens the clinical efficacy of many existing antibiotics (Parekh and Chanda, 2007). *Escherichia coli, Candida albicans, Staphylococcus aureus* and many other ß-lactamase producers have emerged and become a major therapeutic problem in the world today. Multi-drug resistant strains of *E. coli, C. albicans* and *S. aureus* are widely distributed in hospitals (Parekh and Chanda, 2007.)

These strains are increasingly being isolated from community acquired infections (Parekh and Chanda, 2007). *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis (Parekh and Chanda, 2007). This rapid global spread of resistant clinical isolates implies that the need to find new antimicrobial agents is of paramount importance. In addition, the life expectancy of antimicrobial agents remains a challenge to researchers. Widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al*., 2002). Hence, this has led to an increasingly shift of attention by researchers to herbal products in search of new leads to develop better drugs against multiple drug resistant pathogenic microbe strains of clinical origin (Parekh and Chanda, 2007).

The present day use of the term antibiotics was proposed by Naksman in 1945 as those chemical substances of microbial origin which in small amounts exert antimicrobial activity in microbes (Okonko *et al*., 2008). Antibiotics are usually of microbial origin but some have come from higher forms of life and chemotherapeutic agents made synthetically. Their selective toxicity means a low toxicity for host cells and high toxicity for parasites (Okonko *et al*., 2008). For an antibiotic to be effective, it should exhibit selective toxicity and have a high therapeutic index. High therapeutic index implies a high ratio of maximum dose at which antibiotics can be

tolerated to a minimum dose required to cure infections. Such antibiotics do not eliminate the normal microbial flora of the host to avoid an upset of the balance of nature and prevent the development of resistant form of these pathogens (Okonko *et al*., 2008).

In the last few decades, antibiotics have been increasingly exploited by workers in a number of disciplines. For example, their usefulness in agriculture as plant protecting agents or for the promotion of animal growth and metabolic activities; in food industries as a preservative and in basic biochemical research as specific inhibitors of metabolic pathways ( processes) cannot be over emphasized (Florey, 1988). The major groups of antibiotics consist of families of chemically related substances with varying properties, some of which result from the natural manipulations of producing microbes and others from chemical alterations of the products of biosynthesis. The indiscriminate usage of these antibiotics influences its efficacy, resulting in resistance (Okonko *et al*., 2008). It also leads to the growth of abnormal gut flora which inhibits proper digestion and assimilation of food. This undigested food putrefies and produces toxins that lead to the growth of yeast, fungal, bacterial and parasitic infections that damage the gut tissues (Okonko *et al*., 2008). Amongst the more important beneficial bacterial destroyed by this indiscriminate usage include *Lactobacillus, Acidophilus* and *Bifidobacterium bititus.* It also affect many nutrients particularly the ones needed by the immune system to fight infection such as vitamin A and C (Okonko *et al*., 2008.)

The sources in which antibiotics can be obtained include; microorganisms, synthesis and semi synthesis. Thus, antibiotics can be obtained from the culture extracts and filtrates of fungi, for example, penicillin and cephalosporins, bacteria like *Streptomyces* spp., *bacillus* spp., example, rifampicin, aminoglycoside, chloramphenicol, erythromycin, tetracyclines. As the predominance of either the gram-positive or gram negative bacteria isolates is influenced by

geographical location and changes in time (Nwadioha *et al.,* 2010). Most bacteria exhibit remarkable versatility in their behavior towards antibiotics and its capacity to produce human diseases had not diminished even with the introduction of antibiotics (Obiazi *et al*., 2007).

A number of literatures indicate a gradual increase in the emergence of antibiotic- resistant microorganism in hospitals (Obiazi *et al*., 2007). The changing patterns in the etiological agents of clinical pathogens and their sensitivities to commonly prescribe against the antibiotics are reported (Abubakar, 2009). High susceptibility of most pathogens to ampiclox and ciprofloaxin is an indication of effectiveness of the antibiotic against the bacteria (Doughari *et al.,* 2007; Okonko *et al.,* 2009 a, b). Multi-drug resistance to gentamicin, rifampicin and tetracycline in equal magnitude *in vitro* has been reported and, as such, these antimicrobials may not be suitable for treating cases of nosocomial or community acquired infection (Okonko *et al.,* 2009 a, b). In a study by Jamshidi *et al.* (2009), Resistance to ciprofloxacin and gentamicin by *Pseudomonas spp., Klabsiella spp.* and *Escherichia coli* was reported. Similar finding were also recorded in a study on the microbial resistance pattern among intensive care unit patients (Jamshidi *et al.,* 2009).

*Staphylococcus aureus* resistance to cloxacillin, penicillin, ampicillin and tetracycline was reported by Obiazi *et al.* (2007) in Benin City, Nigeria. Although, outbreaks of *S. aureus* resistant to beta-lactam antibiotics have been frequently associated with devastating nosocomial infections (Depardieu *et al.,* 2007), marked resistance to ampiclox which is a beta-lactam antibiotic by *S. aureus* has not been reported in recent studies.(Obiazi *et al.,* 2007).

A previous study combining the data from 25 United Kingdom hospitals has shown that this microorganism is resistant to ofloxacin and ciprofloxacin in 59 and 62% of the cases,

respectively (Jamshidi *et al.,* 2009). In their study, a change in the routine interventions used for empirical therapy of *S. aureus* yielded a decline in resistance of this species against ciprofloxacin from 91.3 to 78.6%, suggesting that a modification of routine antimicrobial treatments can effectively alter the pattern of resistance of this pathogen to these drugs (Jamshidi *et al.,* 2009). Resistance of intensive care unit (ICU) –acquired pathogens against ciprofloxacin can be attributed to its high usage in inpatient and outpatient settings (Jamshidi *et al.,* 2009). *Escherichia coli* sensitivity to ciprofloxacin and gentamycin was also reported by Nwadioha *et al*. (2010). However, gentamicin, erythromycin and tetracycline among others with relatively higher susceptibility can be used for management of clinical conditions in our locality (Obiazi *et al*., 2007).

The emergence of antibiotics resistance in the management of most infections are serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of substandard drugs and spurious drugs of questionable quality in circulation (Nwadioha *et al*., 2010). Much of the current discourse on infectious diseases and drug resistance as it affects sub-Saharan Africa is limited to the pressing problems associated with emerging and reemerging resistant organisms (Nwadioha *et al*., 2010).

Resistance, however, equally compromises the management of acute respiratory infections, sexually transmitted diseases and diseases spread by the fecal-oral route, such as typhoid fever, cholera, dysentery and other diarrheal diseases (Okeke *et al*., 2007; Okonko *et al.,* 2009 b). The negative health and socioeconomic impact of indiscriminate usage of antibiotics and of fake drugs cannot be over emphasized. These poor quality or substandard drugs could be responsible for the increasing number of resistant strains of microorganisms in the country. The general

concept here is that the active ingredients in these antibiotics may be less than what is indicated on the drugs and it calls for serious concern, because the quality of a drug is dependent on the correctness of its active ingredient (Okonko *et al.,* 2008). The potencies of these antibiotics could also be affected by deterioration of the active ingredients due to expiration of the drugs and or storage conditions (Okonko *et al.,* 2008). Further implication is that many bacterial and parasitic diseases that could, until recently, be treated with inexpensive antimicrobial agents, has recently been made more expensive and less successful by the emergence and spread of resistant organisms (Okeke *et al*., 2007; Okonko *et al.,* 2009 a, b).

Bacterial resistance to beta-lactam antibiotics is primarily due to the production of beta-lactam ring of the antibiotics rendering them inactive (Okonko *et al.,* 2008). Resistance by microorganisms to antibiotics may be an indication of the presence of resistance factors such as R plasmids and enzymes such as beta-lactamase and of recent, extended beta- lactamase (ESBL) (Doughari *et al.,* 2007). The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomical infections caused by drug resistant microbes (Okonko *et al.,* 2008).

Multi drug resistance and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge. Also, the rising prevalence of drug resistance such as Methicillin Resistant *S. aureus* worldwide mandates selective susceptibility testing and epidemiological investigations during outbreaks (Okonko *et al.,* 2008). However, strategies for addressing antimicrobial drug resistance stress the need for new drugs (Okonko *et al.,* 2008) and yet the rate of drug development is in decline (Okonko *et al.,* 2008.) Knowledge of etiological agents of infections and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing

policies. The changing spectrum of microorganisms causing infections and the emerging resistance to many of the older and cheaper antibacterial agents require continuous monitoring (Abubakar, 2009). Regular monitoring of the pattern of resistance of common pathogens in the hospitals and the assessment of the efficacies, potencies and qualities of antibiotics sold in a particular area is critical in planning the best routines for empirical treatment of infectious patients.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study area

The study was conducted at Maseno University in the Botany Laboratory. Maseno University is located along Kisumu – Busia highway 20 kilometers Northwest of Kisumu and lies on latitude $0^{o}$ 00’ 60.00’’N and latitude $34^{0}$35’59.99’’E.

## 3.2 Collection, preparation and preservation of plant extracts

Plant materials of *C. papaya* L. Var. Wainamalo which included fruits, leaves and bark were collected from a farm in Kiboswa, Kisumu County and were taken to Maseno University, Botany Laboratory for processing.

### 3.2.1 Seeds

Seeds were extracted from ripe pawpaw fruits (plate 1). The fruits were cut open to extract the seeds which were washed with tap water, rinsed in sterile distilled water and dried for 30 days at room temperature. The dry seeds were then ground to powder using a Ramtons grinder as shown in plate 2.

Plate 1: Pawpaw tree with ripe (yellow) and unripe (green) fruits

Plate 2: *Carica papaya* seed powder.

### 3.2.2 Leaves

Green leaves of *C. papaya* were collected from a farm in Kiboswa. They were washed in tap water, rinsed in sterile distilled water and dried under room temperature for 20 days. They were ground into a green powder using a Ramtons grinder (Plate 3).

Plate 3: *Carica papaya* leaves powder.

### 3.2.3 Bark

Diseases free bark of pawpaw was cut from a tree using a sharp kitchen knife. They were washed in tap water and rinsed in sterile distilled water then dried under room temperature for 30 days. They were then ground into brown powder using a Ramtons grinder (Plate 4).

Plate 4: *Carica papaya* bark powder.

### 3.3 Extractions using organic solvents

### 3.3.1 Ethanol extraction of Seed

One hundred grams powder of dried seeds was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical

flasks. Five hundred mls of 95% ethanol was poured into the conical flask containing the seed powder and stirred. The black mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The resulting filtrate was light yellow in color. The filtrate was concentrated with a rotary evaporator at $79^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al.*, 2012). The resulting concentrate was golden brown in color.

### 3.3.2 Water extraction of seeds

One hundred grams of powdered dried seeds were weighed using an electronic weighing balance (Denver instrument, Model XL-31000).The powder was transferred into 500mls glass conical flask. Two hundred millimeters distilled water was poured into the conical flask containing the powder and stirred. The black mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The resulting filtrate was black in color.

### 3.3.3 Acetone extraction of seeds

One hundred grams of powdered dried leaves were weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five millimeters of 95% acetone was poured into the conical flask containing the seeds powder and stirred. The black mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The resulting filtrate was light yellow in color. The filtrate was concentrated with a rotary evaporator at $45^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al.*, 2012).The resulting concentrate was a golden brown in color.

### 3.3.4 Ethanol extraction of leaves

One hundred grams powder of dried leaves was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five hundred millimeters of 95% ethanol was poured into the conical flask containing the leaves powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The resulting filtrate was dark green in color. The filtrate was concentrated with a rotary evaporator at $79^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al*., 2012).

### 3.3.5 Water extraction of leaves

One hundred grams powder of dried leaves was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Two hundred millimeters of distilled water was poured into the conical flask containing the leaf powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1.

### 3.3.6 Acetone extraction of leaves

One hundred grams of powdered dried leaves were weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five millimeters of 95% acetone was poured into the conical flask containing the leaves powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1.The filtrate was concentrated with a rotary evaporator at $45^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al.,* 2012). The resulting concentrate was dark green in color.

### 3.3.7 Ethanol extraction of bark

One hundred grams of powdered dried bark was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five hundred millimeters of 95% ethanol was measured and poured onto the conical flask containing the bark powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The filtrate was concentrated with a rotary evaporator at $79^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al.,* 2012).

### 3.3.8 Acetone extraction of bark

One hundred grams powder of dried bark was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five hundred millimeters of 95% acetone was poured into the conical flask containing the bark powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1. The filtrate was concentrated with a rotary evaporator at $45^0$C and stored in the refrigerator at $4^0$C until required for use (Okunola *et al.,* 2012).

### 3.3.9 Water extraction of bark

One hundred grams powder of dried bark was weighed using an electronic weighing balance (Denver instrument, Model XL-31000). The powder was transferred into 500mls glass conical flask. Five hundred millimeters of distilled water was poured into the conical flask containing the bark powder and stirred. The mixture was allowed to stand for 24 hours after which it was decanted and filtered through a Whatman filter paper No 1.

### 3.4 Test organisms

The test organisms that were used were human pathogenic organisms of clinical origin. These isolates included one strain of Gram negative bacteria *Escherichia coli*, gram positive bacteria *Staphylococcus aureus* and a fungus *Candida albicans.* They were obtained from Centre for Disease Control (KEMRI/CDC) Kisian, Kisumu and were maintained on Mueller Hinton Agar (Oxoid, UK) medium. They were kept as stock cultures in the refrigerator set at $4^0$C.

### 3.5 Phytochemical compounds extraction and screening

#### 3.5.1 Extraction

Twenty five grams of dried leaves powder were extracted in soxhlet apparatus by using 25ml of solvent having polarity of ethanol for 48hrs and then concentrated by evaporation. The procedure was repeated for seeds and bark powder. These prepared extracts were used for phytochemical screening.

#### 3.5.2 Phytochemical screening

Phytochemical screening was done according to Musyimi *et al.* (2008), Mibei *et al*. (2012) and Akinyemi *et al*. (2005) as explained below :-

##### 3.5.2.1 Determination of alkaloids

To 2ml of seed extracts, 2ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicated the presence of alkaloids. The procedure was repeated for leaf and bark extracts.

##### 3.5.2.2 Determination of flavonoids

Five milliliters of dilute ammonia solution was added to 2 ml of the aqueous filtrate of seed extract followed by addition of concentrated $H_2SO_4$. A yellow coloration indicated the presence of flavonoids. The procedure was repeated for leaf and bark extracts.

### 3.5.2.3 Determination of tannin

Tannin was determined by the Folin-Denis colorimetric method described by Harborne, (1998). 0.5 g of dried powder of seed was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. Three drops of 0.1% ferric chloride was added. A blue-black coloration indicated the presence of tannins (Akinyemi *et al*., 2005). The procedure was repeated for leaf and bark extracts.

### 3.5.2.4 Determination of phenols

Ferric chloride test was carried out where the seed extract was diluted with 5ml distilled water. To this, three drops of neutral 5% Ferric chloride solution was added. A blue-black color indicated the presence of phenolic compounds (Mibei *et al*., 2012). The procedure was repeated for leaf and bark extracts.

### 3.5.2.5 Determination of saponins

Two grams of seed powder was boiled in 20ml of distilled water in a water bath and filtered. Ten milliliters of the filtrate was mixed with 5ml of distilled water and shaken vigorously to form a stable froth (Musyimi *et al*., 2008). The procedure was repeated for leaf and bark extracts.

### 3.5.2.6 Test for terpernoids

Five milliliters of seed extract was mixed with 2ml of chloroform and concentrated $H_2SO_4$ was carefully added to form a layer. A reddish brown coloration that formed at the

interface indicated presence of terpernoids (Mibei *et al*., 2012). The procedure was repeated for leaf and bark extracts.

#### 3.5.2.7 Test for anthraquinones

Two grams of seed powder was boiled with 10ml 10% HCl for 3 minutes, then filtered and allowed to cool. This was then partitioned against equal volume of chloroform. Formation of rose-pink color upon addition 2 ml of 10% aqueous ammonium solution indicated the presence of anthraquinones (Mibei *et al*., 2012). The procedure was repeated for leaf and bark extracts.

#### 3.5.2.8 Test for Cardiac glycosides

Five ml of seed extract was treated with 2ml of glacial acetic acid containing a drop of $FeCl_3$ solution. This was then underplayed with 1ml concentrated $H_2SO_4$. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides (Musyimi *et al*., 2008). The procedure was repeated for leaf and bark extracts.

#### 3.5.2.9 Test for anthocyanins

To 5ml of seed extract 2ml of concentrated $H_2SO_4$ was added, yellowish orange color confirmed the presence of anthocyanins (Musyimi *et al*., 2008). The procedure was repeated for leaf and bark extracts.

### 3.6 Determination of the effect of *C. papaya* L. Var. Wainamalo seed, leaf and bark extracts on growth of *C. albicans, E. coli* and *S. aureus*.

The disc diffusion method on Mueller Hinton agar (Yahaya *et al.*, 2017) was used to determine the antibacterial activity of the plant extracts. An overnight culture of the bacteria (*E. coli* and *S. aureus*) and fungi *Candida albicans* was diluted to 105 cells/ml using a spectrophotometer at a wavelength of 625nm. One milliliter of the bacterial suspension was introduced into sterile petri

dishes and 20 ml of Mueller - Hinton agar at 40ºC was poured into the inoculated dishes. The dishes were allowed to cool and solidify. A sterile filter circular disc, 8mm in diameter was cut from Whatman No.1 filter paper using a paper punch and dipped in a known concentration of 25%, 50%, 75% and 100% of the extracts for about 2 minutes, then were gently transferred to the center of the inoculated agar media. Petri dishes inoculated with bacteria and fungi were incubated for 24hrs at $37^0$C and $25^0$C, respectively. The diameter of inhibition zones were measured using 12.5 cm vernier calipers. This was carried out in triplicates.

**3.7 Determination of Minimum Inhibitory Concentration (MIC)**

The MIC helps to measure more exactly the concentration of the extract necessary to inhibit growth of standardized inoculum under defined conditions (Anibijuwon *et al*., 2009). The MIC of the extracts was determined by using the broth dilution (Anibijuwon *et al*., 2009). Muller-Hinton Broth was made and sterilized using autoclave. Serial dilutions of the seed leaf and bark extracts in liquid medium were prepared. 1.0 ml of the prepared broth was dispensed into the test tubes labeled from 1 to 4 using sterile syringe and needle. A stock solution containing 100mg/ml of the seed leaf and bark extracts was prepared. Then, 1.0 ml of the solution was dispensed into the tube 1. Subsequently, from tube 1, solution was serially transferred until 4 of 1.0 ml of the solution were discarded from it. An overnight culture of each of the test isolates was prepared in sterile nutrient broth. 1 ml inoculum was transferred into each tube from tube 1 to tube 4. The final concentration of the extract in each of the test tubes numbered after dilution 100, 50, 25, 12.5 mg/ml was incubated at 37°C and 25ºC for 24 hrs and examined for growth.

To measure the MIC values, various concentrations of the stock, 12.5 25, 50 and 100 mg/ml were assayed against the test bacteria and fungus. The minimum inhibitory concentration was

defined as the lowest concentration able to completely inhibit any visible microorganism growth after overnight incubation with media (Prescott *et al*., 1999: Yahaya *et al*., 2017).

### 3.8 Data analysis

Data obtained for this study was subjected to analysis of variance (ANOVA) using SAS statistical package (version 16). Means were separated and compared at ($p<0.05$) using the least significant difference (LSD) at 95% level of confidence.

# CHAPTER FOUR

# RESULTS

## 4.1 Phytochemical Analysis

The phytochemical screening of *Carica papaya* plant materials were carried out and the results recorded. All the tested plant materials (leaf, seed and bark) indicated the presence of alkaloids, flavonoids, tannins, terpernoids, anthraquinones, phenolic compounds and saponins as shown in table 4.1 and plates 5-10. Anthraquinones were absent in seeds and bark extracts (Table 4.1).

**Table 4.1: Secondary metabolites in plant extracts of *C. papaya* L. Var. Wainamalo.**

| | **Presence or absence in plant part** | | |
|---|---|---|---|
| **Secondary metabolite** | **Leaves** | **Seeds** | **Bark** |
| Alkaloids | + | + | + |
| Flavonoids | + | + | + |
| Tannins | + | + | + |
| Phenols | + | + | + |
| Saponins | + | + | + |
| Anthraquinones | + | - | - |
| Glycosides | + | + | + |
| Anthocyanins | + | + | + |
| Terpernoids | + | + | + |

+ Present

- Absent

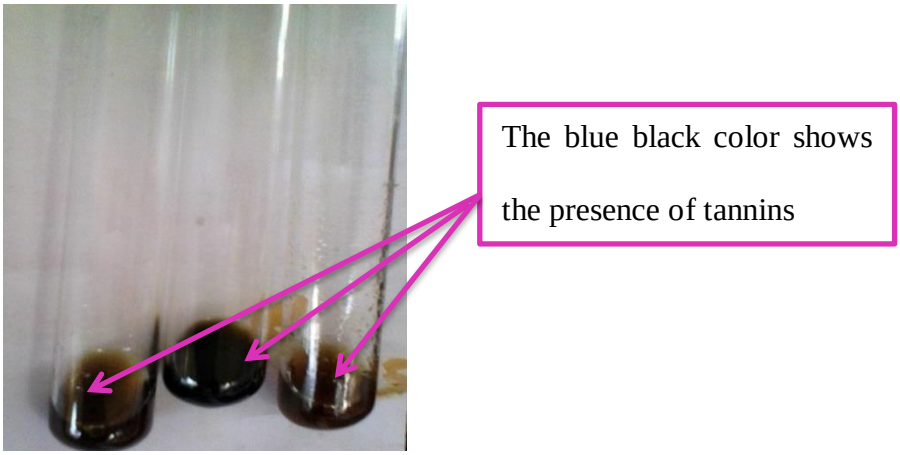



Plate 5: Phytochemical test for tannins in leaves, seeds and bark.

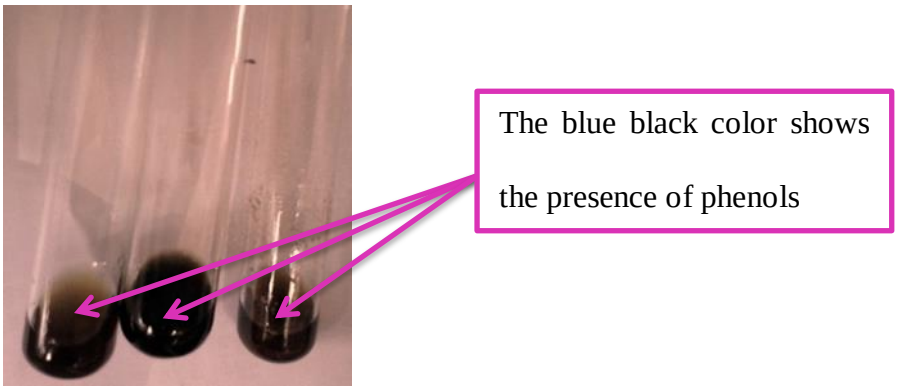



Plate 6: Phytochemical test for phenols in leaves seeds and bark.

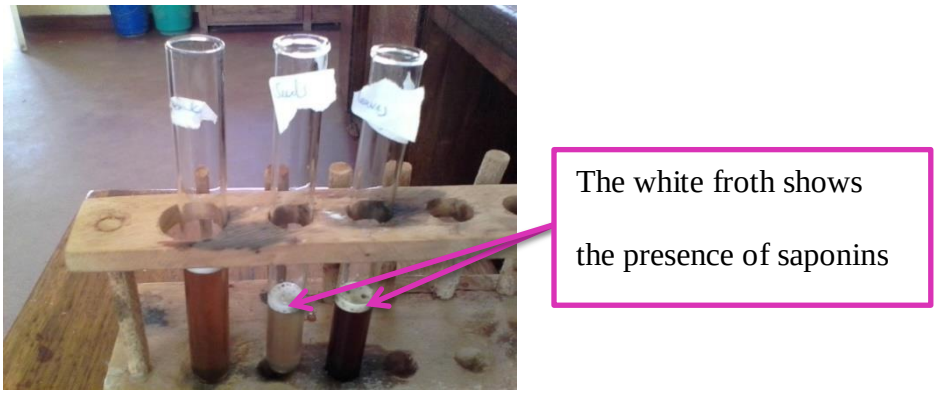



Plate 7: Phytochemical test for saponins in bark, seeds and leaves.

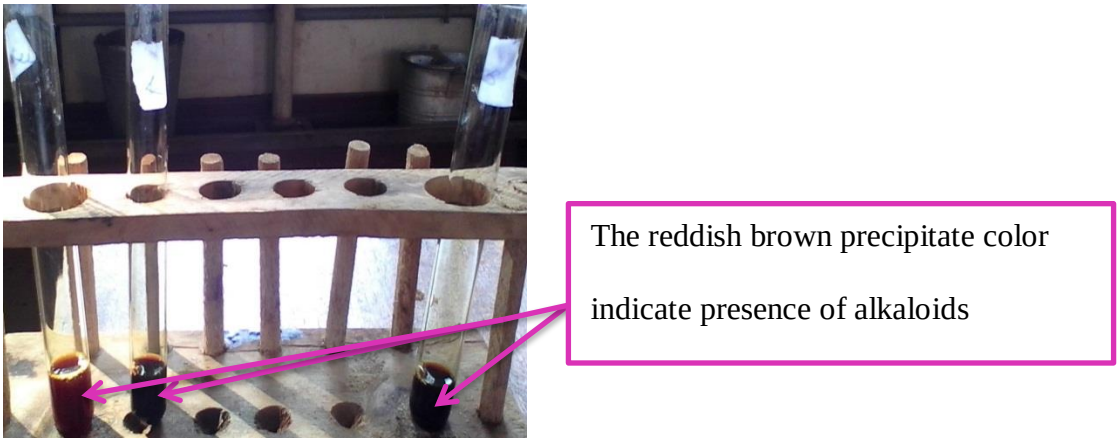



Plate 8: Phytochemical test for alkaloids in bark, seeds and leaves.

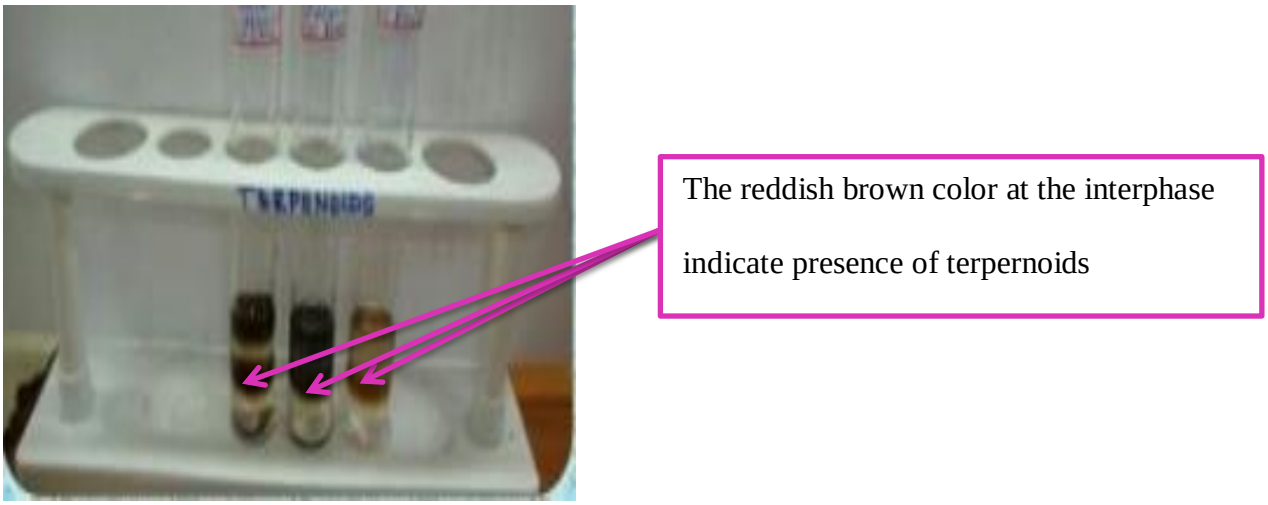



Plate 9: Phytochemical test for terpernoids in bark, seeds and leaves.

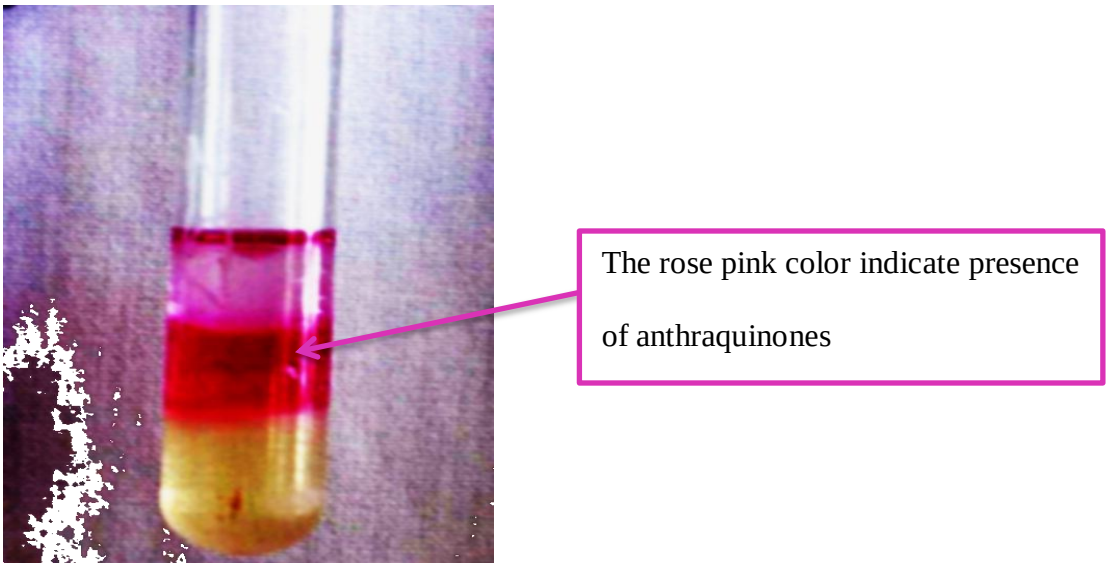



Plate 10: Phytochemical test for anthraquinones in leaves.

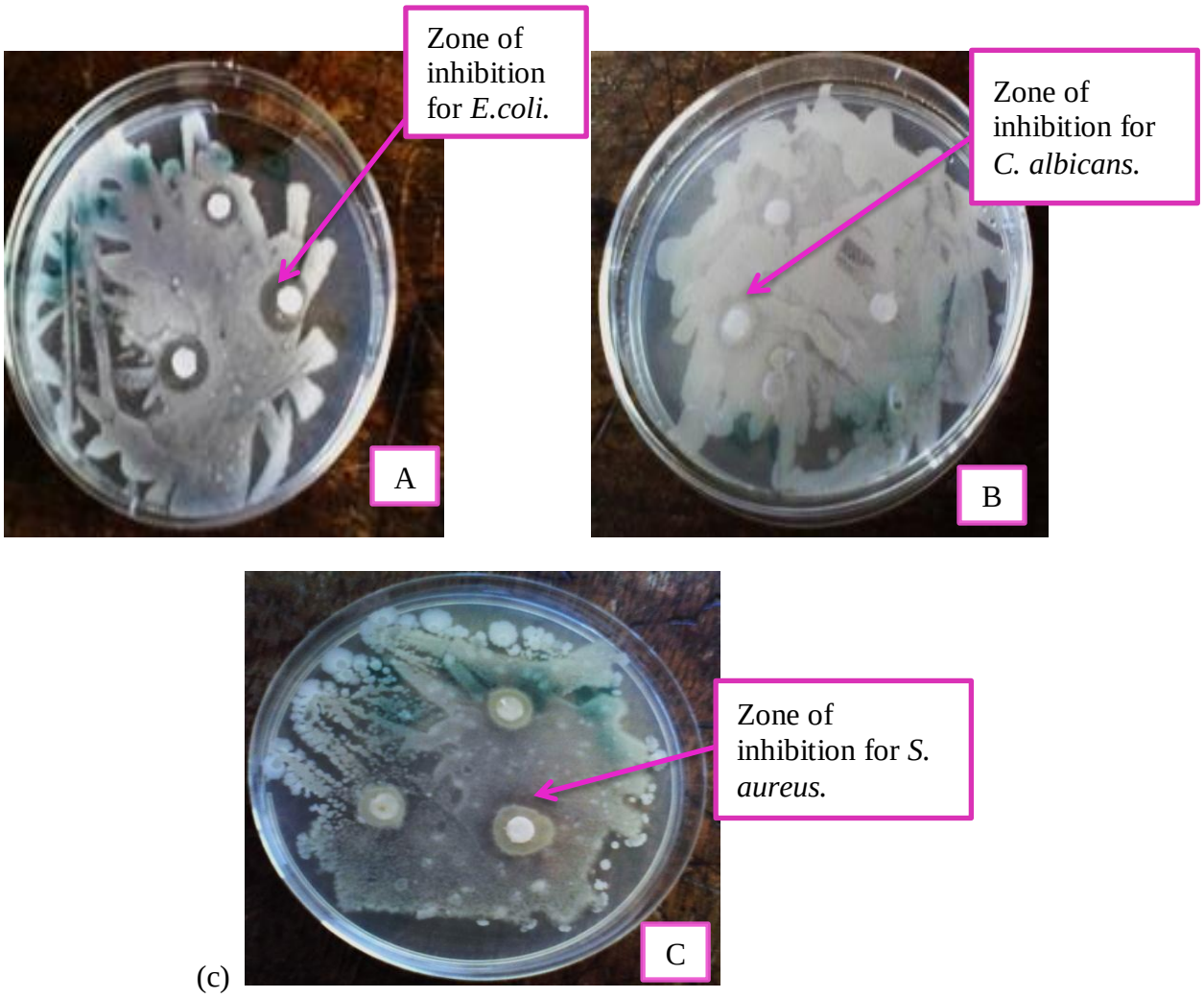
### 4.2 Effect of *C. papaya* L. Var. Wainamalo seed, leaf and bark extracts on growth of *C. albicans, E. coli* and *S. aureus*.

Table 4.2, plates 11, 12 and 13 shows the results of effect of *C. papaya* bark on growth of *E. coli, C. albicans* and *S. aureus.* The highest zone of inhibition was demonstrated against *S. aureus* (9.82mm) by ethanol extracts of bark. The lowest zone of inhibition was demonstrated against *C. albicans* (0.89mm) by water extracts of bark. There was no inhibition exhibited on *C. albicans* by acetone extracts of bark. There was significant difference in growth inhibition among the extracts at $P \leq 0.05$.

**Table 4.2: Diameter of zone of inhibition (mm) exhibited by *C. papaya* L. Var. Wainamalo bark extracts against *C. albicans, E. coli and S. aureus.***

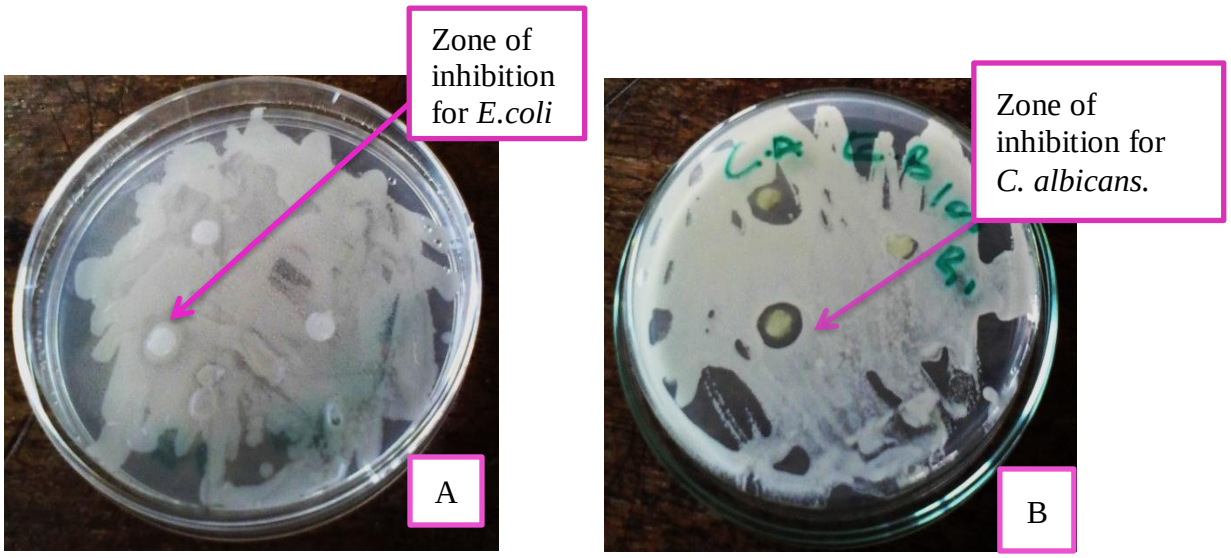
| Test Microorganism | Bark | | |
|---|---|---|---|
| | **Ethanol** | **Water** | **Acetone** |
| *E. coli* | 7.16 a | 5.96 a | 6.29 a |
| *C. albicans* | 8.84 b | 0.89 b | 0 b |
| *S. aureus* | 9.82 c | 3.80 c | 5.13 c |
| **LSD** at **P≤0.05** | **0.35** | | |

Data presented are the means of three replicates. Means with the same letter down the same column are not significantly different at P≤0.05.




(c)

Plate 11; Zone of inhibition for water extracts of bark on *E. coli* A, *C. albicans* B and *S. aureus* C.



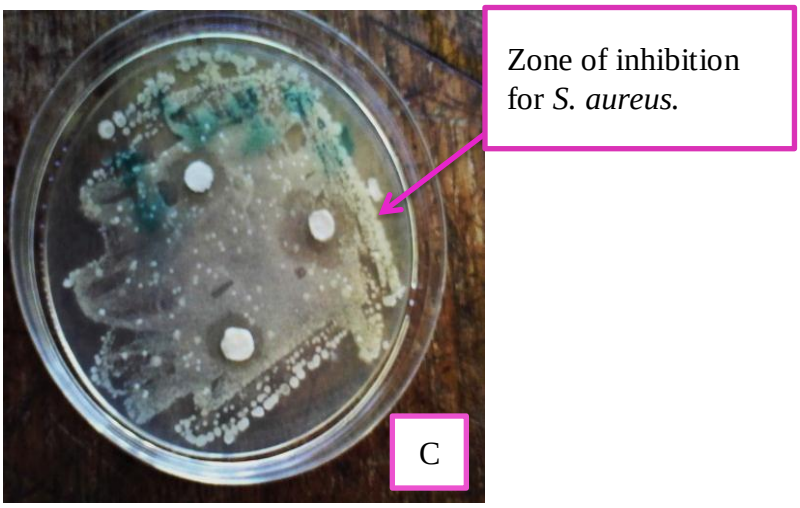




Plate 12; Zone of inhibition for ethanol extracts of bark on *E. coli* A, *C. albicans* B and *S. aureus* C.

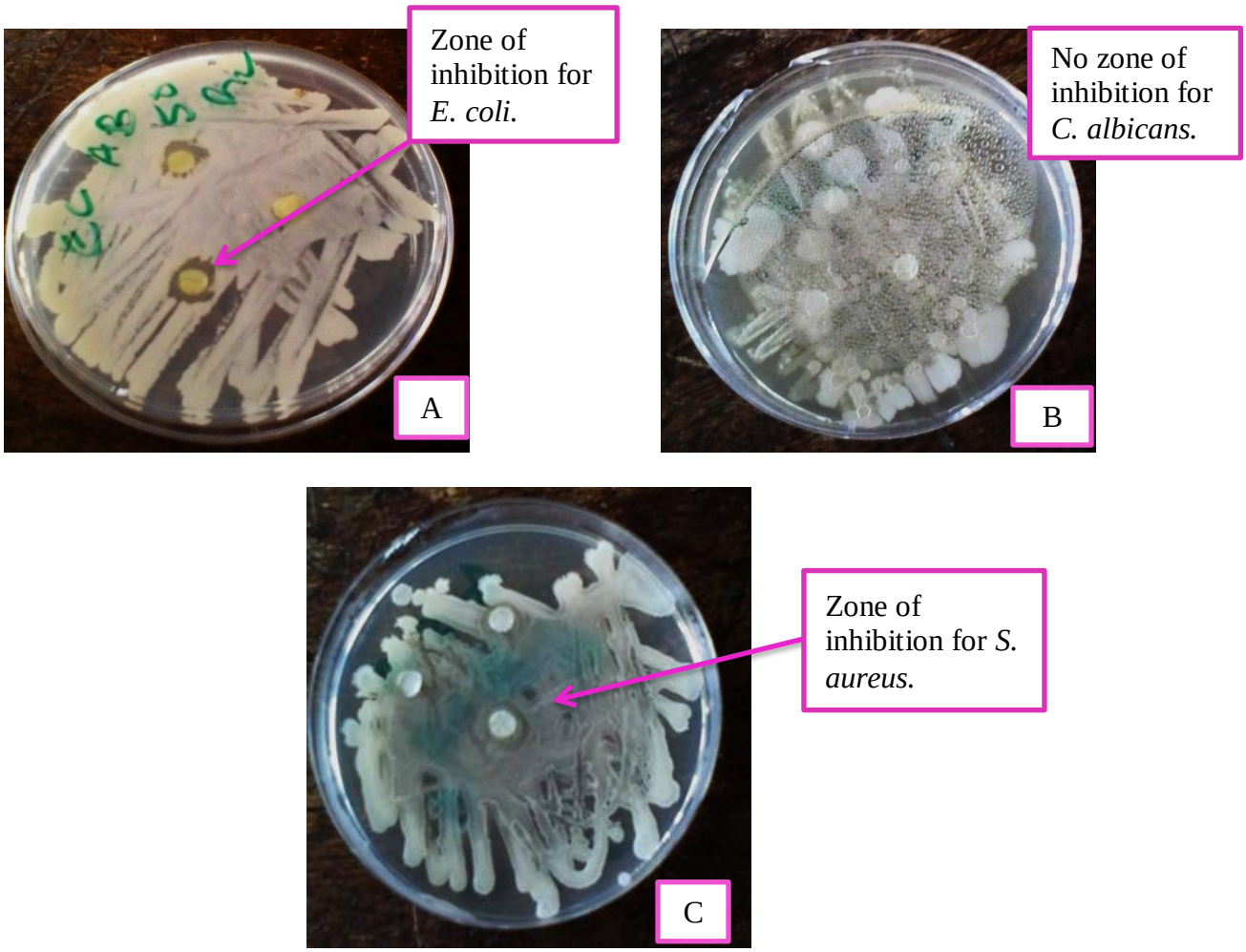



Plate 13; Zone of inhibition of acetone extracts of bark on *E. coli* A, *C. albicans* B and *S. aureus* C.

Table 4.3 and plates 14, 15 and 16 shows effect of *C. papaya* seed on growth of *E. coli, C. albicans* and *S. aureus.* The highest zone of inhibition was demonstrated against *S. aureus* (8.87mm) by ethanol extract of seeds. The lowest zone of inhibition was demonstrated against *E. coli* (2.97mm) by acetone extract of seeds. There was no inhibition exhibited by acetone extract of seeds on *C. albicans*. There was a significant difference among the extracts at $P \leq 0.05$.

**Table 4.3: Diameter of zone of inhibition (mm) exhibited by *C. papaya* L. Var. Wainamalo seed extracts against *E. coli, C. albicans* and *S. aureus***

| Microorganism | Seed | | |
|---|---|---|---|
| | **Ethanol** | **Water** | **Acetone** |
| *E. coli* | 5.13 a | 4.51 a | 2.97 a |
| *C. albicans* | 7.27 b | 5.22 b | 0 b |
| *S. aureus* | 8.87 c | 5.78 c | 5.93 c |
| **LSD** at **P≤0.05** | **0.27** | | |

Data presented are the means of three replicates. Means with the same letter down the same column are not significantly different at P≤0.05

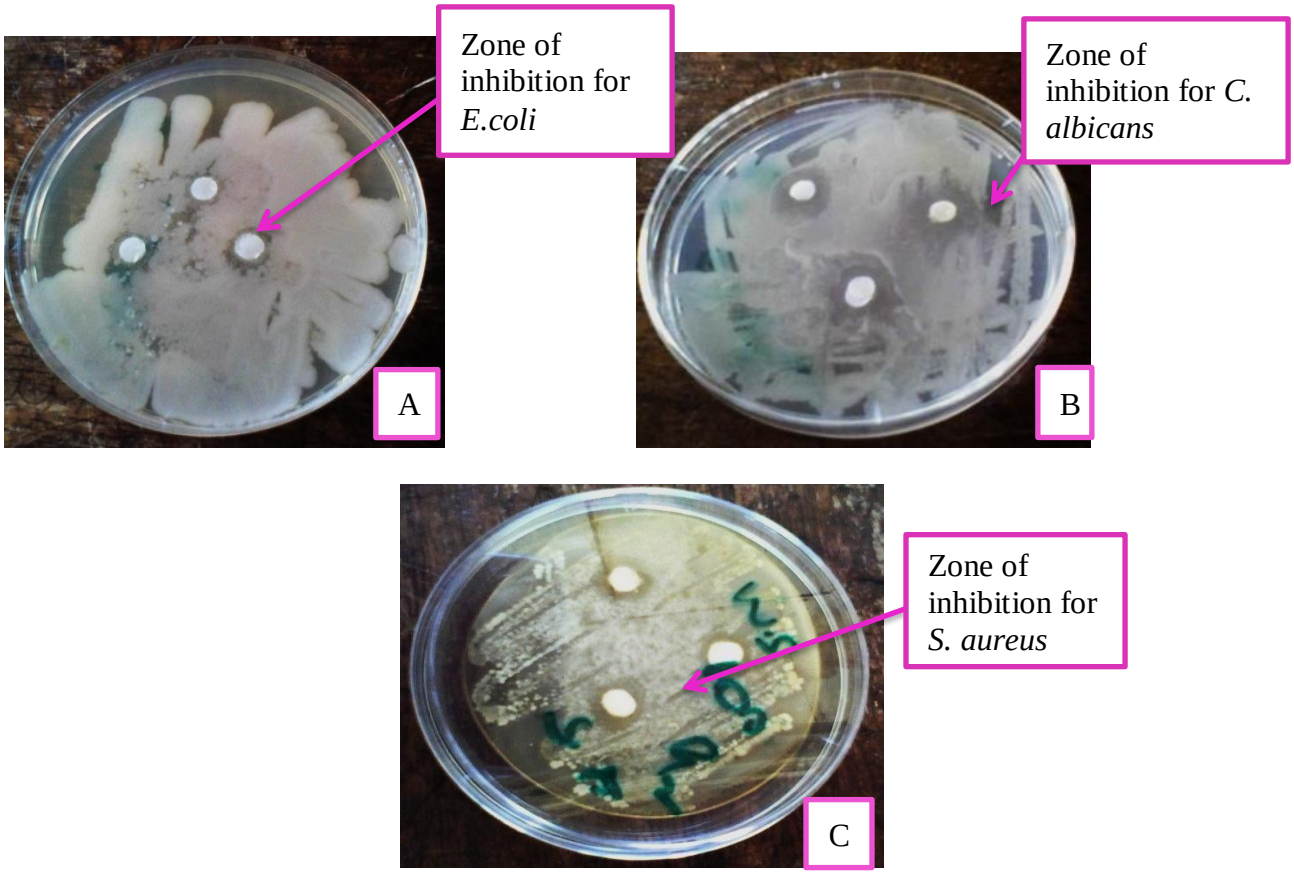



Plate 14; Zone of inhibition for water extracts of seed on *E. coli* A, *C. albicans* B and *S. aureus* C.

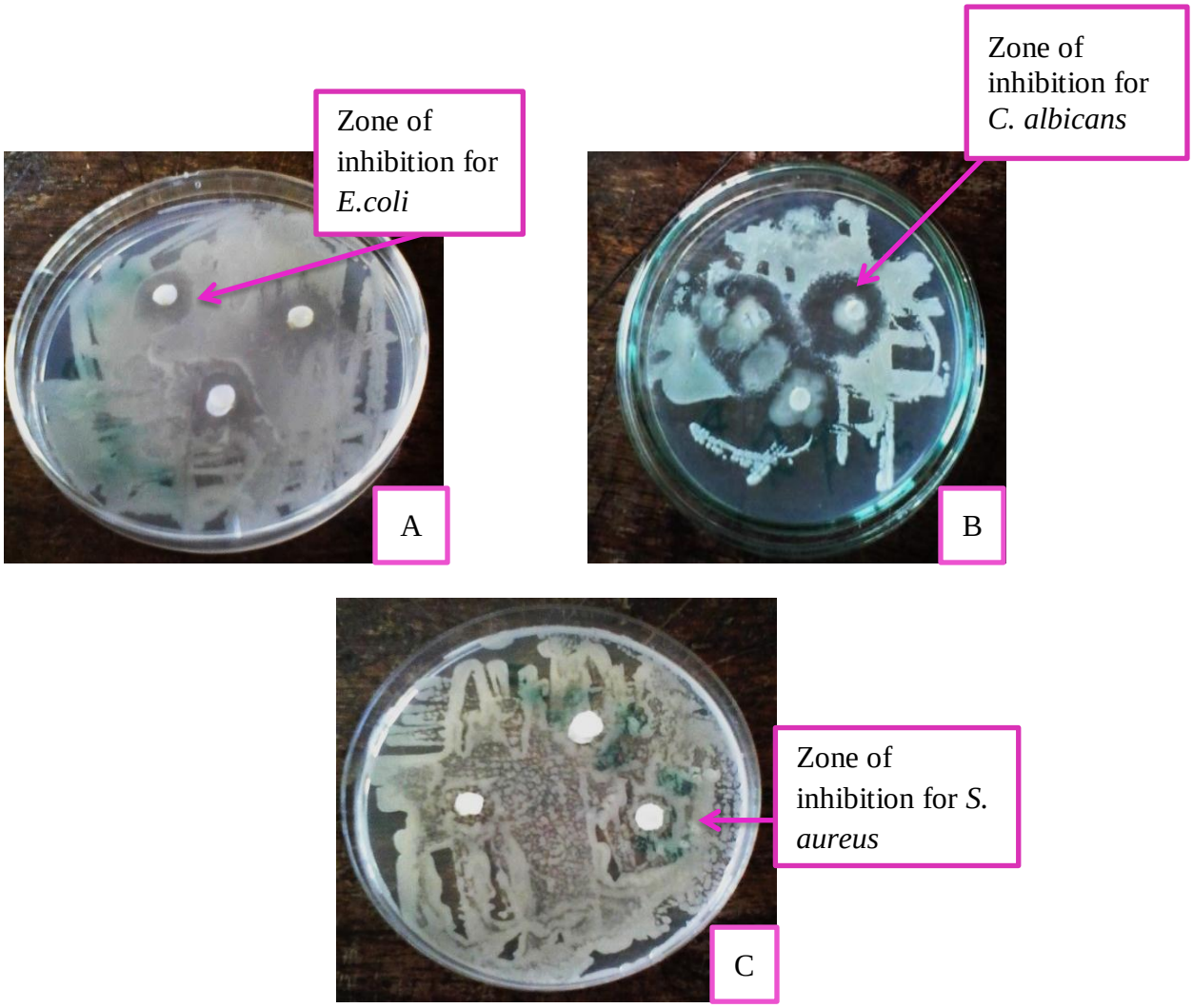



Plate 15; Zone of inhibition for ethanol extracts of seeds on *E. coli A, C. albicans B and S. aureus C.*

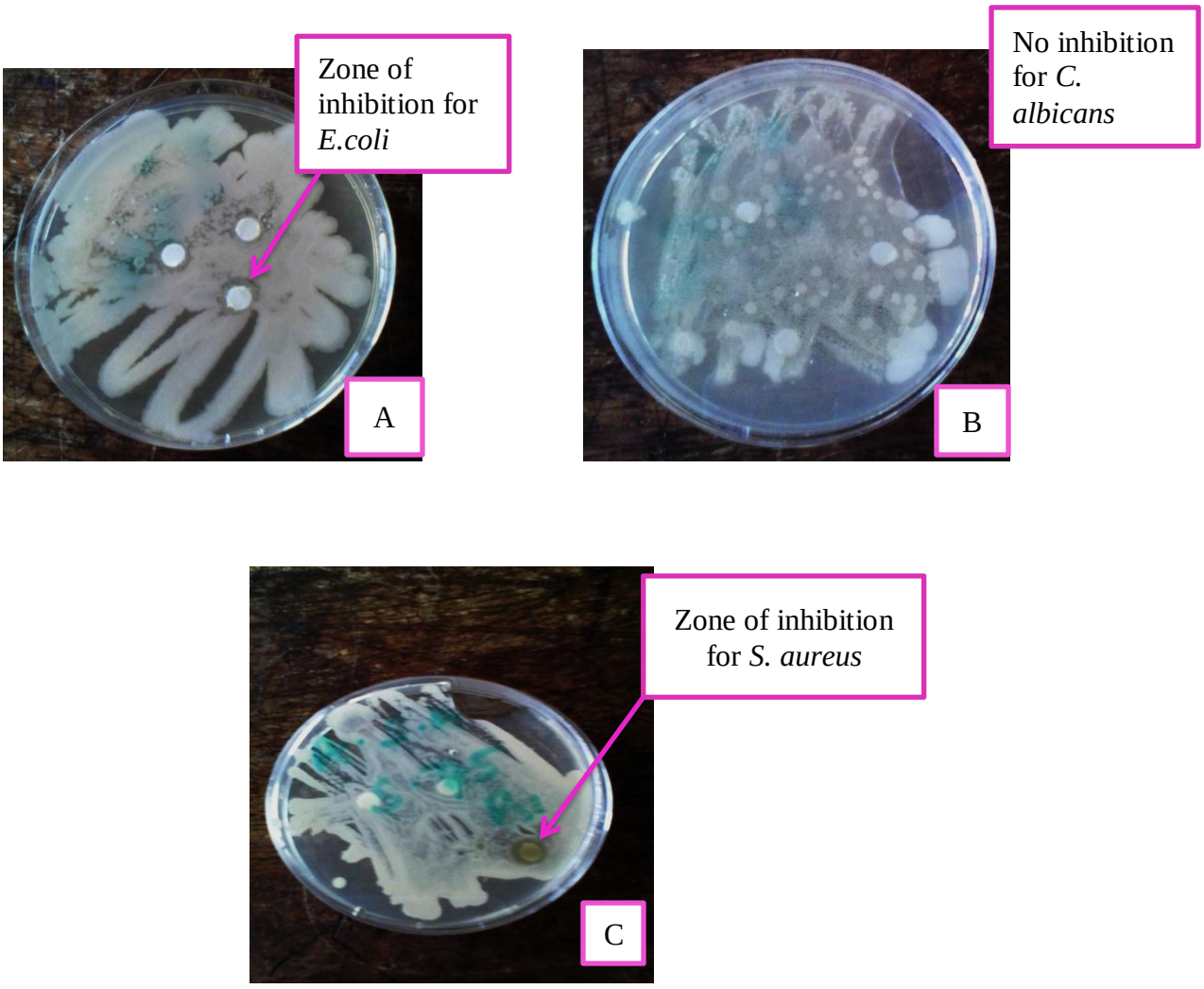



Plate 16; Zone of inhibition of acetone extracts of seeds on *E. coli A, C. albicans B and S. aureus C.*

Table 4.4 and plate 17, 18 and 19 shows the effect of *C. papaya* leaves on growth of *E. coli, C. albicans* and *S. aureus.* The highest zone of inhibition was demonstrated against *E. coli* (8.13mm) by ethanol extract of leaves. The lowest zone of inhibition was demonstrated against *E. coli* (5.09mm) by aqueous extract of leaves. There was no inhibition exhibited by leaves acetone extracts on *C. albicans* and *S. aureus.* There was a significant difference among the extracts at $P \leq 0.05$.

**Table 4.4: Diameter of zone of inhibition (mm) exhibited by *C. papaya* L. Var. Wainamalo leaf extracts against *E. coli, C. albicans* and *S. aureus***

| Microorganism | Leaf | | |
|---|---|---|---|
| | **Ethanol** | **Water** | **Acetone** |
| *E. coli* | 8.13 a | 5.09 a | 5.22 a |
| *C. albicans* | 7.8 b | 6.29 b | 0 b |
| *S. aureus* | 8.11 b | 5.49 c | 0 b |
| **LSD P≤0.05** | **0.27** | | |

Data presented are the means of three replicates. Means with the same letter in the same column are not significantly different at P≤0.05.

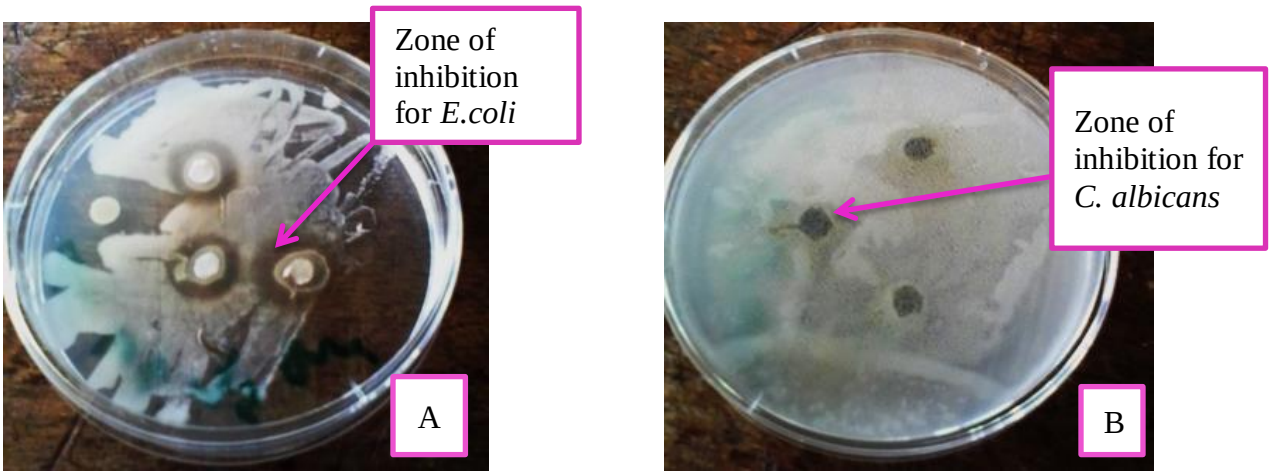



Plate17; Zone of inhibition for water extracts of leaf on *E. coli* A, *C. albicans* B and *S. aureus* C.

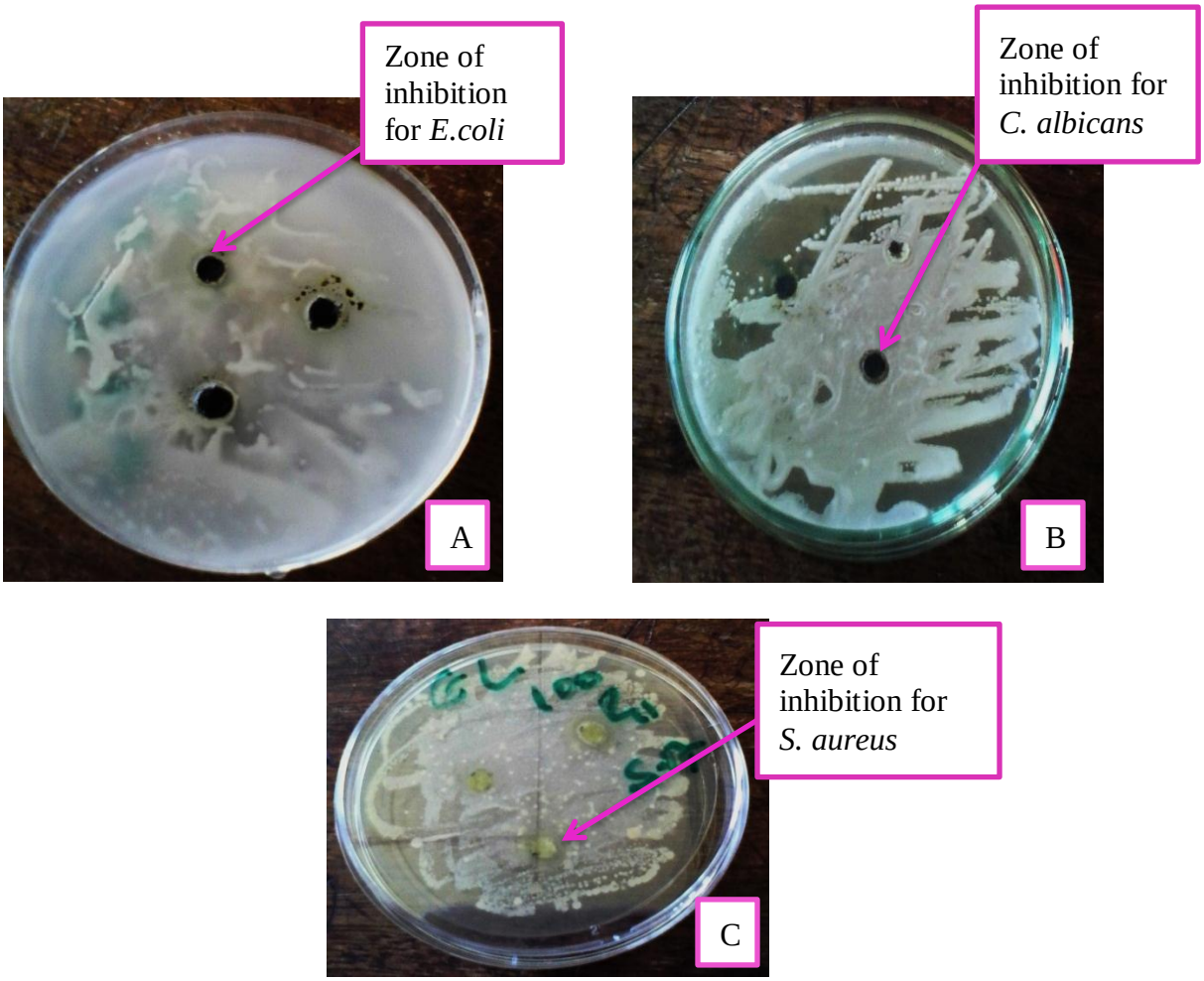


Plate 18; Zone of inhibition for ethanol extracts of leaf on *E. coli* A, *C. albicans* B and *S. aureus* C.

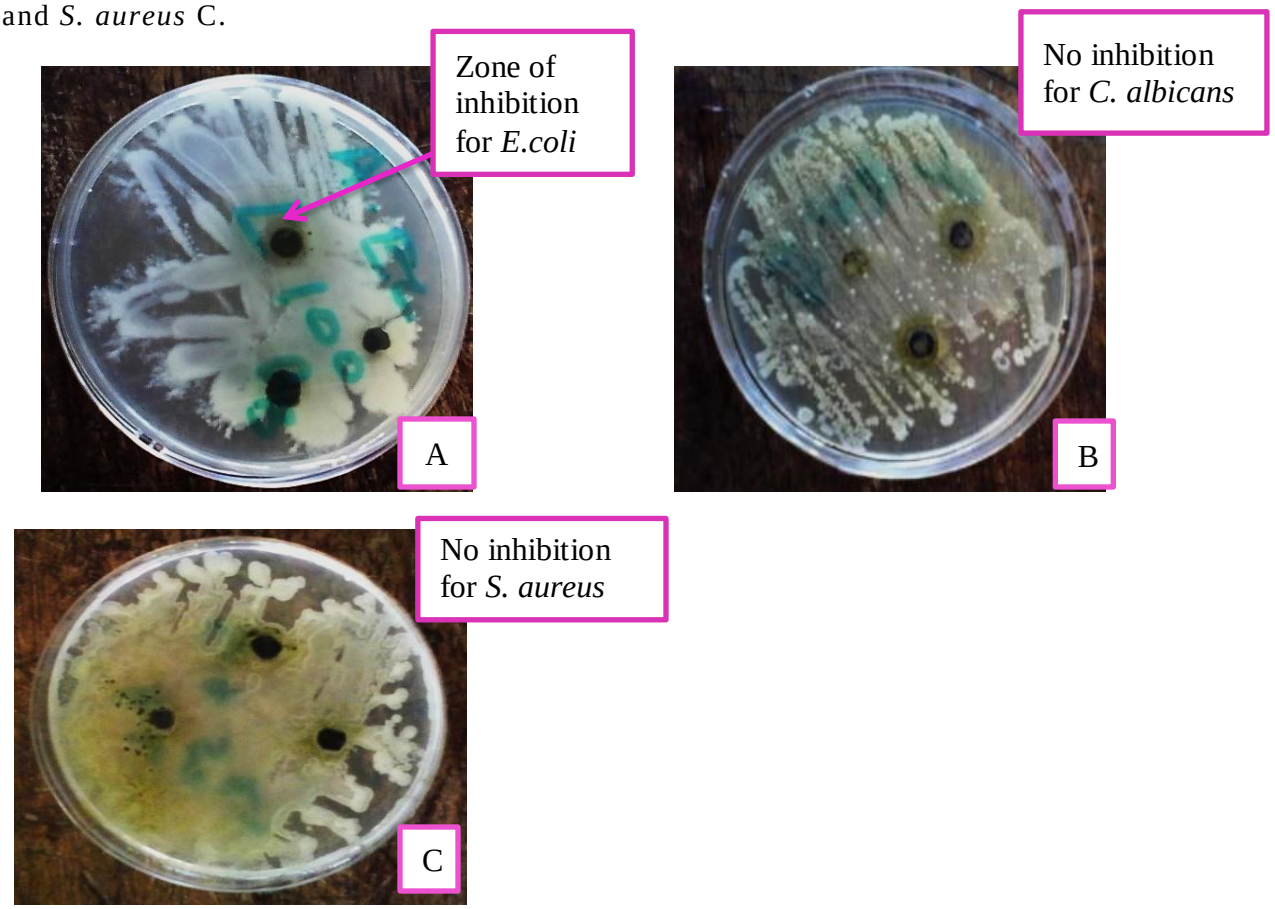



Plate 19; Zone of inhibition for acetone extracts of leaf on *E. coli* A, *C. albicans* B and *S. aureus* C.

The disc diffusion method of Mueller Hinton agar was used to determine the antimicrobial activity of *C. papaya* leaf, seed and bark extracts with different concentrations as shown in table 4.5.

**Table 4.5: Effect of different concentrations of leaf, seed and bark extracts of water, ethanol and acetone on the growth (mm) of *E. coli, C. albicans* and *S. aureus***

| Plant part | Extract | Microorganism | Concentration Mg/ml | | | |
|---|---|---|---|---|---|---|
| | | | 25 | 50 | 75 | 100 |
| | | | Zones of inhibition | | | |
| Seed | Ethanol | *E. coli* | 11.33 a | 10.56 b | 5.78 c | 8.11 d |
| | | *C. albicans* | 10.78 a | 10.67 a | 10.56 a | 12.22 b |
| | | *S. aureus* | 14.22 a | 12 b | 11.78 b | 11.11 c |
| | Water | *E. coli* | 0 | 8.11 a | 8.89 b | 8.67 c |
| | | *C. albicans* | 8.67 a | 9.44 a | 9.67 a | 8.56 b |
| | | *S. aureus* | 10.78 a | 12.44 b | 11.89 b | 9.22 c |
| | Acetone | *E. coli* | 9.60 a | 11.80 b | 9.90 c | 8.83 d |
| | | *C. albicans* | 9 a | 10.44 b | 9.78 c | 9.78 c |
| | | *S. aureus* | 9.78 a | 10.00 a | 10.89 b | 9.89 c |
| Bark | Ethanol | *E. coli* | 8.78 a | 6.44 b | 7.56 c | 7 d |
| | | *C. albicans* | 0 | 0 | 4.44 a | 0 |
| | | *S. aureus* | 0 | 0 | 9.33 a | 9.67 b |
| | Water | *E. coli* | 5.56 a | 5.8 a | 5.33 b | 5.22 b |
| | | *C. albicans* | 9.44 a | 0 b | 7.78 c | 8.70 d |
| | | *S. aureus* | 0 | 10.11 a | 10 a | 8.78 b |
| | Acetone | *E. coli* | 6.78 a | 6.60 a | 5.75 b | 6.22 c |
| | | *C. albicans* | 8.44 a | 7.78 b | 7.44 c | 7.88 d |
| | | *S. aureus* | 2.78 a | 9.78 b | 10.89 c | 4 d |
| Leaf | Ethanol | *E. coli* | 10.33 a | 9.89 b | 5.78 c | 5.44 c |
| | | *C. albicans* | 0 | 0 | 0 | 0 |
| | | *S. aureus* | 6.67 a | 3.56 b | 2.71 b | 5.51 c |
| | Water | *E. coli* | 0 | 6.44 b | 0 | 8.44 b |
| | | *C. albicans* | 0 | 0 | 0 | 0 |
| | | *S. aureus* | 10.56 a | 10.67 a | 0 | 8.44 b |
| | Acetone | *E. coli* | 0 | 8.56 a | 8.33 a | 9.22 b |
| | | *C. albicans* | 0 | 0 | 0 | 0 |
| | | *S. aureus* | 0 | 0 | 0 | 0 |
| **LSD at P=0.05** | | | **0.27** | | | |

Data presented are the means of three replicates. Means with the same letter across the same row are not significantly different at P=0.05.

As shown in table 4.5, increase in the concentration has different effects on the microorganism, plant part and extract used.

Twenty five mg/ml of ethanol seed extracts exhibited a higher inhibition on *S. aureus* (14.22mm) at 25mg/ml compared to the lower inhibition of *E. coli* (5.78mm) at 75mg/ml. There was no inhibition exhibited by 25mg/ml aqueous extract against *E. coli* however the highest inhibition was observed against *S. aureus* (12.44mm) at50mg/ml and lowest inhibition was observed against *E. coli* (8.11mm) at 50mg/ml. Acetone extracts showed a higher inhibition against *E. coli* (11.80mm) at 50mg/ml and lowest inhibition against *E. coli* (8.83mm) at 100mg/ml.

The bark indicated the highest inhibition against *S. aureus* (9.67mm) at 100mg/ml of ethanol extracts, with the lowest inhibition observed against *C. albicans* (4.44mg/ml) at 75mg/ml of ethanol extracts, while there was no inhibition at 25mg/ml and 50mg/ml of ethanol extracts for *S. aureus* and *C. albicans.* Fifty mg/ml aqueous showed a higher inhibition against *S. aureus* (10.11mm), 100mg/ml of aqueous showed lowest inhibition against *E. coli* (5.22mm) and no inhibition exhibited by 50mg/ml of aqueous on *C.albicans.*75mg/ml and 25mg/ml of acetone extracts showed a higher and lower inhibition on *S. aureus* (10.89mm and 2.78 mm) respectively.

The leaf extracts did not show any growth inhibition on *C. albicans* in all the concentrations of the ethanol extract, the highest inhibition was observed in 25mg/ml ethanol extract on *E. coli* (10.33mm) and the lowest inhibition was observed in 75mg/ml ethanol extract on *S. aureus (*2.781mm). *C. albicans* didn't show any inhibition in all the concentrations of aqueous extract, *S. aureus* showed the highest inhibition (10.67mm) at 50mg/ml of aqueous extract while the lowest inhibition was against *E. coli* (6.44mm) at 50mg/ml of aqueous extract. Acetone extracts had no effect on *S. aureus* and *C. albicans*, *E. coli* (25mg/ml) with the highest inhibition observed against *E. coli* (9.22mm) at 100mg/ml of acetone extracts.

### 4.3 Minimum Inhibitory Concentration (MIC)

Table 4.6 shows Minimum Inhibitory Concentration (MIC) of various extracts of leaf, seed and bark extracts of *C. papaya* on the microorganisms, the MIC varies with the microorganism, plant part and extract used. The MIC values varied from 0.025-0.1mg/ml for the three extracts. Lowest MIC value 0.025mg/ml was recorded against *Escherichia coli* and *Staphylococcus aureus* where against *Candida albicans* the lowest MIC observed was 0.05mg/ml. (P≤0.05)

**Table 4.6: Minimum Inhibitory Concentration (MIC) of various extracts of leaf, seed and bark extracts of *C. papaya* L. Var. Wainamalo on *Escherichia coli, Candida albicans* and *Staphylococcus aureus.***

| Microorganism | Plant part | Minimum Inhibitory Concentration MIC (mg/ml) | | |
|---|---|---|---|---|
| | | Water | Ethanol | Acetone |
| *Escherichia coli* P≤0.05 | Bark | 0.1 | 0.05 | 0.1 |
| | Seed | 0.1 | 0.05 | 0.025 |
| | Leaf | 0.05 | 0.025 | 0.1 |
| *Candida albicans* P≤0.05 | Bark | 0.05 | 0.1 | 0.05 |
| | Seed | 0.05 | 0.1 | 0.05 |
| | Leaf | NI | NI | NI |
| *Staphylococcus aureus* P≤0.05 | Bark | 0.025 | 0.025 | 0.025 |
| | Seed | 0.025 | 0.025 | 0.025 |
| | Leaf | 0.025 | 0.05 | NI |

NI=No inhibition

# CHAPTER FIVE

# DISCUSSION

## 5.1 Phytochemical analysis

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Ekaiko *et al*., 2015a). Phytochemical screening of the leaf, seed and bark extracts of the *C. papaya* revealed that the plant contains flavonoids, alkaloids, saponins, tannins, phenolic compounds, glycosides and anthocyanins in agreement with Ekaiko *et al*. (2015a), Sikandar *et al.* (2013) and Ayoola and Adeyeye, (2010). Anthraquinones were only present in leaves but absent in seeds and bark. The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine.

Alkaloids were isolated and identified from the plant extracts and are commonly known to have antimicrobial properties (Sikandar *et al.,* 2013). Alkaloids are most efficient therapeutically significant plant substance. Pure natural and synthetic derivatives of alkaloids are used as a basic medical agent because of their analgesic, antispasmodic and antibacterial properties (Sikandar *et al.*, 2013).

Flavonoids were also present in the plant extracts and they are free radical scavengers and super antioxidants which prevent oxidative cell damage and have strong anticancer activity (Yahaya *et al*., 2017). The biological functions of flavonoids include protection against allergies,

inflammations, platelets aggregation microbes, ulcer, viruses and tumors (Yahaya *et al*., 2017). The presence of flavonoid in the plants extracts suggests that *C. papaya* plant extracts can be used as anti-spasmodic, antifungal and anti-bacterial drugs. This confirms the reason for the use of these plants in the treatment of diarrhea, spasmodic bronchitis and other microbial infections among the local communities. Flavonoids also inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low concentrations (Anibijuwon *et al.*, 2009).

The presence of tannins in *C. papaya* extracts can support its strong use for healing of wounds, ulcers, hemorrhoids, frost-bites and burns in herbal medicine (Igboko, 1983). Tannins are metal chelators and can form complexes with macromolecules. Through this process, essential substrates co-factor and enzymes of micro-organism are depleted leading to cell death. Tannins have astringent properties which hasten the healing of wounds and inflamed mucous membrane (Igboko, 1983). Tannins have the ability to inactivate microbial adhesions, enzymes, and cell envelope transport proteins. Gram positive bacteria are generally more susceptible since their outer peptidoglycan layer is not an effective barrier.

The presence of phenolic compounds in the extracts of *C. papaya* indicates that the extracts have antimicrobial potential and this may explain the use of this plant in treating diarrhea, typhoid fever and some other intestinal problems. Phenols and phenolic compounds have been extensively used in disinfections and they remain the standard to which other bactericides are compared with (Yahaya *et al*., 2017).

The presence of saponins supports the fact that *C. papaya* has cytotoxic effect such as pemearlization of the intestines (Yahaya *et al*., 2017). Saponins also facilitate the entry of toxic material or leakage of vital constituents from the cell; the saponins constituents are known to be responsible for the possession of hemolytic property (Okigbo *et al.*, 2009). The presence of the phytochemicals may be due to ethanol being a polar solvent; it could have extracted more polyphenols (Naomi, 2014).

### 5.2. Effect of *C. papaya* L. Var. Wainamalo seed, leaf and bark extracts on growth of *E. coli, C. albicans* and *S. aureus*

The present study showed that the different parts of *C. papaya* possess antimicrobial potential against *S. aureus, E. coli* and *C. albicans,* this may be due to the disruption of the cell wall formation which consequently causes the leakage of cytoplasmic constituent (Chima *et al*., 2016). In line with the present finding, several other studies have reported *C. papaya* leaves (Baskaran *et al.*, 2012; Anibijuwon and Udeze, 2009), seeds (Ocloo *et al*., 2012) to have antimicrobial potentials. The study by Yahaya *et al*. (2017) has also shown that *C. papaya* leaves and bark has significant antibacterial activity in various extracts from different tree parts.

Several other studies have shown that *C. papaya* have significant antibacterial activity in various extracts from different tree parts (Nirosha and Mangalanayaki, 2013; Doughari *et al*., 2007). Results of this study revealed very significant growth inhibition activity with the extracts demonstrating broad spectrum of activity against both bacteria (*E. coli* and *S. aureus*) and fungi (*C. albicans*).

In the bacterial and fungal test, it was observed that the potency of the activity of *Carica papaya* against microbes depends on the extraction solvent used. *Carica papaya* parts in organic

extracts such as ethanol and acetone was more effective than *Carica Papaya* in aqueous extracts. This may be due to the better solubility of the active components in organic solvents.

The ethanol extracts demonstrated a higher activity than the acetone and aqueous extracts, the better efficacy of the ethanol extract against the acetone may be because different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents (Ruhama, 2014). Based on the limited spectrum of activity of the other extracts (acetone and water) compared with the ethanol extracts, it suggests that the active component is more soluble in ethanol than in the other solvents. This is in agreement with Aruljothi *et al*. (2014) and Ekaiko *et al*. (2015 b).

The result further showed that the bark and seed extracts were more effective on *E. coli* and *S. aureus* while leaf extracts were less effective on them. Seed and leaf extracts were more effective on *C. albicans* while bark extracts were less effective on the fungus. These observations are in agreement with the results of Doughari *et al*. (2007).

Not all the extracts prepared from leaf, seed and bark using acetone, water, and ethanol exhibited antibacterial and antifungal activity against the tested microorganisms. *S. aureus* exhibited the highest inhibition with the ethanol extract of bark, *E. coli* exhibited higher inhibition with ethanol extract of seeds and *C. albicans* exhibited a higher inhibition with ethanol extract of *C. papaya* leaves. There was no inhibition exhibited by acetone extracts of seed, leaf and bark against *C. albicans* and acetone extracts of leaf on *S. aureus.* This may be due to acetone not being able to extract the antibacterial and antifungal compounds present in the plant parts

(Naomi, 2014). These results are in agreement with results of Yahaya *et al*., 2017 and Malik *et al*., 2016.

The present study showed that different parts of *C. papaya* possess antimicrobial potential against *S. aureus, E. coli* and *C. albicans.* In line with the present finding, several other studies have reported *C. papaya* leaves (Baskaran *et al*., 2012; Yahaya, *et al*., 2017), seeds (Ogunjobi and Ogunjobi 2011) to have antimicrobial potentials on the same microbes used in this study. Several other reports also, have shown that parts of *C. papaya* have significant antibacterial activity in various extracts (Ifesan *et al.,* 2013; Nirosha and Mangalanayaki, 2013; Doughari *et al*., 2007).

The result for the effect of acetone, ethanol and aqueous extract of *Carica papaya* bark, seed and leaf showed that the plant parts were effective against the test microorganisms at different concentrations showing varying zones of inhibitions, however the ethanol, water and acetone extracts of the leaf did not have any effect on *C. albicans* and acetone extracts of the leaf did not have any effect on *S. aureus.*

The study showed that increased concentration either increased or decreased the zone of inhibition for the tested microorganisms depending on the plant part and extracts used. Ethanol extracts of *C. papaya* bark, and leaf were found to decrease the inhibition zone of *E. coli* when their concentrations were increased while ethanol extracts of *C. papaya* seed were found to increase the inhibition zone of *E. coli* when their concentrations were increased.

It was also observed that *E. coli* zones of inhibition were decreased by ethanol, acetone and water extracts of bark while increased by ethanol and water extracts of seed. This was also observed in the other tested microorganisms. *Candida albicans* showed no inhibition with ethanol bark extracts and acetone leaf extracts while *S. aureus* showed no inhibition on acetone extracts of leaf. These findings can be attested to other works by Okunola *et al.* (2012) who reported the effect of *C. papaya* on similar microorganisms. However these results are in disparity with those of Sumathi and Gowthami, (2014), who reported that the zone of inhibition was observed only in leaf extracts.

The gram – positive bacteria (*S. aureus*) was more susceptible to the plant extracts. These results disagree with previous studies by Ganjewala *et al.* (2009) who found that *S. aureus* to be resistant to plant extract and suggested that this was due to the outer membrane of the bacteria.

The current results differ with those reported earlier by Jigna *et al*. (2006) indicating that plant extracts are more active against gram- negative bacteria than gram-positive bacteria. Nirosha and Mangalanayaki (2013) also reported gram negative bacteria are more susceptible to the extracts of pawpaw leaf and stem. The fact that the extracts were active against both Gram-positive and Gram-negative bacteria may indicate a broad spectrum of activity. This observation is very crucial because it indicates a possibility of developing therapeutic substances from this plant that will be active against multidrug-resistant organisms. There may be several factors that will predispose bacteria and fungi to antibacterial and antifungal agents such as previous encounters with the agents or the nature of medium used, which may affect the diffusability of the agent.

The demonstration of activity against the test bacteria and fungus provides scientific bases for the local usage of these plants in the treatment of various ailments.

Anibijuwon and Udeze (2009) extracted bioactive compounds from leaf and root of *C. papaya* using water and organic solvents, which were investigated for antibacterial activity against some human pathogenic bacteria. Both leaf and root extracts showed pronounced inhibition against gram positive bacteria (*S. aureus)* than the gram negative bacteria tested. Ocloo *et al*., 2012 studied the efficacies of crude extracts of *C. papaya* seeds against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method. The crude organic (acetone, methanol) extracts inhibited the growth of all three organisms.

Plant products, particularly extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. The presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi and therefore explains the demonstration of antibacterial and antifungal activity by the plant extracts used in this study.

### 5.3 Minimum inhibitory concentration of leaf, seed and bark extracts of *C. papaya* L. Var. Wainamalo on *E. coli, C. albicans* and *S. aureus*

Minimum Inhibitory Concentration (MIC) is the lowest concentration able to completely inhibit any visible microorganism growth after overnight incubation with media (Chima *et al*., 2016). The MIC result showed that increasing concentration has an increasing effect in inhibiting the organisms used. Since the MIC values indicated the definite nature of the antimicrobial activities of this plant, the inhibition zones values, only, indicated extent of effectiveness of the extract with increasing concentration.

It has been proposed that the leaf's action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract (Ekaiko *et al*., 2015 a).

High Minimum inhibitory Concentration observed for *Candida albicans* at 0.005mg/ml may be an indication of higher concentration of the extract required to inhibit the organism's growth (Chima *et al*., 2016). It may also indicate low efficacy or that the organism has higher potential for developing resistance to the bioactive compounds in the plant, which is said to be related to the thick murein layer in their outer membrane which prevents the entry of inhibition substances (Chima *et al*., 2016). This may be an indication that the bioactive compounds are heat stable and explains the ethno- botanical process of the plants where boiling at very high temperatures for extended time periods are often practiced without the concoctions losing their efficacies.

The low MIC value observed for *E. coli* and *S. aureus* is a good indication of high efficacy against these bacteria. This also means that lower concentration of the extract is required to inhibit the organism's growth (Chima *et al*., 2016). This outcome is remarkable considering that diarrhea caused by *E. coli* is on the rise among people and *S. aureus* which causes boils, abscesses and impetigo which are becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Kenya. On the other hand disparity in Minimum Inhibitory Concentration may be due to variable sensitivity to the chemical substances related to different resistant levels among strains (Chima *et al*., 2016).

The therapeutic value of medicinal plants has been reported to lie in the various chemical constituents in it. Active principles singly or in combination inhibit life processes of microbes, by binding with their protein molecules, acting as chelating agents, altering their biochemical

systems and preventing utilization of available nutrients to the microorganisms (Orchue *et al.*, 2013). Aravind *et al*. (2013) reported that the many benefits of pawpaw, is due to the high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain, that have antiviral, antifungal and antibacterial properties.

# CHAPTER SIX

# CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

## 6.1 Conclusions

i. *Carica papaya* L. Var. Wainamalo leaves, seeds and bark contain alkaloids, saponins, tannins, glycosides, phenols, terpernoids, anthocyanins and flavonoids. Anthraquinones were absent in seeds and bark.

ii. The plant extracts showed both antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli* and *Candida albicans,* thus an indication that the plant can be a potential source for production of drugs with a broad spectrum of activity.

iii. Minimum inhibitory concentration for *Staphylococcus aureus* and *Escherichia coli* was 0.0025mg/ml observed in acetone and ethanol extracts of seed and aqueous, ethanol and acetone extracts of seed and aqueous extracts of leaf. *Candida albicans* was 0.05mg/ml.

## 6.2 Recommendations

Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibiotics from this plant should be explored. *Carica papaya* L. Var. Wainamalo may be recommended as useful antimicrobial agent for pathogenic microorganisms tested in this study. In the search for new pharmaceuticals, screening of such various natural organic compounds and identification of active agents must be considered as a fruitful approach.

### 6.3 Suggestions for further studies

For further research on antimicrobial activity of *Carica papaya* L. Var. Wainamalo extracts against *E. coli, S. aureus* and *C. albicans*, the following should be considered.

i. Use of different microorganisms that causes diseases in plants and animals that weren't used in this study.

ii. Use of different varieties of *C. papaya* L. and parts of *Carica papaya* L. Var. Wainamalo apart from the ones used in this study.

iii. Using different isolation, purification and characterization methods antimicrobial principals can be obtained and thus the activity of antimicrobial compounds can be improved for further pharmaceutical uses.

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# APPENDICES

Appendix 1: Analysis of Variance of Antimicrobial activity of *C. papaya* seed, leaf and bark extracts on *C. albicans, E. coli* and *S. aureus.*

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Model** | **102** | **25117.05** | **246.25** | **62.80** | **<.0001** |
| **Error** | **1112** | **4359.97** | **3.92** | | |
| **Corrected Total** | **1214** | **29477.00** | | | |

| | |
|---|---|
| R-Square | 0.85 |
| Coeff Var | 38.27 |
| Root MSE | 1.98 |
| Inhibition Mean | 5.17 |

| SOURCE | DF | TYPE I SS | MS | F value | P≥F |
|---|---|---|---|---|---|
| Concentration | 4 | 8279.31 | 2069.83 | 527.91 | <.0001 |
| Extract | 2 | 5287.75 | 2643.88 | 674.32 | <.0001 |
| Pathogen | 2 | 804.60 | 402.30 | 102.61 | <.0001 |
| Plant part | 2 | 13.53 | 6.77 | 1.73 | <.0001 |
| extract*concentration | 8 | 1843.95 | 230.49 | 58.79 | <.0001 |
| pathogen*concentration | 8 | 501.99 | 62.75 | 16.00 | <.0001 |
| Plant part *concentration | 8 | 496.62 | 62.08 | 15.83 | <.0001 |
| Pathogen *extract | 4 | 1308.46 | 327.11 | 83.43 | <.0001 |
| Extract* plant part | 4 | 757.57 | 189.39 | 48.38 | <.0001 |
| Pathogen *plant part | 4 | 928.78 | 232.20 | 59.22 | <.0001 |
| Pathogen* extract* concentration | 16 | 1728.36 | 108.02 | 27.55 | <.0001 |
| Extract *plant part *concentration | 16 | 1014.94 | 63.43 | 16.18 | <.0001 |
| Pathogen *plant part *concentration | 16 | 1439.81 | 89.99 | 22.95 | <.0001 |
| pathogen*extract*plant part | 8 | 711.37 | 88.92 | 22.68 | <.0001 |

Appendix 2 LSD for inhibition of concentration.

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 1112 |
| **Error Mean Square** | 3.92 |
| **Critical Value of t** | 1.96 |
| **Least Significant Difference** | 0.35 |

LSD separates the means between the concentrations Means with the same letter are not significantly different

| Mean | Concentration |
|---|---|
| **6.78 a** | 100% |
| **6.72 a** | 75% |
| **6.56 a** | 50% |
| **5.81 b** | 25% |

Appendix 3 LSD for inhibition of extract

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 1112 |
| **Error Mean Square** | 3.92 |
| **Critical Value of t** | 1.96 |
| **Least Significant Difference** | 0.27 |

LSD separates means between extracts. Means with the same letter are not significantly different.

| Mean | N | Extract |
|---|---|---|
| 7.90a | 405 | Ethanol |
| 4.78b | 405 | Water |
| 2.84c | 405 | Acetone |

Appendix 4 LSD for inhibition of microorganisms

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 1112 |
| **Error Mean Square** | 3.92 |
| **Critical Value of t** | 1.96 |
| **Least Significant Difference** | 0.27 |

LSD separates means between microorganisms. Means with the same letter are not significantly different.

| Mean | Microorganism |
|---|---|
| 5.88 a | *S. aureus* |
| 5.61 b | *C. albicans* |
| 4.03 c | *E. coli* |

Appendix 5 LSD for inhibition of plant part.

| | |
|---|---|
| **Alpha** | 0.05 |
| **Error Degrees of Freedom** | 1112 |
| **Error Mean Square** | 3.92 |
| **Critical Value of t** | 1.96 |
| **Least Significant Difference** | 0.27 |

LSD separates the means between plant parts. Means with the same letter are not significantly different.

| Mean | Plant part |
|---|---|
| 5.32a | Bark |
| 5.13a | Seeds |
| 5.08a | Leaves |

**Appendix 6:** Means of Antimicrobial activity of *C. papaya* seed, leaf and bark extracts on *C. albicans*, *E.* coli and *S. aureus*.

EFFECT EXTRACT*CONCENTRATION

| Extract | concentration | Mean of inhibition | Std. Dev. of inhibition | Std. Error of inhibition |
|---|---|---|---|---|
| Ethanol | 25% | 9. 35 | 3.98 | 0.44 |
| Ethanol | 50% | 10.58 | 2.77 | 0.31 |
| Ethanol | 75% | 10.06 | 2.84 | 0.32 |
| Ethanol | 100% | 9.5 | 3.13 | 0.35 |
| Water | 25% | 4.72 | 4.18 | 0.46 |
| Water | 50% | 5.15 | 4.03 | 0.44 |
| Water | 75% | 7.64 | 2.71 | 0.30 |
| Water | 100% | 6.40 | 3.44 | 0.38 |
| Acetone | 25% | 3.37 | 4.89 | 0.54 |
| Acetone | 50% | 3.95 | 4.69 | 0.52 |
| Acetone | 75% | 2.47 | 3.86 | 0.43 |
| Acetone | 100% | 4.41 | 4.55 | 0.51 |

EFFECT EXTRACT*PLANT PART

| EXTRACT | PLANT PART | Mean of inhibition | Std. Dev of inhibition | Std. error of inhibition |
|---|---|---|---|---|
| Ethanol | Bark | 8.61 | 5.34 | 0.46 |
| Ethanol | Seed | 7.09 | 4.72 | 0.41 |
| Ethanol | Leaf | 8.01 | 4.52 | 0.39 |
| Water | Bark | 3.55 | 4.22 | 0.36 |
| Water | Seed | 5.17 | 4.11 | 0.35 |
| Water | Leaf | 5.62 | 3.86 | 0.33 |
| Acetone | Bark | 3.81 | 4.65 | 0.40 |
| Acetone | Seed | 2.97 | 4.44 | 0.38 |
| Acetone | Leaf | 1.74 | 3.54 | 0.30 |

EFFECT PATHOGEN*CONCENTRATION

| Microorganism | Concentration | Mean of inhibition | Std. Dev of inhibition | Std. error of inhibition |
|---|---|---|---|---|
| *E. coli* | 25% | 5.89 | 4.66 | 0.52 |
| *E. coli* | 50% | 8.20 | 3.10 | 0.34 |
| *E. coli* | 75% | 6.56 | 3.77 | 0.42 |
| *E. coli* | 100% | 7.40 | 3.38 | 0.38 |
| *C. albicans* | 25% | 5.15 | 4.82 | 0.54 |
| *C. albicans* | 50% | 4.26 | 4.94 | 0.55 |
| *C. albicans* | 75% | 5.52 | 4.65 | 0.52 |
| *C. albicans* | 100% | 5.25 | 5.06 | 0.56 |
| *S. aureus* | 25% | 6.40 | 5.61 | 0.62 |
| *S. aureus* | 50% | 7.22 | 5.34 | 0.59 |
| *S. aureus* | 75% | 8.10 | 4.64 | 0.52 |
| *S. aureus* | 100% | 7.69 | 3.91 | 0.43 |

EFFECT PATHOGEN*EXTRACT

| Micro organism | Extract | Mean of inhibition | Std. Dev of inhibition | Std. error of inhibition |
|---|---|---|---|---|
| *E. coli* | Ethanol | 6.81 | 5.27 | 0.45 |
| *E. coli* | Water | 5.19 | 2.92 | 0.25 |
| *E. coli* | Acetone | 4.83 | 4.59 | 0.40 |
| *C. albicans* | Ethanol | 7.97 | 4.39 | 0.38 |
| *C. albicans* | Water | 4.13 | 4.27 | 0.37 |
| *C. albicans* | Acetone | 0 | 0 | 0 |
| *S. aureus* | Ethanol | 8.93 | 4.81 | 0.41 |
| *S. aureus* | Water | 5.02 | 4.96 | 0.43 |
| *S. aureus* | Acetone | 3.69 | 4.71 | 0.40 |

EFFECT PATHOGEN*PLANT PART

| Microorganism | Plant part | Mean of inhibition | Std. Dev. of inhibition | Std. error of inhibition |
|---|---|---|---|---|
| *E. coli* | Bark | 6.47 | 4.80 | 0.41 |
| *E. coli* | Seed | 4.21 | 3.80 | 0.33 |
| *E. coli* | Leaf | 6.15 | 4.38 | 0.38 |
| *C. albicans* | Bark | 3.24 | 5.05 | 0.43 |
| *C. albicans* | Seed | 4.16 | 4.64 | 0.40 |
| *C. albicans* | Leaf | 4.70 | 4.62 | 0.40 |
| *S. aureus* | Bark | 6.25 | 5.42 | 0.47 |
| *S. aureus* | Seed | 6.86 | 5.16 | 0.44 |
| *S. aureus* | Leaf | 4.53 | 5.09 | 0.44 |

EFFECT PLANT PART*CONCENTRATION

| Plant part | Concentration | Mean of inhibition | Std. Dev. of Inhibition | Std. error of Inhibition |
|---|---|---|---|---|
| **Bark** | 25% | 7.21 | 5.51 | 0.61 |
| **Bark** | 50% | 5.51 | 5.33 | 0.59 |
| **Bark** | 75% | 7.04 | 4.27 | 0.47 |
| **Bark** | 100% | 6.85 | 5.17 | 0.57 |
| **Seed** | 25% | 5.07 | 4.86 | 0.54 |
| **Seed** | 50% | 7 | 4.52 | 0.50 |
| **Seed** | 75% | 5.95 | 4.72 | 0.52 |
| **Seed** | 100% | 7.36 | 3.26 | 0.36 |
| **Leaf** | 25% | 5.15 | 4.50 | 0.50 |
| **Leaf** | 50% | 7.17 | 4.54 | 0.50 |
| **Leaf** | 75% | 7.19 | 4.40 | 0.49 |
| **Leaf** | 100% | 6.12 | 4.20 | 0.47 |

## Appendix 7 Morphological characteristics of test organisms

*Candida albicans* had colonies with a smooth texture, circular, white in color and a raised elevation; *Escherichia coli* had colonies with a smooth texture, circular, white in color and a raised elevation while *Staphylococcus aureus* had colonies with a rough texture, circular, tan in color and a convex elevation.

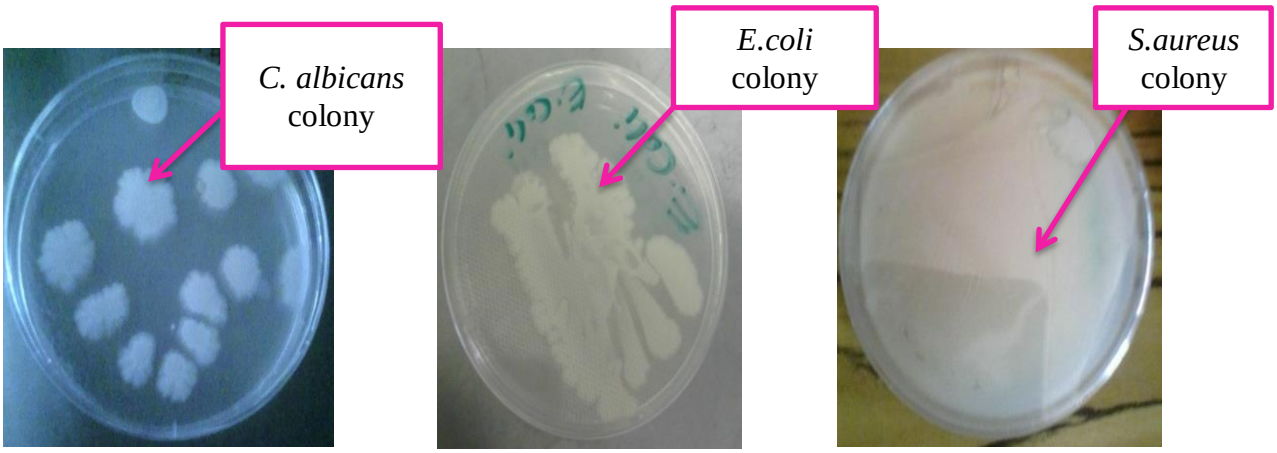


Plate 20: *C. albicans*, *E. coli* and *S. aureus* grown on media