COMPARATIVE ANTIMICROBIAL ACTIVITIES OF PAWPAW LEAF EXTRACT ON BACTERIA ISOLATED FROM MEAT

BY

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CERTIFICATION

We hereby certify that this work title "Comparative Antimicrobial Activities of Pawpaw Leaf Extract on Bacteria Isolated from Food" was carried out by **Unuigbokhai Naomi Ikheme** with Matriculation Number **AST/2382070272**undxer our supervision in the Department of Biological Science Laboratory Technology (Biology/Microbiology Option), Auchi Polytechnic Auchi, Edo State.

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DEDICATION

This project work is dedicated to God Almighty who has been my source of help and giver of all wisdom, knowledge and understanding for making this project a success.

ACKNOWLEDGEMENT

I give thanks to God Almighty the maker of the universe for our lives and also making us to accomplish our goal in life, also for giving us the strength and determination to put this project work together.

Special regards to my parent **Mr. and Mrs.Unuigbokhai**for their love support throughout my stay in school. And to my friends, siblings who have made this project a great success.

I will never fail to acknowledge the effort of my project supervisor, my dear father in the person of **Mr. Audu J.** for the love and time spent in supervising this project work, I say may Almighty God bless you sir (Amen).

And lastly I wish to acknowledge the effort of all the entire Biological Science Laboratory Technology lecturers, for their contribution towards my academic pursuit in this great citadel of learning.

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ABSTRACT

Microbial contamination of meat may result in spoilage and infection / intoxication to consumers hence the need to eliminate microorganisms from meat. This can be achieved by physical and chemical means, as well as the use of natural and cheaper antimicrobial substances such plant extracts. In this study, the antimicrobial activity of pawpaw(Carica papaya)leaf extract on bacteria isolated from meat was evaluated. The antimicrobial potentials of hot water extract of pawpaw(Carica papaya)leaf on bacteria isolated from meat was measured and compared with antimicrobial activity of azithromycin (antibiotic) using agar well diffusion test. The results showed that the bacteria isolated from meat were identified as Escherichia coli, Staphylococcus aureus, Staphylococcus sp; Klebsiella sp; Pseudomonas aeruginosa; and Bacillus sp. The zones of inhibition on Escherichia coliranged from 2.0 to 3.3mm; Klebsiella species ranged from 2.0 to 2,8mm; Pseudomonas aeruginosa ranged from 2.2 to 3.0mm; Staphylococcus species ranged from 2.3 to 3.7mm; Staphylococcus aureus ranged from 1.7 to 3.2mm; and Bacillus species ranged from 2.0 to 2.8mm. The zones of inhibition of azithromycin ranged from 11 to 15.5mm.The minimum inhibitory concentration (MIC) of Escherichia coliand Staphylococcus species is 30mg/ml; Pseudomonas aeruginosa is 40mg/ml; Staphylococcus aureus is 50mg/ml; and Klebsiella and Bacillus is 60mg/ml. The findings of this work showed aqueous leaf extract of pawpaw posses' antimicrobial activity similar to but not as strong as that of the antibiotic, azithromycin. Based on the MIC and zones of inhibition of the extract as well as its broad-spectrum activity against meat bacteria, they could be used to control meat spoilage and prevent food poisoning and other food borne diseases.

CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

We preserve food by using preservative, and in most cases chemical preservatives are used and some of these chemical preservative are poisonous hen**ce** the need for plant extract is important.

In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. Therefore, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire. 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, 2004).

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. 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, 2001).

Some of the active principles singly or in combination inhibit greatly the life processes of microbes, especially the disease causing ones. They do this by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available

interests to the microorganisms, other causes inflammation analysis of microbial cells (Garrod *et al.,* 2005). The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent. Since medicinal plants do not merely save people from feeling pain but also permit them to emerge unscathed, then they deserve investigation (Jigna and Sumitra, 2006).

The active components in these medicinal attribute are expected to be inimical to the growth of at least some microorganisms especially the disease causing ones e.g. *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* etc. therefore, many studies and researches had been done on the antimicrobial properties of many plants but for this study, the leaf of *Carica papaya* will be discussed

Carica papaya belongs to the family Caricaceae. It has the following common names; pawpaw tree, papaya, papayer, tinti, pepol, chich put, fan kua, wan shou kuo, kavunagaci, kepaya etc. The parts that are usually used include the leaves, fruit, seed, latex, and root. The plant is described as a fast growing, erect, usually unbranched tree or shrub, 7-8m tall with copious latex, trunk of about 20cm in diameter. The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. It is distributed throughout Asia, Nigeria etc (Afolayan, 2003). Carica papaya contains many biochemically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain is used in the treatment of arthritis. The leaves of Carica papaya is used as soap substitute which are supposed to remove stains. (Omojasola and Awe, 2004).

It has milk-clotting (rennet) and protein digesting properties. Active over a wide pH range, papain is used in medicine, combating dyspepsia and other digestive orders. In liquid preparations, it has been used for reducing enlarged tonsils. Nearly 80% of American beer is treated with papain, which digests the precipitable protein fragmented and then the beer remains clear on cooling (Oyagade *et al.,* 2009). Papain is also used for degumming natural silk. But most of the papin imported in the U.S is used for meat-tenderizers and chewing gums. Also used to extract the oil from tuna liver cosmetically, it is used in some dentifrices, shampoos and face-lifting preparations. Use to cleat silks and wools before dying and to remove hair from hides during tanning. Itis also used in the manufacture of rubber from heaven (Sofowora, 2008).

1.2 Statement of the Problem

Chemical preservatives are expensive and they are also toxic hence the need for the research to help screen for new preservative that are not poisonous and expensive just like pawpaw leaf extract.

1.3 Purpose of the Study

1. To know the nature attached of microorganisms we have in food.
2. To increase the shelf life of food.
3. And to prevent the transmission of food borne diseases.

1.4 Research Questions / Hypothesis

1. Does pawpaw leaf extract has an antimicrobial activities?
2. How beneficial is pawpaw leaf extract to human?

Ho: There is no difference in the antimicrobial activities of paw – paw leaf extract and the antibiotic, azithromycin

Hi: There is difference in the antimicrobial activities of paw – paw leaf extract and the antibiotic, azithromycin

1.5 Scope of the Study

1. To isolates and identify bacteria from pawpaw leaf extracts from food
2. To determine the percentage occurrence of the isolates and
3. To determine antibiotic susceptibility of the isolates.

1.6 Significance of the Study

Pawpaw leaf extractfrom isolated food is often consumed as an extract, tea, or juice and has been found to treat symptoms related to dengue fever. Other common uses include reducing inflammation improving blood sugar control, supporting skin and hair heath and preventing cancer.

CHAPTER TWO

2.0 Literature Review

2.1 Description of Papaya

Carica papaya is an herbaceous succulent plant popularly known as pawpaw, and belongs to the Caricaceae family. It is native to the tropics of the Americas but now is widely cultivated in other tropical regions of the world for its edible melon-like fruit, which is available throughout the year. Different parts of the plant are employed in the treatment of different human and veterinary diseases in various parts of the world (Attar *et al.*, 2004)

Carica papaya belongs to the family Caricaceae. It originated from Central America, and is now grown in all tropical countries and many subtropical regions of the world. It lives for about 5–10 years, and normally grows with a single unbranched trunk. The leaves are palmately-lobed, up to 75 cm across, on long, hollow petioles. The blades are divided into five to nine main segments, bearing prominent yellowish ribs and veins. The flowers are born on inflorescences which appear in the axils of the leaves. Generally, the fruit is melon-like, round or long, and may contain more than 1000 seeds. The skin is smooth and green, but turns yellow or orange when ripe (Auria *et al.*, 2002).

Figure 1: Carica papaya
Source: (Attar *et al.*, 2004).

2.2 Nutritional Value of Caripa Papaya Leaf

The papaya, papaw, or pawpaw is the fruit of the plant *Carica papaya*, the only species in the genus Carica of the plant family Caricaceae. It is native to the tropics of the Americas. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The leaves are large, 50–70 cm in diameter, deeply palmately lobed, with seven lobes. The tree is usually unbranched, unless lopped. The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has attained amber to orange hue (Baybutt *et al.*, 2005).

These nutritional values of papaya help to prevent the oxidation of cholesterol. Papaya is rich in iron and calcium; a good source of vitamins A, B and G and an excellent source of vitamin C (ascorbic acid). The extracts of unripe C. papaya contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids (Bayer and Stockigt, 2004).

2.3 Health Benefits of Papaya Leaf

Papayas offer not only the luscious taste and sunlit colorof the tropics, but are rich sources of antioxidant nutrientssuch as carotenes, vitamin C and flavonoids; the Bvitamins, folate and pantothenic acid; and the minerals, potassium and magnesium; and fiber. Together, thesenutrients promote the health of the cardiovascular systemand also provide protection against colon cancer. Inaddition, papaya contains the digestive enzyme, papain,which is used like bromelain, a similar enzyme found inpineapple, to treat sports injuries, other causes of trauma,and allergies (Cho *et al.,* 2004).

Protection against Heart Disease: Papaya's may be very helpful for the prevention of atherosclerosis and diabetic heart disease. Papayas are an excellent source of vitamin C as well as a good source of vitamin E and vitamin A (through their concentration of pro-vitamin A carotenoid phyto nutrients), three very powerful antioxidants. These nutrients help prevent the oxidation of cholesterol. Only when cholesterol becomes oxidized it is able to stick to and build up in blood vessel walls, forming dangerous plaques that can eventually cause heart attacks or strokes. One way in which dietary vitamin E and vitamin C may exert this effect is through their suggested association with a compound called Paraoxonase, an enzyme that inhibits LDL cholesterol and HDL cholesterol oxidation Binns CW (Cho *et al.,* 2004).

Promotes Digestive Health: The nutrients in papaya have also been shown to be helpful in the prevention of colon cancer. Papaya's fiber is able to bind to cancer-causing toxins in the colon and keep them away from the healthy colon cells. In addition, papaya's folate, vitamin C, beta-carotene, and vitamin E have each been associated with a reduced risk of colon cancer. These

nutrients provide synergistic protection for colon cells from free radical damage to their DNA. Increasing your intake of these nutrients by enjoying papaya is an especially good idea for individuals at risk of colon cancer Baybutt RC (Cantin *et al.*, 2009).

Immune Support: Vitamin C and vitamin A, which is made in the body from the beta-carotene in papaya, are both needed for the proper function of a healthy immune system. Papaya may therefore be a healthy fruit choice for preventing such illnesses as recurrent ear infections, colds and flu Rebecca (Ensminger, 2006).

Protection against Macular Degeneration: Mother's especially mentions to children that taking carrots would keep your eyes bright as a child, but as an adult, it looks like fruit is even more important for keeping your sight. Data reported in a study published in the Archives of Ophthalmology indicates that eating 3 or more servings of fruit per day may lower your risk of age-related macular degeneration (ARMD), the primary cause of vision loss in older adults, by 36%, compared to persons who consume less than 1.5 servings of fruit daily (Clifford, 2009).

Protection against Rheumatoid Arthritis: While studies suggested that high doses of supplemental vitamin C makes osteoarthritis, a type of degenerative arthritis that occurs with aging, worse in laboratory animals, another indicates that vitamin C-rich foods, such as papaya, provide humans with protection against inflammatory polyarthritis, a form of rheumatoid arthritis involving two or more joints. The findings, presented in the Annals of the Rheumatic Diseases were drawn from a study of more than 20,000 subjects and focused on subjects who developed inflammatory polyarthritis and similar subjects who remained arthritis-free during the follow-up period. Subjects who consumed the lowest amounts of vitamin C-rich foods were

more than three times more likely to develop arthritis than those who consumed the highest amounts (Clifford, 2009).

2.3.1 Other Benefits of Papaya and its Contents

Industrial Applications of Papain: The papain present in the latex of Carica papaya has been extensively studied and was found to be an enzyme of industrial use and of high research interest. Among the major applications of papain are its use in the food industry, beer clarification, meat tenderizing, Preparation of protein hydrolysates and others Jonas Contiero (Guorong *et al.*,2009).

Wound Healing Activity of Carica Papaya: Carica Papaya is used as food or as medication in folk medicine; papain is the active principle in carica papaya that exerts the ulcer-protective effect reduces gastric acid secretion induced by histamine in rats. Carica papaya fruit extract possess antibacterial antioxidant anti-inflammatory activity antifertility antihypertensive agent chronic skin ulcer therapy and diuretic effects (Gayosso *et al.*, 2010).

2.3.2 Cosmetic Benefits of Papaya

Rubbing the white pulp of raw papaya improves pimples as well as wrinkles. Papaya works as a good bleaching agent. It is an important ingredient in bath soaps, astringents, detergent bars and hand washes. Home Recipe for Papaya Skin Lightner Experts suggest that papaya can help in removing dead worn-out skin cells and replace it with healthy new cells, thereby lightening the color of our skin. For this, one can prepare a paste of raw papaya and apply it on the skin once for few days (Clifford, 2009).

2.4 Allergies and Side Effects of Caripa Papaya Leaf

Papaya is frequently used as a hair conditioner, but should be used in small amounts. Papaya releases a latex fluid when not quite ripe, which can cause irritation and provoke allergic reaction in some people. The latex concentration of unripe papayas is speculated to cause uterine contractions, which may lead to a miscarriage. Papaya seed extracts in large doses have a contraceptive effect on rats and monkeys, but in small doses have no effect on the unborn animals. Excessive consumption of papaya can cause carotenemia, the yellowing of soles and palms, which is otherwise harmless. However, a very large dose would need to be consumed; papaya contains about 6% of the level of beta carotene found in carrots (Chavez, 2011).

a. **Toxicity:** Externally the papaya latex is an irritant to the skin and internally it causes severe gastritis. Some people are allergic to various parts of the fruit and even the enzyme papain has its negative properties.
b. **Skin Discoloration:** Eating too much of a yellow, green or orange colored food that contains beta carotene can cause a benign form of skin discoloration called carotenemia. The palms of the hands and soles of the feet are the most visible areas of the body affected by carotenemia. Cutting back on your papaya consumption will resolve the discoloration of the skin.
c. **Free Radical Scavenging Acivity:** Papaya has many phenolic groups which may scavange free radicals. Aqueous extract of papaya leaves shows anti-oxidant activity.
d. **Respiratory Distress:** Papain is also a potential allergen, according to Purdue University, people who eat too much papaya and ingest high levels of papain may develop symptoms consistent with hay fever or asthma, including wheezing, breathing difficulties and nasal congestion.

e. **Gastrointestinal Symptoms:** Ironically, the same papain that calms your stomach can cause an upset stomach when taken in large amounts. The high fiber content of papaya can also contribute to unrest of the digestive system. The latex of the fruit's skin can also cause irritation of the stomach.

2.5 Anti-Microbial Substances in Papaya Leaves

The bioactive compound of leaf extracts of Carica papaya was extracted, using water and organic solvents, and was investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The aqueous extracts of the root extracts did not show significant activity, but the organic extracts had significant activity with the methanol extracts demonstrating the highest activity against the test bacteria. The root extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against Pseudomonas aeruginosa while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (4.2 mm zone of inhibition) demonstrated against Pseudomonas aeruginosa. Increase in temperature enhanced the activity of the extracts, while alkaline pH decreased the activity (Junaid *et al.*, 2006).

2.6 Microbial Contamination of Food

Contamination refers to a condition of being inappropriate for use due to presence of undesirable elements. Food contamination can occur due to the existence of foreign particles, such as chemicals, insects, and microbes in the food.The presence of microbes in certain foods is

necessary and obviously not obnoxious. Microbial food contamination can be more precisely explained as some unwanted microbes present in a particular food. The common microbial contaminants are Pseudomonas, Listeria monocytogenes, Salmonella sp., Shigella flexneri, Vibrio cholerae, Bacillus sp., and Campylobacter jejuni. Microbial biofilms are a great threat to the food industry because most of the microbes are capable of forming biofilms in the presence of even minimal amount of moisture and nutrients. The source of food contaminations is due to clinical infections resulting from the biofilm by pathogenic microbes of food industry. Prevention of the microbial contamination is essential to decrease the rate of food-borne diseases. The contamination can be controlled by proper cleaning and sanitization (Latha and Kannabiran, 2006).

2.6.1 Types of Food Contamination

There is some dispute regarding how many different types of food contamination there are, with some saying there are three and others declaring four. Both cover the multiple incidences that could occur. The three types of contamination are biological, physical, and chemical. However, for the purpose of this article, we will discuss four categories. These include chemical contamination, physical contamination, microbial contamination, and allergen contamination (Aruna, 2021).

2.6.1.1 Chemical Contamination: Chemical contamination is when the food becomes contaminated by some form of chemical. It is the most difficult type of contamination to control, and it could potentially result in acute poisoning and long-term diseases. Symptoms of chemical contamination can range drastically. In most cases, the consumer will experience some form of mild gastroenteritis, but in some situations, chemicals in food can be lethal.In recent years, there

has been a wider interest in the impact of chemicals in our food and the potential effects on consumer health and wellbeing. For example, studies have shown that prolonged exposure to low levels of carcinogens can increase a person's likelihood of being diagnosed with cancer.

2.6.1.2 Natural chemical contamination: This refers to the existence of chemicals that occur naturally in food. These are regulated, and the government has prescribed minimum limits for those considered harmful. Thus, food production and manufacturing businesses have a responsibility to ensure there are measures in place to prevent products from exceeding these limits.

2.6.1.3 Artificial Chemical Contamination: This results from food being contaminated with a chemical that is not a natural by-product of the food. This could include chemicals used for cleaning and disinfection, fertilisers, and pesticides, amongst others.

2.6.1.4 Physical Contamination: Physical contamination refers to food that has been contaminated by a foreign object. Finding random objects in our food is certainly off-putting, and it is definitely something that causes concern for consumers. Food that has been contaminated by a physical object could directly pose as a choking risk and cause serious injury. Furthermore, the object may also carry bacteria, which could cause microbial contamination at the same time.The most common objects to contaminate food include glass, hair, metal, jewellery, dirt, and fingernails. Physical contamination of food may also be from the environment including the building and the equipment you are using, such as plaster, flakes of paint, and screw fixings. Moreover, physical contamination could also occur because of issues with packaging, such as staples, string, polythene, and cardboard. However, some physical contamination can occur naturally, like insects entering fruit and vegetables or bones in boneless

fish. However, regardless of whether it is a natural component of the food, businesses must still find out how it got there and how to avoid reoccurrence of the incidents.

2.6.1.5 Microbial Contamination: Microbial contamination, also known as biological, is the most common cause of food poisoning. It is basically the existence of harmful pathogens in food, like microorganisms, bacteria, viruses, mould, fungi, and toxins. This is the leading cause of food-borne illness and food poisoning, and food spoilage or waste is the most common cause of it. Chilling food causes the pathogens to become dormant but does not necessarily prevent the growth of bacteria. To ensure bacteria are destroyed during the cooking process, foods should be cooked thoroughly to the right temperature. However, it is noted that many microbial toxins are heat resistant and spoiled food should not be cooked and consumed. The most common food-borne illnesses include norovirus, salmonella, listeria, e.coli, and campylobacter, and symptoms can range from mild gastro issues to fatal and long-term diseases.Microbial contamination can occur due to either direct or cross-contamination. Direct contamination is a result of the pathogens already produced in the food reaching unsafe levels. An example of this would include the bacteria and toxins found in spoiled meat. Whereas cross-contamination is when pathogens enter food from other sources and multiply to unsafe levels.

2.7 Implications of Microorganisms in Food

Microbial activities during the last 5,000 to 10,000 years a variety of techniques (such as drying, salting, heating, fermentation, refrigeration, or freezing) evolved empirically and contributed to the increased shelf-life of plant and animal foods. These techniques, which controlled microbial activity to a greater or lesser extent, were applied before the mechanism of their effect was understood. In the early 1800s Francois Nicholas Appert was awarded a patent for a practical

method of food preservation, namely, "canning." Since that time, and particularly during the last 40 years, new processes have been developed to extend shelf-life of foods. Although some others may have suggested microbial involvement in food spoilage at earlier dates, it was Louis Pasteur who in the mid-1800s first established a scientific basis for the direct relationship between food spoilage and microbial activity. Microorganisms responsible for foodborne diseases were first recognized around 1880. Since that time, the number of microbial agents recognized as involved in foodborne illness has increased steadily (ICMSF, 2005)

Microorganisms are of great significance to foods for the following reasons:

1. Microorganisms can cause spoilage of foods
2. Microorganisms are used to manufacture a wide variety of food products, and
3. Microbial diseases can be transmitted by foods.

2.7.1 Food Spoilage

Food can be considered as a medium for microbial growth. Considering the vast array of sources, substances, and methods with which food is produced, practically every kind of microbe is a potential contaminant. Given a chance to grow, microbes will produce changes in appearance, flavour, odour, and other qualities of the food. The changes vary according to the type of food degraded but can be summarized by examining the fates of the major nutrients found in food: proteins, carbohydrates, and fats.Protein-containing foods, particularly meats, are putrefied by organisms (e.g., Proteus, Pseudomonas, and Clostridium bacteria) that break down the long peptide chains of proteins into amino acids and foul-smelling compounds such as amines, ammonia, and hydrogen sulfide (H2S).

Carbohydrates (sugars and starches) are fermented into acids (e.g., the acetic acid in vinegar), alcohols, and gases, especially carbon dioxide. This process is responsible for the bursting of spoiled chocolate cream candies by yeasts (Garcha, 2018).

2.7.2 Food Intoxication

Botulism: Botulism, a neuroparalytic disease paralyzing or weakening skeletal muscle in the body, is caused by the ingestion of food containing toxin produced by the bacterium Clostridium botulinum. It is associated with the consumption of poorly preserved and improperly handled foods. C. botulinum is a grampositive, rod shaped, spore-forming and anaerobic bacterium that is commonly found in soils, aquatic sediments, rotting vegetation and digestive tracts of animals and birds. It is a common contaminant of fishes, occurring in their intestinal tracts. Many vegetables and fruits can easily become contaminated with spores of bacterium because they are often in contact with soil. The toxin (an exotoxin) produced by the bacterium is a protein neurotoxin, called botulinum neurotoxin (BoNT) or simply botulinum toxin, which is most potent toxic substance in nature. There are four types of botulism:

a. Food-borne botulism, an intoxication caused by ingest
b. Wound botulism, which occurs due to the production of BoNT in vivo after growth of C. botulinum in an infected wound
c. Infant botulism, which results from the production of BoNT in vivo in the intestinal tract of an infant colonized with C. botulinum, and
d. Botulism due to the intestinal colonization in children older than infants and in adults in which no food or wound source is implicated (Hatheway and Johnson, 2008).

Staphylococcal Intoxication: Staphylococcal food poisoning (SFP) or intoxication is one of the common types of food-borne disease which is caused by the ingestion of foods containing the thermostable enterotoxins produced by certain strains of the bacterium Staphylococcus aureus. S. aureus is a facultative anaerobe, gram-positive coccus, non-motile, and catalase and coagulase positive. It is widely distributed in air, dust, sewage, water, foods, humans and animals. Humans and animals are the primary reservoirs for this microbe. Itis present as normal microflora of human beings and colonizes skin. But, it can cause various infections and diseases such as boils, carbuncles, bullous impetigo, toxic shock syndrome, scalded skin syndrome, enterocolitis, osteomyelitis and food poisoning. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipments used in food handling and environmental surfaces can also be sources of contamination with S. aureus. It is usually transferred to the foods and food products due to their poor handling. The growth of the bacterium is favoured by proteinrich foods with high salt content. Foods that are frequently incriminated in staphylococcal food intoxication include meat and meat products, poultry and egg products, fish and fish products, salads, milk and milk products, and cream-filled bakery products. If the contaminated food is stored at temperature that encourages the growth of these organisms, production of enterotoxins occurs in the food and after ingestion of this food it will spread rapidly in humans. Staphylococcus aureus is able to grow in a wide range of temperatures (7 to 48.5°C with an optimum of 30 to 37°C), pH (4.2 to 9.3 with an optimum of 7 to 7.5) and sodium chloride concentrations up to 15% (Schmitt *et al.*, 2012).

Bacillus cereus Intoxication: Bacillus cereus is a gram-positive, spore-forming, motile, aerobic, rod-shaped bacterium that is commonly found in soil and on vegetation. In addition to food poisoning, it causes opportunistic infections. It easily spreads and colonizes a wide range of

foods and food products of both plant and animal origin (e.g. meat, eggs and dairy products). Due to the significant difference in the amount of enterotoxin produced by different strains of B. cereus, the total infective dose seems to vary between about 105 and 108 viable cells or spores (Granum, 2009). Spores of B. cereus generally survive cooking. B. cereus is known to produce one emetic toxin and three different enterotoxins. Two types of B. cereus food poisoning have been reported. The first type, caused by an emetic toxin, produces nausea and vomiting, while the second type, caused by enterotoxins, results in diarrhea (Agata, 2007).The diarrhoealtype of food-poisoning is characterized by abdominal cramps and pain with diarrhea.

2.8 Control of Microorganisms in Food

Food contaminated by microorganisms (bacteria and yeasts), viruses, and protozoa can cause severe disease in humans. There are two categories of foodborne diseases.First, food poisoning is caused by the presence of microbial toxins in food products, e.g. by Staphylococcus aureus, Clostridium perfringens (both produce enterotoxins which elicit enteric disease such as diarrhea), and Clostridium botulinum(botulism is the most severe type of food poisoning).

Second, the growth of microorganisms in the body after eating contaminated food, e.g. by Salmonella spp. (salmonellosis) and Campylobacter jejuni (high fever, abdominal cramps). Many human pathogens are transmitted by fecal contaminated water, the most important being Salmonella typhi (typhoid fever) and Vibrio cholerae (cholera).

Hygiene: Generally refers to the set of practices associated with the preservation of health and healthy living. The focus is mainly on personal hygiene that looks at cleanliness of the food.Improvements in personal knowledge, skill and practice that modify an individual's behavior towards healthy practice are the focus of hygiene promotion. Safe hygiene practice

includes a broad range of healthy behaviors, such as handwashing before eating and after cleaning a child's bottom, and safe faeces disposal. When you carry out hygiene education and promotion the aim is to transfer knowledge and understanding of hygiene and associated health risks in order to help people change their behaviour to use better hygiene practices.

Environmental Hygiene: Thorough environmental hygiene is important for the prevention of transmission of microbial contaminant within food settings. Environmental hygiene encompasses effective cleaning of surfaces using appropriate products, decontamination of equipment and devices used in food production procedures, safe and appropriate handling of sharps, blood and body fluid spills, waste and linen.

2.8.1 Chemical Preservation

There are three classes of chemical preservatives commonly used in foods:

1. Benzoates (such as sodium benzoate)
2. Nitrites (such as sodium nitrite)
3. Sulphites (such as sulphur dioxide)

Benzoates (such as sodium benzoate)

Sodium Benzoate is used as a preservative to prevent food from molding. It helps keep our products shelf-stable for at least two years from the date of purchase and is used in concentrations of less than 0.5% by volume.

While sodium benzoate is considered safe, scientists have shown that negative side effects occur when it's mixed with ascorbic acid (vitamin C). Their studies indicate that it then turns into benzene, a known carcinogen that may cause cancer.

Uses of Sodium Benzoate

Food. In the food industry, sodium benzoate is used to prevent spoilage from harmful bacteria, yeasts, and molds. It also helps maintain freshness in food by helping to slow or prevent changes in color, flavor, PH, and texture.

Nitrites (such as sodium nitrite)

Nitrates and nitrites are chemicals found naturally in soil and water as part of the earth's nitrogen cycle. We find these ingredients naturally in many vegetables, and we add them to meat products to help keep them fresh.

Chemically they are expressed below:Subscribe for weekly updates_ go.msu.edu/cris-connect

1. Nitrate: 1 nitrogen atom, bonded to 3 oxygen atoms: NO_3-
2. Nitrite: 1 nitrogen atom, bonded to 2 oxygen atoms: NO_2-
3. Nitrates can be transformed into nitrites through digestion when bacteria in the mouth and enzymes in our body breakdown the nitrates into nitrites.
4. Nitrites typically transform into two distinct compounds in our body:
5. Nitric oxide, which is good for human health.
6. Nitrosamines, which can cause harm to human health depending on the level of exposure.

Sulphites (such as sulphur dioxide)

Sulfur dioxide (added to wine as potassium or sodium metabisulphite or as gaseous SO2) is an antimicrobial compound and antioxidant that has for centuries been used to preserve wine by preventing the growth of undesired microorganisms.

Sulfur dioxide is a common combustion product from the burning of coal or oil products and as a result it has been a major contributor to atmospheric corrosion in urban and industrial areas. It is moderately water soluble and forms sulfurous acid in water:

(1) Sulfurous acid is a moderately strong acid and, in addition, tends to react with air or ozone to form sulfuric acid

(2) Both of these acids are very corrosive to metals including steel, zinc, and nickel. Limits have now been established for sulfur dioxide emissions in much of the world so that the effect of sulfur dioxide has been progressively decreasing in most of the industrialized nations. Electric power generation is a major source of sulfur dioxide emissions in spite of a variety of technologies to minimize this problem. Sulfur dioxide is absorbed in dew and tends to keep the pH of the dew below its natural value of 5.2 (from CO2 in the air).

Sulfur dioxide in air can be measured by conventional techniques such as infrared absorption. It is also possible to use a lead peroxide absorbent to determine a deposition rate of sulfur dioxide on a surface.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

The materials used for this project work include test tubes, petri dishes, nutrient agar, MuellerHinton agar,MuellerHintonbroth, peptone water, bunsen burner, autoclave, blender, incubator, hot air oven, refrigerator, microscope, inoculating loop, Pipette, measuring cylinder, test tubes, pipettes, aluminum foil, cork borer,Whatman filter paper, membrane filter, water bath, cotton wool, chemical balance, conical flask, beaker, distilled water, metre rule, meat(beef), *Carica papaya* leaves, ethanol (95%), ethanol (70%),azithromycin disc (25µg)

3.2 Sterilization of Glass Wares and Apparatus

All glass wares were properly washed and rinsed with clean water. The method of sterilization adopted was the use of hot air oven at 170°C for 2hours.The inoculating loop was sterilized using the flame from a Bunsen burner. The flaming of the wire loop was repeatedly done at the end of every inoculation. The table top was disinfected using ethanol (70%). Media used were sterilized using an autoclave.

3.3 Media Preparation

3.3.1 Preparation of Nutrient Agar

Dehydrated nutrient agar powder (14g) was weighed with a chemical balance and poured into a conical flask containing 500ml of distilled water. The mixture was stirred with sterile spatula and placed over a bunsen burner flame on a tripod stand to fully dissolve the entire powdered agar.

The dissolved mixture in the flask was covered with cotton wool and aluminum foil , placed in an autoclave and was sterilized at 121^{o}C for 15 minutes. Once the sterilization was complete the autoclave was allowed to cool and the molten agar was brought out and allowed to cool to about 45^{o}C.

3.3.2 Preparation ofMueller-Hinton agar

Mueller-Hinton agar base powder (16.8g) was weighed with a chemical balance and transferred into a conical flask containing 500ml of distilled water. The mixture was stirred with sterile spatula and placed over a Bunsen burner flame on a tripod stand to fully dissolve the entire powdered agar. The dissolved mixture in the flask was covered with cotton wool and aluminium foil and placed in an autoclave and was sterilized at 121^{o}C for 15 minutes.

3.4 Collection of Meat Samples

Freshly slaughtered meat (beef) samples were collected Jattu abattoir in sterile wide-mouthed flask previously plugged with cotton wool wrapped with aluminum foil to prevent possible contamination during transport to the laboratory. The samples were properly labeled, kept in icebox, and transported. The *Carica papaya* (pawpaw) leaves used in this project work was collectedinasterilepolythenebag,rinsed,sundried for 4daysandmadeintoapowderyform with electric blender beforeuse.

3.5 Isolation and Identification of Bacteria

In the laboratory, ten gram (10g) of the meat sample was weighed and aseptically taken into a sterile jar containing 90 mlsterile normal saline and homogenized with sterile blender (Retsch, GM 200,Australia) at 3000 rpm for 5-10 min. A 1.0ml aliquot of homogenate was successively

transferred to series of test tubes containing 9.0 ml sterile distilled water to make dilutions up to 10^{-5}.

From each dilution, 1.0 ml was transferred aseptically into the appropriately labeled sterile petri dishes using different pipette for each dilutions. Then about 20 ml of the cooled molten agar (45^{o}C) was poured aseptically into the labeled dishes containing the samples and was gently swirled to evenly distribute the sample. The plates were inverted and incubated at 37^{o}C for 24 hours. Coloniesidentified asdiscreteonnutrientagarwerecarefully examined macroscopically (using hand lens) for cultural characteristics such as the shape, colour, size, and consistency.Gramstainingaswellasappropriatebiochemical tests was carried out according to the standard procedure(Oyeleke and Manga, 2008). The isolates were identified by comparing their morphological and biochemical characteristics with standard referenceorganismsofknowntaxa,asdescribedinBergey's Manual for Determinative Bacteriology (Buchanan and Gibbons, 1984).

3.5.1 Gram Stain

Test organisms were heat-fixed on slides and flooded with crystal violet for about 60 seconds and rinsed with water for about 5 seconds. The slides were then flooded with iodine solution for about 60 seconds and rinsed with water. Ethanol was then added as a decolourizer and rinsed with water afterwards. Finally, the slides were flooded with saffranin for about 60 seconds, rinsed with water, blotted dry, and viewed under a microscope. Gram-positive organisms appeared blue/purple under the microscope while Gram-negative organisms appeared red/pink. The cell shapes were also viewed under this procedure.

3.5.2 Capsule stain

Make a smear of the bacterial isolates, air dry, place the slide on a beaker of boiling water and cover the smear with 1.0% aqueous solution of crystal violet for one minute. Wash with 20% copper sulphate solution, blot dry and view under oil immersion of microscope. Capsules appear as faint blue-violet zone surrounding purple bacterial cell.

3.5.3 Spore stain

Make smears of the isolates culture, air dry and heat fixed properly. Flood the slide with malachite green and heat over a beaker of boiling water for 5 minutes. Wash with clean water and counterstain with safranin for about 30 seconds. Wash with water, blot dry and examine the slide under oil immersion objective of the microscope. The presence of endospores is indicated by green spot in a red background.

3.5.4 Motility test

This is a test done to differentiate motile from non-motile organisms. A wire loop was used to inoculate a motility medium by making a stab to the bottom of the tube and afterwards incubated for 24-48 hours. If the organism is motile, the tube will appear cloudy the organisms will spread out of the stab line. Non-motile organisms will grow along the streak line only and the media will not be cloudy.

3.5.5 Oxidase Test

Fresh growth is removed from the agar plate using a non-metallic instrument such as a sterile plastic inoculating loop or a sterile swab or wooden splint. The oxidase test strip is moistened slightly with oxidase reagent and the growth is rubbed into the moistened paper of the strip. If the microbe has cytochrome oxidase, it will add electrons to the reagent, changing it from its colourless appearance to a deep indigo blue in a matter of 10-20 seconds. Waiting any long than

this increases the likelihood that the reagent turns blue due to natural chemical changes caused by exposure to air. If the colour does not turn blue within 20 seconds, the test is negative for the presence of oxidase.

3.5.6 Coagulase Test

A drop of sterile distilled water was placed on each end of a sterile slide. A colony of the test organism was emulsified on each spot to make two thick suspensions. A loop-full of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension, which served as control.

3.5.7 Citrate utilization test

Aseptically prepare slants of Simmons's citrate agar in capped tubes, inoculate with each isolate and incubate at 370C for 3-4 days. A positive result is indicated by change in medium colour from green to blue.

3.5.8 Indole Test

Tryptophan broth was inoculated with test organism and incubated for 24 hours. Drops of Kovacs Reagent were then added to the broth. Formation of a red ring at the surface of the broth signified a positive result.

3.5.9 Catalase test

Two drops of hydrogen peroxide was place on the surface of clean grease free glass slide A and B (control).Using a clean glass rod the test organism was transferred to A; and the gas bubbling or effervescence indicates a positive reaction.

3.5.10 Sugar Fermentation Test (glucose, lactose sucrose and manitol)

The basal medium used was sterilized peptone water with a drop of phenol red added as p^H indicator. Also added is 1.0% of sugar prepared in 99ml of peptone water and sterilized in the autoclave at 121^0C for 15minutes. A loopful of pure culture of the test organism was inoculated into the sterile solution of sugar and incubated at 37^0c for 48 hours. At the end of the incubation, the formation of yellow colour due to acid production and gas production in the Durham tube immersed in the medium indicates a positive result.

3.6 PreparationofPawpaw (*Carica papaya)* Leaf Extracts

The pawpaw (*Carica papaya)* leaf was separately extracted with hotwater using the method as described by Oyagade *et al* (1999). Extraction was carried outby suspending 25grams of the powdered leaves in 125milliliter of distilled water. The hot water extraction was done at 80°C in a water bath for 30 minutes. Theextracts werethen decanted and filteredthrough a Whatman filter paper, the filtered extract was then sterilized using a membrane filter andevaporatedtodrynessat45°C.Theresiduesobtainedwerereconstitutedin95%ethanol by dissolving 20g in 100ml of ethanol to formstockconcentrationof 0.2g/ml (200mg/ml). The extract solution were then stored in the refrigerator at 4°C until required(Omojosola andAwe, 2004). Prior to use, the stock is diluted two-fold by successive transfer of 1.0ml to three tubes containing 1.0ml of 95% ethanol to yield concentrations of 100mg/ml, 50mg/ml and 25mg/ml.

3.7 Antimicrobial Assay of Pawpaw (*carica papaya)*Leaf Extracts

Theantibacterialactivityofcrudeextract was determined by agar-well diffusion method described byIrobi *et al* (1994). The test organisms were standardized to 0.5 McFarland standards by subculture into fresh nutrient broth, incubated at 37^0C and compared with reference

solution to achieve a turbidity containing about 1.5×10^{8} cells/ml (Albert *et al*; 1991). One millilitre (1.0ml) ofthe different standardized organisms were introduced separately andthoroughlymixedwith20mlofmoltenMuellerHinton agareachina sterilePetridishandallowedtoset.Sterilecorkborer of 6mm was used to make 5 wells in the MuellerHinton agar plates. Four of the wellswere filled with 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml concentrations of the extract; while the 5^{th} well contained distilled water as negative control and as positive control, commercially available 6mm single antibiotic disc of azithromycin (25µg) was aseptically placed on the surface of the inoculated agar plates with sterile forceps. These were then left on the work benchfor 1hour for adequate diffusion of the extracts and thereafter incubated at 37°C for 24hours. After incubation, thediameterofthe clearzonesaroundeachwell and discsweremeasuredtothenearestmillimetresalongtwoaxis i.e. 90°toeachotherandthemeanofthetworeadings werethencalculated. The zones of inhibition werethencalculated by subtracting 6.0mm from the diameterofthe clearzones.

3.8 Determination ofMinimumInhibitoryConcentration(MIC) Of Leaf Extracts

The estimation of MIC of the aqueous leaf extract was carriedoutbyusingthemethodofAkinpeluandKolawole(2004).Different concentrations ranging from 20 – 60mg/ml of the extracts by dissolving 2-6g of extract in 100ml of solvent. The different concentrations were introduced intotest tubecontaining9mlof the MuellerHintonbroth.About 1.0ml of the 18 hours standardized organism wasalso introduced into test tubes containing brothand extract.Control test tube containing brothand extract but without organism was also set up. All thetest tubes were incubated for 24 hours

at 37 ^{o}C. Theleast concentration of the extract that did not permit anyvisiblegrowth inthebroth wastakenastheMIC.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Results

A total of sixty-four (64) bacterial were isolated from the beef samples and they were identified as *Escherichia coli*, *Staphylococcusaureus*, *Staphylococcus* sp; *Klebsiella* sp; *Pseudomonasaeruginosa*; and *Bacillus* sp(Table 1).

Table 2 showed that *Escherichia coli*, *Pseudomonasaeruginosa*, *Klebsiella* sp; *Staphylococcusaureus*, *Bacillus* sp; and *Staphylococcus* sp constitute 29.7%, 25.0%, 15.6%, 14.1%, 10.9% and 4.7% respectively of the total bacteria isolated from the meat samples.

The zones of inhibition showed that the antimicrobial activity of the leaf extract on the test organisms increases with concentration. The zones of inhibition on *Escherichia coli*ranged from 2.0 to 3.3mm; *Klebsiella* species ranged from 2.0 to 2,8mm; *Pseudomonasaeruginosa* ranged from 2.2 to 3.0mm; *Staphylococcus* species ranged from 2.3 to 3.7mm; *Staphylococcus* aureus ranged from 1.7 to 3.2mm; and *Bacillus* species ranged from 2.0 to 2.8mm. It also showed that the antibiotic, azithromycin had much higher antimicrobial activity on the organisms than the leaf extract. The zones of inhibition of azithromycin ranged from 11 to 15.5mm (Table 3).

Table 4 showed the minimum concentration of the leaf extract that will inhibit the growth of test organisms. The minimum inhibitory concentration (MIC) of *Escherichia coli*and *Staphylococcus*

species is 30mg/ml; *Pseudomonasaeruginosa* is 40mg/ml; *Staphylococcus* aureus is 50mg/ml; and *Klebsiella* and *Bacillus* is 60mg/ml.

Table 4.1: Characterization of bacteria isolated from meat samples

Tests	***E.coli***	***Klebsiella*** sp	***Pseudomonas****aeruginosa*	***Staphylococcus aureus***	***Staphylococcus sp***	***Bacillus sp.***
Gram stain	- rod	- rod	- rod	+ cocci	+ cocci	+ rod
Capsule stain	-	+	-	-	-	-
Spore stain	NT	NT	NT	NT	NT	+
Oxidase	-	-	+	-	-	+
Catalase	+	+	+	+	+	+
Coagulase	NT	NT	NT	+	-	NT
Citrate	-	+	+	-	-	+
Motility	+	-	+	+	-	+
Indole	+	-	-	+	-	-
Glucose	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+

Lactose	+	+	-	-	-	NT
Manitol	NT	NT	NT	+	-	NT
Growth at 42^0C	NT	NT	+	NT	NT	NT

NT = Not tested + = Positive - = negative

Table 4.2: Distribution of bacterial isolates in meat samples

Bacterial isolates	**Numbers**	**Percentage (%)**
Escherichia coli	19	29.7
Klebsiella species	10	15.6
Pseudomonas species	16	25.0
Staphylococcus aureus	9	14.1
Staphylococcus species	3	4.7
Bacillus species	7	10.9
Total	**64**	**100**

Table 4.3:Antibacterial activityofaqueousextractof*Caricapapaya*leaves againstbacteria isolates

Inhibition zone diameter (mm)				**Bacterial isolate**
200mg/ml	100mg/ml	50mg/ml	25mg/ml	azithromycin
*Escherichia **coli***	3.3	2.8	2.6	2.0	12.4
Klebsiella species	2.8	2.6	2.5	2.0	13.0
Pseudomonasaeruginosa	3.0	2.7	2.5	2.2	12.0
Staphylococcus species	3.7	3.3	2.9	2.3	15.5

Staphylococcus aureus	3.2	2.5	2.3	1 .7	11.0
Bacillus species	2.8	2.3	2.1	2.0	11.6

---------- Negative control (water) = 0mm

Table 4.2 Distribution of bacterial isolates in meat samples

	Inhibition Zone				
Diameter (mm) Bacterial Isolates	**200mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**Azithromycin**
Escherichia coli	3.3	2.8	2.6	2.0	12.4
***Klebsiella* species**	2.8	2.6	2.5	2.0	13.0
***Pseudomonas* species**	3.0	2.7	2.5	2.2	12.0
Staphylococcus aureus	3.7	3.3	2.9	2.3	15.5
Staphylococcus aureus	3.2	2.5	2.3	1.7	11.0
***Bacillus* species**	2.8	2.3	2.1	2.0	11.6

Negative control (water) = 0mm

Table 4.3: Minimum Inhibitory Concentration (MIC) of aqueous leaves extract of Carica papaya on bacterial isolates

Bacterial Concentration (mg/ml)	Minimum inhibitory
Escherichia coli	30
***Klebsiella* species**	60

***Pseudomonas* species**	40
Staphylococcus aureus	30
Staphylococcus aureus	50
***Bacillus* species**	60

4.2 Discussion

The detection of various bacteria and fungi in the meat samples is an indication that meat supports the growth of diverse organisms. Meat is an ideal medium for the growth of microorganisms because of its high moisture content, nitrogenous components, minerals, growth factors, fermentable carbohydrate, and favourable pH. These and other factors such as temperature, oxygen, and the biological structure of the meat influence the type and number of bacteria it can support (Frazier and Westhoff, 2004).

Majority of microorganisms associated with meat are derived from the environment (extrinsic bacteria) and from the gut(intrinsic bacteria).These extrinsic bacteria find their way into meat surfaces through processes such as skinning, cutting,eviscerating,washing, handling and packaging (Baines, 2000); while the intrinsic ones reach meat tissue exclusively by an internal route before or after death and are harboured in organs like the lymph nodes, bone marrow and muscles(Gill,1998).The inner flesh of animals slaughtered under normal hygienic condition are usually sterile, so that spoilage at chill and room temperature will result from growth of organisms on meat surfaces only(Frazier and Westhoff, 2004).

In this study *E.coli* is the most frequently isolated bacteria from meat samples. This supports the findings of Abebe *et al* (2019), who obtain similar results. The presence of *E.coli* and other

coliforms such as *Klebsiella* sp may have been introduced into meat by the failure of handlers to observe basic hygienic and packaging rules. They may also have come from the air, dust, flies and other insects. The use of packaging materials, such as trays, basins and other containers are also possible sources of contamination.The presence of Staphylococcus, especially *Staphylococcus aureus* in some meat samples is a pointer to poor personal hygiene and improper handling (Nwachukwu and Osuofia, 2014). The frequent isolation of these bacteria from the nose and hands of humans supports the findings of previous workers that people carrying *Staphylococcus aureus* in their noses and hands are the main sources of meat contamination via contact and respiratory secretions (Le Loir and Gautier, 2003). The occurrence of *Bacillus* species in the meat samples is most probably due to contamination from dust and air droplets because it is a common inhabitant of soil and it produces endospores which are more resistant to unfavourable environmental conditions.The presence of *Pseudomonas* species in meat samples may be due to contamination with soil and vegetation, because these organisms occur in soil; and surfaces of plants, animals and humans (Field, 2002) .They are versatile bacteria with minimum nutritional requirements and resistance to many antimicrobial agents.

The presence of these organisms in meat may result in spoilage and infection and intoxication to consumers hence the need to eliminate these agents from meat. This can be achieved by physical and chemical means, especially the use of natural and cheaper antimicrobial substances such plant extracts. In this study, the antimicrobial activity of pawpaw leaf extract on meat bacteria was evaluated. The plant extracts show varying degree of activity on Gram positive and negative organisms; they were neither more nor less active in Gram positive than Gram negative organisms. This contradict previous reports that they were more active against Gram-positive bacteria than Gram-negative bacteria (Jignaand Sumitra, 2006); and less active against Gram-

positive bacteria than Gram-negative bacteria (Anibijuwon and Udeze, 2009). The fact that the extracts were active against both Gram-negative and Gram-positivebacteria tested may indicate a broad spectrum of activity. This observation is very significant because of thepossibilityofdevelopingtherapeuticsubstancesthatwillbeactiveagainstmultidrug-resistant organisms.

Based on the inhibition zones and concentration of the extract and azithromycin, the antibiotic was significantly more active on the test organisms than the extract. For example, against *Staphylococcus aureus* 25µg azithromycin produced inhibition zone of 11.0mm, while 25mg extract produced inhibition zone of 1.7mm. The superior activity of azithromycin is because it is a standard antibiotic and it is in pure state (Anibijuwon and Udeze, 2009). The poor activity of the extract could be due to the following reasons;

1. Dryingmethod.Sometimes,activeagentsofleavesaredestroyedbydirectexposuretosunlight, especially iftheactive agentisvolatile.
2. Itcouldalsobeduetothe solventused for extractionandthemethodusedtoobtaintheactivecomponent. Though earlier report that bioactivecompounds in plant extracts are heat stable (Doughari, 2006); hot water used for extraction in this study may have destroyed some active compounds in the leaf. Previous studies have showed that the organicextracts (methanol, acetone and ethanol) were more effective than aqueous extracts. This may be due to the better solubility of the active components in organic solvents (de Boer *et al*.,2005 and Anibijuwon and Udeze, 2009).

Thebasicparameterforthedeterminationofantimicrobial agents with antimicrobial potential is theminimum inhibitory concentration (MIC). The MIC of theextract for test organisms ranged between 30-50 mg/ml. The demonstration of activityagainst the test bacteria provides

scientific bases for the local usage of these plants in the treatment ofvarious ailments. The presence of bioactive substances have been reported to confer resistance to plantsagainst bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by theplant extracts used in this study (Srinivasan *et al*., 2001). Various chemicals such as alkaloids, tannins, saponnin,glycosides, oleic acid and stearic acids whichare naturally present in plants have been implicated intheantimicrobialactivitiesontheplantcontaining them (Popoola *et al*;2007).

CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

The findings of this work have showed that beef samples were contaminated with microorganisms, including coliform bacteria that are of public health concern. It also harbours pathogenic organisms which can cause food poisoning and other food borne diseases. The findings also showed that aqueous leaf extract of pawpaw posses' antimicrobial activity similar to but not as strong as that of the antibiotic, azithromycin. Based on the MIC and zones of inhibition of the extract as well as its broad- spectrum activity against meat bacteria, they could be used to control meat spoilage and prevent food poisoning and other food borne diseases.

5.2 Recommendations

In order to enhance the antimicrobial activity of aqueous leaf extract of pawpaw the following practices are recommended.

1. Sun drying of leaves should be replaced with drying at room temperature for longer duration.
2. Hot water extraction should be replaced by organic solvent extraction using ethanol or methanol or acetone.
3. Locationof plantharvestshouldneverbe fromareastreatedwithinsecticides.
4. Timeofcollection of leavesshouldbewhentheleaforplantsproutsmostandcollectionshouldalsobeduringtheday.
5. Oncedried,the leaves shouldbedated,labeledandstoredinareanotexposedtolight,moistureorheat.
6. All these practices have been speculated to enhance the antimicrobial activity of plant extracts (Bernice, 1997); and further research is recommended.

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