SCREENING OF GINGER AND MORINGA OLIFERA EXTRACTS FOR ANTIMICROBIAL ACTIVITY AGAINST COMMON POST-HARVEST BACTERIAL PATHOGENS ISOLATED FROM STORED YAM (*DIOSCOREA ALATA)*

BY

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CERTIFICATION

I certify that this work was carried out in partial fulfillment of the requirement for a Master of Science Degree in Microbiology under my supervision by Taiwo Kupolati in the Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Osun State, Nigeria.

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DEDICATION

I dedicate this project to the Almighty God, my keeper, sustainer and preserver.

ACKNOWLEDGEMENT

This project would not have been a success without the help of God, my supervisor, Dr. Femi Ayoade, who dedicated so much time to correcting, editing and proofreading and my wife, Mrs. Oluwateniola Kupolati and Kayode Adeyemi, who helped in compiling and proofreading. I will be ever grateful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Yam (*Dioscorea* spp.) is a tuber crop belonging to the family *Dioscoreaceae* with six economically important species. *Dioscorea alata* is the most widely cultivated species in West Africa especially Nigeria. However, pre- and post-harvest spoilage by microorganisms result in the loss of a significant percentage of cultivated yam. Therefore, this study is aimed at isolating and characterizing microbial pathogens of post-harvest rot in yam. Moreover, the antimicrobial effects of *Moringaolifera* and *Ginger officinale* extracts on the isolates was also targeted. Serially diluted samples from healthy, dry and soft rotted yam were inoculated onto nutrient agar and blood agar using spread plate technique. Pure cultures were obtained and identified using biochemical tests. These were further characterized by amplification of 16S rDNA. Isolates were tested for antimicrobial activities by measuring zones of inhibition on Mueller-Hinton agar. Results obtained, showed the presence of known bacterial pathogens of yam rot such as *Streptococcus* sp, *Staphylococcus aureus, Aeromonas* sp, *Enterococcus* sp and *Micrococcus* sp. The antimicrobial effect of *M. olifera* methanoic extracts was low in 12.5% isolates while 87.5% were completely resistant. Antimicrobial effect of *G. officinale* methanoic extracts was low in 12.5% isolates and intermediate in 37.5%. Also, 12.5% isolates showed complete resistance while another 12.5% were susceptible. Furthermore, 62.5% of the isolates were susceptible to the ethanoic extracts of *G. officinale,* while in 25% antimicrobial activity was intermediate and in 12.5%, there was complete resistance.*Moringaolifera* ethanoic extract had low antimicrobial effects in 50% of the isolates, intermediate in 25%, 12.5% were completely resistant, while 12.5% were susceptible. There was no antibacterial activity with water extracts of *M olifera* and *G officinale* against isolates. This study further confirms the efficacy of *Molifera* and *Gofficinale*as antimicrobial agents of known pathogens associated with pre- and post-harvest spoilage of yam.

Keywords: Yam rot, *Dioscorea alata, Ginger officinale, Moringer olifera*, antimicrobial activity, pathogen, spoilage.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 Economic and social impact of yam and world production

The crop yam (*Dioscorea* spp) is one of the common food crops in the tropics and plays vital roles in food security as a staple food in the regions where it is being cultivated (Maroya *et al*, 2012). *Dioscorea* sppoccur in Asia, East Africa, the Caribbean, India and Tonga Kingdom (Figure 1), south pacific as well as West Africa (Okigbo, 2004). (Maroya *et al*, 2012) estimated that yam consumption yearly is over 48million tones globally. As shown in Table 1, out of the 48million tons of yam (95% global supply) that are produced on 4million hectares annually, Nigeria alone produces 67.7% of global yam supply (FAO, 2010). This makes Nigeria the highest consumer of this staple crop after Cassava and Maize. However, demand for this commodity is increasing (Figure 2); as incomes increase consumers shift from substitutes to yam especially when the price of yam relative to price of its substitutes declines (Maroya *et al*, 2012).

Tuber yam, among many other staple foods, is susceptible to postharvest diseases caused by bacteria and fungi under poor storage conditions. In Nigeria, pathogens constitute the major factor to rot in yam production (Nari, 2004). Losses caused by pathogens attack vary from 20-30% generally in some crops (Park *et al*, 2008).

Yam plays an outstanding function in social cultural lives of some producing regions like the celebrated Moon festival and also the popular Yam festival in West Africa, an act that is well observed. In Nigeria for instance, the meals offered to

deity and associates consist mainly of mashed yam (Ogunleye, 2005). Yam storage in comparison with some other staple crops has relatively longer life span, so stored tuber symbolizes stored wealth, which can be sold throughout the year by the marketers. Also, tuber yam in West Africa particularly Nigeria can be converted into different staple transitional and end product forms (Okaka and Aajekwu, 1990; Okaka *et al,* 1991) which can be consumed by human beings and animals, used as the essential ingredient of snacks and flour that is now used in instant puree making (Coursey, 1983; Okaka and Okechukwu, 1987).

Yam can be eaten in a variety of ways as it can be roasted, fried, grilled, baked, barbecued, smoked and most commonly boiled. Yam is also used as food for livestock. Tuber yam can be dried, ground into flour and stored fpor use. However, in Nigeria, a country known to produce a large percentage of yam around the globe, it is believed that the supply of yam tuber is lower than its high demand, a problem currently facing the country (Ogundana, 1971; Okigbo *et al,*2000;Okigbo and Emoghene, 2004; Okigbo and Ogbonnaya, 2006).

1.0.2 Types of Yam

Over 300-600 types of yams are accessible, however, there are a few species that are grown principally for human consumption, while others might be grown for medicinal purposes (Okigbo, 2004). The cultured species in West Africa particularly Nigeria are the white yam (*D. rotundata*), yellow yam (*D.cayenesis),* water yam (*D. alata),*Cush cush yam(*D.trifida*), Bitter yam (*D.dumetorum),* Aerial yam (*D.bulbifera*), Chinese yam (*D.esculenta)* (Onwueme, 1978). *Dioscorea cayenesis* and *D. rotundata* are rare in West Africa while *D. alata and D. esculenta are* indigenous to Asia. In addition, there are various numbers of wild yams that are

also harvested as food (Wilson, 1982). *D.alata*, *D. cayenesis*, *D. bulbifera and Ddumentorum* function as medicinal and nutritional purposes, while others indicated in Table 3 are for nutritional purposes (Okwu,2006)

1.0.3General Morphology and composition of yam tuber

Tuber yam shape ranges greatly due to environmental and genetic factors. Yam tubers are more or less cylindrical in shape and mostly weight 3-5kg (Omwueme and Charles, 1994).

A mature yam tuber has four concentric layers:

a. Corky periderm- the outer portion of the yam tuber that provides effective barrier against pathogenic attack and water loss.
b. Cortex- a layer located immediately beneath the cork that assists in storage of starch.
c. Meristematic layer – elongated thin-walled cells that give cover to the cortex
d. Ground tissue- the central portion of the tuber that house yam nutrients (Omwueme, 1978).

Table 1: World production of yam (*Dioscorea* spp*)* (FAO, 2010)

Location	Cultivated area ('000 ha)	Yield (t/ha)	Production ('000 t)	Percentageof world
World	4,928	10.5	51,778	100.0
Africa	4,443	10.8	49,833	96.3
West Africa	4,178	10.6	48,101	93.0
Nigeria	3,045	11.5	35,017	67.7
Cote d'Ivoire	820	8.5	6,933	13.4
Ghana	299	11.9	3,550	6.9
Benin	205	8.8	1.803	3.5
Togo	63	10.2	638	1.2

Figure 1: Change of yam production, cultivation area, yield and consumption of Africa (FAO, 2010).

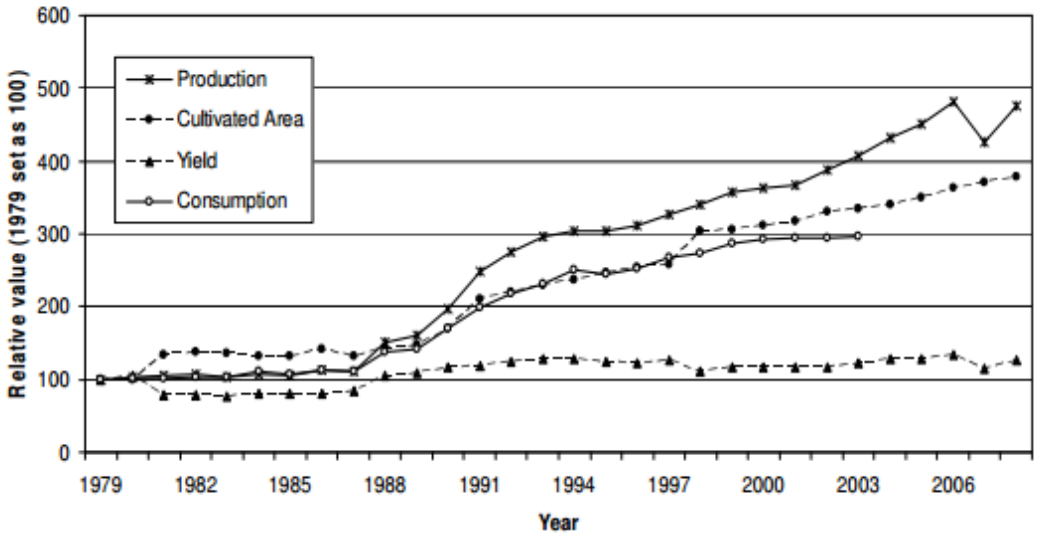

Figure 1: Yam and other root crops on sale in the kingdom of Tonga at a local market (FAO, 2003)

1.0.4 Nutrients in Yam

The nutrients in yam are minerals such as magnesium, calcium, phosphorus and also vitamins such as riboflavin, thiamine and vitamins A, C, E and K including carbohydrates, fats, crude proteins (Table 2)(Opara, 1999; Okigbo and Ogbonnaya, 2006). These nutrients make yam one of the high valued food crops (Okigbo, 2000). The benefits of yam cannot be overemphasized. Oyenuga (1981) reports that yam is rich in calcium, which strengthens the bones, phosphorus, riboflavin, niacin, and as well as vitamins that aid immune function, slow down aging and improves wound healing.

Yam contains potassium, which serves as an important compound of the cell and body fluid and also helps in controlling heart rate so as to avoid hypertension as well as regulating blood pressure. *Dioscuri*, a storage protein found in yam can also be used to cure people suffering from hypertension (Niba, 2003).

Yam also serves as source of energy and dietary fiber. Energy is vital in human life as well as dietary fibers which help to reduce constipation, decrease bad (LDL) cholesterol level by binding it to the intestines and prevent colon cancer by preventing toxic compounds in the food adhering to the colon mucosa (Niba,2003). Osuji (1981) also finds that due to the *allantoin* contained in yam, it helps to speed the healing process of ulcers, boils and abscess. Aside these, it stimulates appetite, relieves bronchial infections, irritation and cough.

Chen*et al.,* (2008) examined the neuro-protective effect of yam on mice and found that "yam at the dose of 100 or 500 mg/kg significantly improved the cognitive ability as well as increased activities of endogenous antioxidant enzymes in the brains of mice". The report concluded that yam possesses the potential to serve as an anti-aging agent or as a treatment of neurodegenerative diseases.

Ihediohanma *et al.,* (2012) studied the Glycemic Indices (GI) of three yam cultivars and found that because of the low GI contained in water yam for instance, it is ideal for people with diabetics and obese (fat) people.In addition, due to the high proportion of antioxidant compounds found in yam, people who consumed yam for 6weeks were observed to show a significant increase in cognitive abilities (fast rate in learning and memory building). In view of this, compounds extracted from yam demonstrate potentials for development of therapies in the treatment of Alzheimer's disease (Chen*et al*, 2008).

The benefits of Yam consumption are numerous. For instance, it delays ageingdue to the presence of vitamin C, vitamin B6 and antioxidants, which help to prevent wrinkles and other signs of ageing. The high level of vitamin A in this crop makes it one of the staple foods that help in cell growth, particularly growth of hair.

Yam intake has also been reported to aid the process of digestion, by dilating vessels and stimulating bile flow (Okigbo, 2004).

In spite of the numerous nutritional and health benefits of yam, there remains high rates of malnutrition and diseases such as hypertension and diabetes in developing countries linked directly to inadequate sources of dietary protein, high glycemic index carbohydrate found in the staple foods such as yam, maize and cassava consumed in these countries. The quality of staple food that is regularly consumed in most of these areas can be improved biotechnologically. The use of biotechnology in developing counties has been targeted at increasing yields of cash crops and food quality (Niba, 2003).

1.0.5 Post-Harvest Food Loss (PHL)

PHL is the measurable qualitative and quantitative **food loss** along the supply chain, starting at the time of **harvest** till its consumption or other end uses (Hodges *et al*,2011). The loss of edible and viable food due to the actions of man or inaction such as throwing away flaccid produce, not consuming available food before its expiry date is regarded as post-harvest spoilage (Buzby and Hyman, 2012).

Post-harvest food loss is most commonly found in developing countries (Kitinoja and Gorny, 2010).Reports indicate that volume of lost and wasted food in developed countries are higher in downstream phases of the food chain, but the reverse is the case in developing countries where more food is lost and wasted in upstream phases (FAO, 2013).

There are many factors that can contribute to food loss, ranging from mechanization of practices, for example, harvesting to handling, processing, weather conditions, production practices, transportation, consumer choice as well as the availability of financial market (Hodges *et al*, 2011). In line with this, there are many stages it takes to get products from harvested to the final consumers. Losses incurred in each stage vary depending on the system and technologies adopted in the food supply chain. In developing regions for instance, supply chain is less mechanized; large amount of losses is incurred during storage, drying, transportation and processing (Hodges *et al*,2011).

1.0.6 Food losses

Food losses occur as a result reduction in the quantity in edible food (weigh and volume), or qualitative reduction in nutrient of food originally designed for the consumption of humans (FAO, 2013). This reduction in nutrient causes unwanted changes to texture, color and taste which can as well directly affect the food viability for human consumption (Buzby and Hyman, 2012). The losses often take place during production, storage and processing stage in the food supply chain (Parfit *et al*, 2010). However, reports show that considerable reduction in food loss is basically as a result of poor infrastructure and logistics, lack of technology in developing countries, lack of skills, lack of knowledge and management capacity of supply chain actors, and lack of right markets (FAO, 2013). In contrast, developed countries are low in middle stages of the supply chain. This is attributed to more proficient systems, opulent infrastructure and logistic, better transport, efficient storage and improved management capacity that ensures that a larger proportion of harvest output reach the markets (Figure 2, Table 3) (Hodges *et al*, 2011).

1.0.7. Food waste

Food waste is a subset of food losses; food wastage refers to food lost by deterioration or waste (Buzby and Hyman, 2012). Food waste may also include quality considerations, residual and waste flows (Waarts *et al*, 2011). Food waste is defined as any food material the holder discards or intend or required to discard as a result of spoilage or other considerations (Quested and Johnson, 2009).

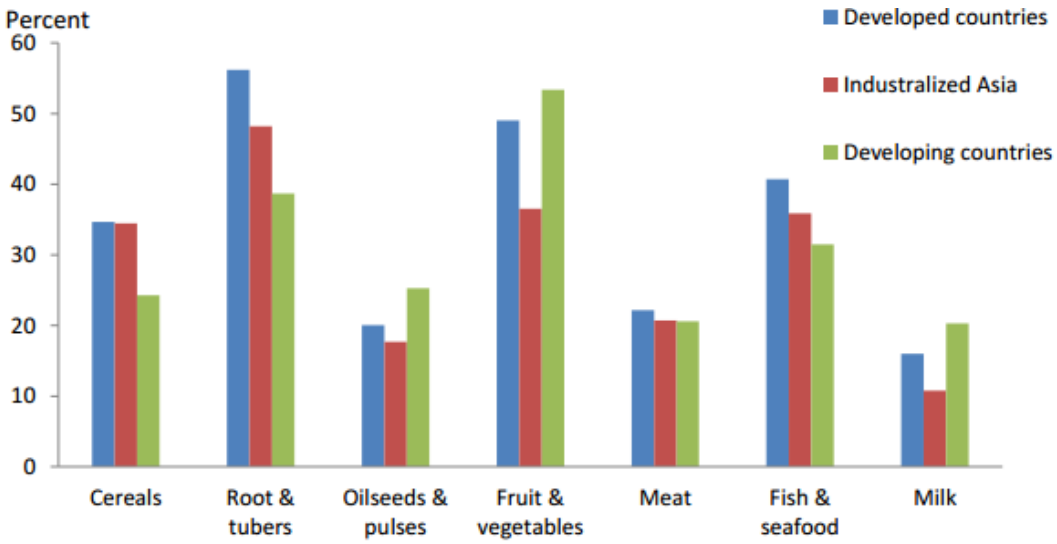

Figure 3: Statistical report of food losses across the world (Gustavsson *et al*., 2009, 2011)

**Please note,bar denotes percent of production loss of respective commodity*.

Table 2: Nutrient content of yam species *Dioscorea spp*. 100-g edible tuber position**(Opara, 1999)**

	D.spp.	*D. alata* Water Yam	*D. bulbifera* Potato Yam	*D. cayanesis* Yellow Yam	D. dumentorum Bitter Yam	D. esculenta Lesser Yam	D. rotundata White Yam	D. trifida Cush-cush Yam
Water (ml)	69	65 76†	71 (79) ‡	80	67	70 74†	80	80.7
Calories	119	135 87	112 (78)	71	124	112 102	71	
Protein (g)	1.9	2.3 1.9	1.5 (1.4)	1.5	3.2	3.5 1.5	1.5	2.54
Fat (g)	0.2	0.1 0.2	0.1 (0.2)	0.1	0.1	0.1 0.2	0.1	0.44
Carbohydrate (g)	27.8	31 20	26 18	16	28	25 24	16	38
Fibre (g)	0.8	1.5 0.6	0.9 (1.2)	0.6	0.8	0.5 0.6	0.6	
Calcium (mg)	52	28 38	69 (40)	36	52	62 12	36	8
Phosphorus (mg)	61	52 28	29 (58)	17	45	53 35	17	38
Iron (mg)	0.8	1.6 1.1	2.0	5.2		0.8	5.2	0.52
Vitamins								
β-carotene equiv (μg)	10	10 5						
Thiamine (mg)	0.11	0.05 0.10				0.10		
Riboflavin (mg)	0.02	0.03 0.04				0.01		
Niacin (mg)	0.3	0.5				0.8		

Table 4: Food supply chain and example of food chain

Stage	Examples of waste
1. Harvesting, handling of harvesting	Edible crops left in field, ploughed into soil eaten by pests, timing of harvest not optimal crop damaged during harvesting
2. Threshing	Loss due to poor technique
3. Drying, transportation and distribution	Quality and quantity loss of during drying, poor transport infrastructure
4. Storage	Pest and disease attack
5. Primary processing, cleaning classification, hulling, pounding, grinding, packaging, soaking, winnowing, sieving, drying , milling	Process losses: contaminant in process causing loss of quality
6. Secondary processing, mixing, cooking, frying, molding, cutting, extrusion	Process losses: contaminant in process causing loss of quality
7. Product evaluation and quality control	Product disregarded
8. Packaging	Inappropriate packaging damages produces; grain spillage from sacks; attack by pest
9. Marketing, selling, distribution	Damage during transport and losses due to pure storage
10. Post-consumer	Poor storage and stock management

Source: Parfitt *et al*, 2012

1.0.8 Post Harvesting in tuber yam

Yam harvesting is basically attained by hand using sticks, spades or diggers. Spades and sticks of wood are often used instead of metallic tools that are likely to damage the fragile tuber yam. Yam harvesting is a severe labor operation, which consists bending, standing and sitting on the ground depending on the size and depth of tuber penetration. For instance, during harvesting tubers more often than not experience physical damage as a result of hand manipulation during harvesting (Onayemi, 1983). Mechanized yam harvesting especially for *D. composite*, which is the species for pharmaceutical uses, has been reported but machines used are still for exploration (Nystrom *et al*, 1973).

Yam harvesting which is usually single or double helps in gathering early and late harvest produce. Single harvesting involves "digging around the tuber so as to loosen it from soil, lifting, cutting from the vine with the corm attached to the tuber". It is done only with mature crops at the end of every planting season (FAO 1991). The age of a tuber, which usually determines post-harvest quality, is between either 6 – 7 months or even 6 – 10 months. However, the "periods of 8–10months and 4-5 months from planting or emergence to maturity have been recommended for double harvesting" (Onwuewe, 1977; Martin, 1984). Bencini (1991) records a harvest regime at 5-6 months for the first harvest after planting. *D. rotundata* and other species such as *D. cayenensis* and D. *alata* would be better harvested using double harvesting. Onwueme and Charles (1994) have reported better eating quality for single rather than double harvested tubers. One of the major differences between

single and double harvesting in yam is that the harvesting in the former is completed in one procedure. It is completed in the space of 1-2 months so as to prevent losses due to tuber rot (Onwueme, 1978). On the other hand, the latter is completed in two phases. The first phase begins 4-5 months after the initial growth of the plants. It involves uncovering and separating the tubers from the plant without damage and re-preparing the bed. Onwueme (1978) highlights that "the tubers from the second harvest have pronounced 'planting features' and are less suitable for eating" thus necessitate the vigorous work in this kind of harvesting.

The tubers from the first phase, which have high prices in the markets, are available early (Onwueme, 1978).

1.0.9Agronomy of Yam and Storage process

To successfully store yam, the importance of regulation of temperature and regular inspection cannot be undermined. It is pertinent for the temperature in storage barns to be minimal so as to prevent rotting and sprouting and at the same time, a cold temperature at about 12-15°C must be maintained so as to avoid chilling injury (Thompson, 1996). There must also be proper ventilation in storage barns as this prevents moisture condensation and removes heat (Figure 4). Since rotting cannot be 100% attained regular inspection is needed to prevent diseases and pests and to remove rotted tubers. Martin (1984) and McGregor (1987) agree that freshly harvested yam tubers will thrive and survive if stored in temperatures between 12-16^{o} while 15^{o}C or 16^{o}C at 70-80% relative humidity will suffice for cured tubers. Stored yams begin to sprout in cases of inadequate ventilation. For example, during storage at ambient condition (20^{o} – 29^{o}C, 46 – 62% relative humidity), *D. trifida* begins to sprout within 3 weeks (Thompson, 1996). At higher humidity yam decay occurs and like most staple crops, they are susceptible to

chilling injury at low storage temperature. To avoid tuber damage, minimal temperature of 10^oC, 12^oC and 13^oC are therefore recommended (Martin, 1984; McGregor, 1987). Studies have revealed that *D. rotundata* will suffer from chilling injury if stored at 12.5^oC (Opara,1999). In the same vein, storage of *D. alata* at either $3\text{-}12^0C$ will lead to a total physiological breakdown within 3-4 weeks (Czyhrinciw and Jaffe, 1951). Storage of *D. alata*, at 5^oC for 6 weeks gave good results but chilling injury symptoms developed rapidly when tubers were subsequently put in ambient 25^oC conditions (Coursey, 1961). Earlier reports have also indicated varying storage conditions for different varieties of yam, specifically however, it has been reported that optimally, *D alata* tubers should be stored at 12.5^oC for a maximum of eight (8) weeks (Opara, 1999).

There are several other methods that can be used to store yam locally. Opara (1999) lists these as: leaving the tubers in the ground until required, the yam barn and the underground structure.Local farmers majorly practice leaving the tubers in the ground until required and these prevent the use of the farmland for further cropping.Yam barn is one of the oldest and major local storage structures in the major areas where yam is produced. Barns are more or less shaded area constructed to create adequate ventilation while protecting yam from flood and pest attack.Tubers are kept under this wooden framework, depending on the amount of yam to be stored, frame of 42m or more in length.Bencini (1991) has recommended that instead of tying yam tubers to poles, the use of open sided shelves made from poles, bamboo poles or sawn wood will ensure careful handling and easy inspection.

In barn storage, yams have a maximum storage life of 6 months and are therefore most suited for long-term varieties (FAO, 1991). Storage losses can be high and up to 10-15% in 3 months, and 30-50% after 6 months if tubers are not

treated to prevent from rotting using fungicides such as Benlate, Captan or Thiabendazole. It has also been suggested that well ventilated, weather proof and stronger shelters can be built as to improve the performance of local shelters described above.

It is against this backdrop that this study seeks to investigate the control of post-harvest deteriorations in yam which are caused by a wide variety of factors, ranging from growing conditions to pathogen invasion which serves as one of the key reasons of decline in production and state precisely methods that can be used in stored yam preservation.

1.1.0 Plant extracts as antimicrobial agents.

The emergence of antibiotics has greatly inhibited pathogenic organisms and reduced postharvest losses in crops, which has been a serious burning predicament (Abimbola *etal*,1993). Fungicides such as Dichloronitroanline, sodium orthiophenylphenate, Borax, Captan, Thiobendazole, bleach among many other anti-microbial drugs have been used to protect and preserve tubers against *Rhizopus* soft rot (Clark and Moyer, 1988).

However, the use of anti-microbial fungicides is not affordable for most of the farmers besides it constitutes danger to these farmers and their surroundings. As a result of this, there is an urgent search for alternative chemotherapeutic drugs with considerable less effect or no side effect to farmers and also easily accessible (Khulbe and Sati, 2009). The use of plant and their extracts to treat infections by plant pathogenic organisms has long been practiced in many parts of the world (Sofowora, 1984). In Nigeria for example, plants extracts have been used to inhibit diseases of crops such as yam (Okigbo and Nmeka, 2005), banana (Okigbo and Emoghene, 2004). The occurrence of plant extracts to treat microorganisms had

rendered some of the current synthetic antimicrobial agents unused for controlling bacterial diseases because of their accessibility and non-severe damages (Gustavo *et al*, 2010).

Figure 4: Typical Yam Barn in West Africa**(**Opara, 2003)

Medicinal plants extracts can be used in different forms, which include: powder, liquid, mixtures, which could be raw, boiled such as liniments, ointments and incisions (Apata, 1979).

Moringa and *Ginge*r have been successfully applied in minimizing the growth of the organisms implicated in tuber yam rots. This agrees with the reports that had used the same plant extracts to control diseases in potato and yam (Okigbo and Ikediugwu 2000; Okigbo and Emogbene, 2004). Also, it is important to state that extracts contain antibacterial compounds, which conferred the antimicrobial properties on these plants. Thus, extracts of these plants can serve as alternative ways of reducing and controlling rot by farmers especially those caused by multi-drug resistant bacteria.

1.1.1 *Moringaolifera*

The anti-microbial plant *Moringa olifera*(Figure 5) has been widely used for treatment of some ailments and also promotes digestion, infectious disease, diarrhea, as stimulant in paralytic afflictions, epilepsy and hysteria (Farooq *et al*,2012)

It is experimentally stated that *Moringa*roots, leaves, steam bark, and seed possesses some anti-inflammatory and therapeutic properties (Anwar and Rashid, 2007). Successes have been reported that the use of *M. olifera* extract to control pathogens (Okigbo *et al,* 2010; Devendra *et al,* 2011).

Studies have reported the presence of some phytochemical constituents in extracts of this plant, which has been observed to have antimicrobial activities (Ochola *et al,* 2015). *Moringa olifera* leaf extract for instance, has antibacterial and antifungal properties against pathogens such as S*taphylococcus aureus, B. substiilis, Streptococcus pyrogenes, Aspergillus niger, Escherichia coli, Pseudomonas and Candida albicans* (Devendra *et al,* 2011).

The medicinal and anti-microbial properties of *Moringa* are numerous, for example, *Moringa*seed powder works as a natural coagulant, which clarifies very turbid water (Broin *et al,* 2005). In addition to this, *M. olifera* seeds have been reported to contain anti-tumor promoting activity and also wound healing property (Guevera *et al,* 2007). In Nigeria for instance, *M. olifera* is an edible plant, often consumed in the Northern part of the country (Lar *et al,* 2011). *M. olifera* among many other plants extracts are alternative, cheap and effective antimicrobial agent in addition to its nutritional and medicinal use

1.1.2 Ginger

Zinigiber officinale (family Zinigiberacea) (Figure 6) is an herbaceous perennial plant which has a vertical stem, narrow medium, green leaves arranged into double ranks on each stem (Okigbo and Nmeka, 2005). *Z. officinale* plant is commonly used to treat ailments including rheumatism, sprains, sore throats, muscular aches, pains, constipation, arthritis, vomiting, hypertension, indigestion, dementia, fever, and infectious disease (Ali, 2008).

Ginger can inhibit bacterial infections because of the anti-microbial compounds it contains (Tan and Vanitha, 2004). It also has an aromatic and medicinal properties characterized by their tuberous or non-tuberous rhizomes

(Chen, 2008). In line with all antimicrobial qualities this perennial plant possesses, it stands the chance of inhibiting or eliminating food borne pathogenic bacteria (Kamrul *et al.*, 2014).

Figure 5: *Moringer Olifera* Plant (Price, 2007)

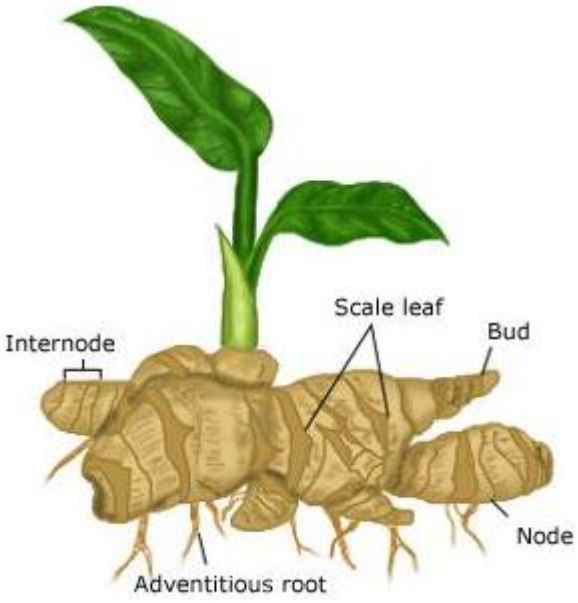

Figure 6: Ginger plant (Ahmed,2012)

1.2.0 Storage pathogens

Tuber yams are usually harvested in Nigeria within 6- 9months after planting and the product is often amassed in various storage facilities depending on the custom and belief of such community (Amusa, 2000) until consumption.Throughout yam storage, up to 50% of the fresh matter are lost to microbial attack (Amusa,2000). The presence of pathogens that contaminate the inner yam tissues and the improper handling of yam during and after harvest is a serious threat to yam.

In their research at barns near Idah, Kogi state Nigeria, Morse *et al*, (2000) found that yam rot caused by fungi were likely to have previously encountered post-harvest attacks from insects especially storage beetles (Coleopteran), mealy bug (*Planococcus citri*) and scale insect (*Aspidiella hartii*) in the process of storage.

1.2.1 Emergence of post-harvest pathogen in yam

Arya (2010) categorized postharvest pathogens into two; "those that penetrate the produce on farm, but develop in their tissue only after harvest, during storage or marketing on one hand and those that initiate penetration and colonization during or after harvest on the other hand." Mammoth post-harvest losses have been attributed to fungal deteriorations (Okigbo, 2003; 2004, Shukla *et al*., 2012). Studies on yams also revealed that *Aspergillus niger* was the main frequently occurring species associated with yam tuber deterioration in the same location (Okigbo and Ikediugwu, 2000). However, based on the causal agent and

signs of the microbe, the diseases of yam can be classified into 3 different types which are wet rot, dry rot (Figure 7), and soft rot (Figure 8) (Amusa and Baiyewu, 1999)

1.2.2 Wet rot

Wet rot is among the pathogens that cause yam infections and the invading pathogens can be identified through the yam tissue becoming hard and dry as well as changing colors from its original color to another color (IITA, 1993). Studies have shown that yam tubers contaminated with *Penicillium oxalicum* and *P. cylopium*, turn brown and become hard and dry maintaining their veracity. If the tubers tissue is also invaded by *S. marcescens*, it is covered with green mycelia of the fungus and if the infection is caused by *Aspergilius niger* and *A. tamari* such tissues are brown with a yellowish margin (IITA, 1993). Also, when a tissue is and when it is pressed there is a release of a whitish fluid, then it may be concluded that the disease symptom is generally related with a bacterium called *Erwinia carotovora PV carotovora*(IITA, 1993; Amusa and Baiyewu, 1999).

1.2.3 Dry rot

According to IITA (1993), *Posellinia bunodes*, and *Botryodiplodia theobramae*, are the major causes of dry black rot (Figure 7). Infected yam tubers turn grey and then black, become thin and eventually break into small dry particles (IITA, 1993). Ogundana *et al*, (1970) and Morse *et al*, (2000) report the presence of *Fusarium species* in tubers with dry rot in Nigeria, which make the infected tuber tissue pink with yellow edges.

(Amusa and Baiyewu,1999) identified species of *Fusarium* found in dry tuber yam rot as *F. oxysporium, F. moniliforme* and *F. Solani*. IITA (1993) further lists *Scutellonema bradys*(a yam Nematode) as one of the deadly pathogens of yam that decays yam tubers and causes dry rot. The consequential outcome of this pathogenic disease causes light yellow lesions below the outer system of the yam tuber and as the disease advances, it extends into the tuber, and eventually turns light brown, and finally turns the brown color to black. A key confirmation of this would be a crack emerging from the outer surface of the yam.

One of the most dangerous symptoms of this deadly disease (dry rot) is seen in full-grown yam tubers particularly during storage. However, this causes weight disparities between healthy and contaminated yam tubers harvested from the farms. For instance, weight differences in this part of the country have been estimated to be 0 to 29% (Wood *et al,* 1980). This infection also reduces the water level of tuber yam, which continues during storage (Adesiyan *et al*, 1975). Dry rot in yam causes losses of whole tubers, which can be as high as 80-100%, and it also reduces the marketability. Researches on yam tubers in Nigeria have revealed a high rate pre-harvest injury by *S. bradys* and wet and dry yam rot diseases (Bridge 1973; Adesiyan and Odihirin, 1977; Wood *et al.*, 1980).

1.2.4 Soft rot

The occurrence of fungal mycelium in yam tubers results in swollen yam tissue, which makes it brown or soft, at times and then finally collapses the cell wall. Fungi associated with this soft rot (Figure 8) include *Rhizopus* spp, *Mucor circinelloides, S. rolfsii, Rhizoctomia solani* and *Armillariella mellea* (Ikotun, 1983, 1989; Green *et al*, 1995; Amusa and Baiyewu, 1999).

Figure 7: Pictorial representation of a tuber of yam showing lesions of dry rot disease (Gugino and Abawi 2009)

Figure 8: Pictorial representation of a tuber of yam showing lesions of soft rot disease (Abawi and Gugino, 2009)

1.2.5 Post-harvest stored yam organisms

Numerous researchers have reported several microbial etiologies associated with post-harvest spoilage of yam. These include *Penicillium chrysogenum, Geotrichum* spp, *Penicillium digitatum, Collectotrichum tamari, Cladosporium herbarium, C. sphacrosperunum, Cylindrocapon radicicola, Fusarium poa, Penicillium expansum, P. halicum, P. oxalicum, Serratia* spp, and *Bacillius* spp, *Pectobacteria,* and*Lactobacillus plantarum.*(Okigbo *et al.*, 2009; Arya, 2010)

1.2.6 Management of post-harvest pathogens of stored yam

In order to meet the food demand challenges of our teeming economies, and attain sufficiency and security in food production, food production must match adequately with protection from spoilage and rots attacking organism during transit or storage (Shukla *et al*, 2012). Control of pathogens associated with yam diseases has been comprehensively studied with the goalof managing these pathogenic organisms and the destructive rots resulting from these infections. Consequently, more than a few procedures have been recommended. These include:

a) Biological and chemical control of rot organisms

Biological control of plant disease involves the practice whereby the growth, survival and activity of a pathogen is reduced via the agency of any other living organism. The result that there is reduction in the evidence of the disease caused by the pathogenic strains of *Bacillus subtilis* and *Trichoderma viridae* are potent biological agents with controlled postharvest and storage rots of yam tubers. An application of this biological control agents protect tuber in storage for up to 6 months (Okigbo and Ikediugwu, 2000). Also, using fungicides such as Benlate and captan have been proven to be effectual in the reduction of yam infected with

fungus during storage (Ogundana, 1981). There have also been alternative methods used to reduce fungal infection in yam. For example, the use of locally made dry gins, wood ash, and tecto (thiabendazole) which have little or no mammalian toxicity have been recommended for use during storage due to the toxicity of many chemicals. (Ogundana, 1971; Osai, 1993).

However, Okigbo (2004) expresses that the need for repeated spray application as in synthetic fungicides is unnecessary with biological control agents. Although the mechanisms of action of these bio control agents have not been fully explained, competition for space and nutrients, antibiosis, direct parasitism, as well as rapid and effective colonization of wound sites against the invading pathogens have been presumed and suggested for their activity (Okigbo, 2004; Arya, 2010).

b) Use of plant – derived pesticides in the control of postharvest rot diseases.

Shukla *et al*. (2012) report numerous *in vitro* studies that have validated the efficacy of plant – derived pesticides in many branches of agriculture.*Zinigiber officinale*or ginger has been used in Asia for relief from arthritis rheumatism coughs, fever and infectious diseases (Anonymous, 2004).

Plant extracts have been used to control diseases in potatoe (Amadioha and Obi, 1998) and yam (Okigbo and Emoghene, 2004). Pesticides of plant origin are specific, biodegradable, cheap, readily available and environmentally safe than synthetic chemicals. Ginger (*Z. officinale*), is effective against the fungi and bacteria for example growth inhibition of ginger on some organisms in percentage are *A.niger* 33.3% and *F. solani* 31.5% (Okigbo and Nmeka, 2005). The extracts reduced the growth of these fungi and bacteria in culture and reduced rot development in yam tubers**.**

c) Good agronomic practices, field sanitation and store hygienic

Research has revealed that the process of rotting usually begins at the field and eventually matures during storage (Okigbo and Ikediugwu, 2000; Okeigbo 2004). Arya (2010) observed that postharvest storage diseases could be minimized if cultural practices are adopted during pre-harvest. In the evaluation of yams, Shukla *et al*, (2012) notes that the high moisture content in yam causes a lot of deterioration by fungal pathogens in storage.

d) Thermal and physical control

Okigbo *et al.,* (2009a) describe curing as the process of "exposing harvested tubers to high temperatures and relative humidity (RH) for a short time usually about 24hrs." They further highlight that during this process which helps to thicken the tuber skin and allow for quick healing of wounds sustained during harvest, harvested tubers are exposed to 25 to 35°C and 80 to 90% RH for about 7 to 14 days.

1.3.0 Rationale for research

Studies such as Taiga and Onyeka *et al.* (2011) have identified different microorganisms associated with various forms of rot and deterioration in postharvest yam tuber and this is a major factor that reduces its commercial value in Nigeria. From the foregoing, yam harvesting methods and yam storage processes used in Nigeria and Africa as a whole predispose yam to mechanical damage which in turn serve as an infection route to stored yam. Furthermore, in the South Western part of Nigeria, it has been observed that rot pathogens affect the quality of yam tubers making them unpleasing and unsellable to buyers and highly perishable, as

they get rotten if stored for more than six months (Ogundana *et al*, 1970; Adesiyan and Odihirin, 1975). There is hence a need to identify rot-causing pathogens in stored yam and to find cheap means of protecting stored yam against these pathogens

1.4.0 Aims and objectives of study

The broad aim of this study was to isolate and identify rot-inciting pathogens of yam and to screen extracts of the Ginger (*Z.offinale*) and Moringa (*M. olifera*) plants for antimicrobial activity against the isolated pathogens.

The specific objectives of this study were to:

I. Isolate some rot-inciting pathogens of yam.

II. Characterize pure cultures of isolates using a battery of biochemical tests and confirmation using molecular techniques.

III. Establish the antimicrobial effect of Methanoic and Ethanoic extracts of Ginger (*Z.offinale*) and Moringa (*M. olifera*) plants on pathogen growth.

IV. Compare the antimicrobial effects of Methanoic and Ethanoic extracts of Ginger (*Z.offinale*) and Moringa (*M. olifera*) plants on pathogen growth, with known antibiotics.

CHAPTER TWO

Materials and Methods

2.1 Sampling

Discorea alata (water yam) with symptoms of infection by post-harvest pathogens (Dry rot and soft rot) and (Healthy yam) were procured locally from Osogbo market in Osogbo metropolis of Osun state. These yam tubers were identified through the local names given by the yam dealers in the market and later confirmed from literature using the local names and the morphological traits as indicated on the plant identification keys (Binns *et al*,1988).

2.2 Sterilization of materials

Kits and glassware were washed thoroughly, rinsed as well as sterilized in a dry ventilated oven (Gallen Kamp, Model NYC-101) at 160^{o}C for 2 hours. All media were sterilized by autoclaving at 121^{0}C for 15 minutes(Cheesbrough, 2000; Jawetz *et al,* 2004). Thereafter, these were allowed to cool to 45-50^{o}C, poured and then allowed to cool further to room temperature in the Petri plates before inoculation.

2.3 Sterilization of Sample

Complete water yam tuber was sliced to visualize rotten tissue. The rotten tissues were then cut into 3mm pieces and were surface sterilized by 1ml of 10% concentration or lactic acid solution to suppress bacterial contaminants. The tuber

and rotten tissue pieces were placed on sterile disposable petri dishes beside lighted spirit lamp to dry for 10 minutes.

2.4Preparation of Media

Throughout this study, the assayed culture media used were Nutrient agar (NA), Muller-Hinton (MHA), Blood agar (BA), Nutrient Broth (NB). These media were used for growth and also to maintain bacteria isolates. NA was used to culture bacteria while Blood agar (BA) was used for blood hemolysis test. MHA was used for antimicrobial activities All culture media were prepared according to their manufacturer recommendations.For NA, 28g was weighed and was dispensed into 1litre of distilled water and autoclaved at 121^0C for 15 minutes. 39g of MHA was dispensed into 1liter of distilled water and autoclaved at 121^0C for 15 minutes. 37g of blood agar was dispensed into 1liter of distilled water and autoclaved at 121^0C for 15 minutes(Cheesbrough, 2000; Jawetz *et al.,* 2004). These media were allowed to cool to temperature 42-45^0C before pouring into petri plates. The media were allowed to solidify in the plate at room temperature.

2.5 Isolation Microorganisms

The isolation technique by Onyike and Maduewesi (1985) was employed in this study. A small section 3mm of yam tissue containing the rots and adjoining healthy water yam was cut using sterilized scalpel and cork borer surface. Surface sterilization was done and the peeled portions of yam were placed using cork borer into the culture medium. Three (3) slices of rotten portionsin a single yam sample was obtained and placed inappropriate culture medium plate with equal distance

between them. The plate was incubated at about 37^{o}Cfor bacteria for 24hrs and pure culture was observed from the organisms earlier incubated. The pure culture on the plates was transferred into 10ml of nutrient broth and incubated at 37^{o}C for 24hrs and growth was observed. The organism in the nutrient broth was used for serial dilution. Dilution 10^{-1} to 10^{-10} was made while dilution 10^{-1},10^{-4},10^{-7} and $10^{-10}$was used for inoculation using spread plate method in which 0.5ml of diluents was put on the nutrient agar plate using sterile pipette and was spread using a sterile swab. The plates were incubated for 24hrs at 37^{o}Cto observe growth of organisms.

2.6.1 Identification of microbial isolates

Cultural characteristics and Cell morphology

The plate was examined microscopically daily to check bacteria growth. Colony color, type, texture, shape as well as growth pattern was observed. Light microscope (low power) was used to observe the culture by careful preparation of slides, staining with 10% ethanol for 2mins. Using cell morphological, colony/ cultural characteristics and biochemical tests, identification of the isolated organisms was made using the Bergey's manual (2003).

2.6.2 **Biochemical tests foridentification of bacterial isolates**

2.6.2.1 Gram-staining

The bacterial smear obtained from pure cultures was prepared and then flooded with crystal violet stain for 60 seconds. The dye (stain) was drained off quickly and washed with Lugols's iodine solution. The iodine solution was left for 30 seconds, and drained off afterwards. Excess dye was rinsed gently with a running tap water. The smear was de-colorized with 95% ethanol until the slide appeared

free of the violet stain (10-15 seconds) and rinsed under the tap. The smear was flooded with Safranin O stain for 30 seconds, blotted dry and observed under a light microscope. Gram-positive microorganism appeared purple while gram-negative appeared red (Atlas *et al.*,1995)

2.6.2.2 Urease Activity

Pure culture isolate was inoculated into test tubes containing urea broth (one was left un-inoculated to serve as control) and labeled appropriately. The test tubes were incubated for 24- 48 hours at 37^{o}C. Pink color indicated a positive test while a yellow-orange color indicates a negative test (Atlas *et al,* 1995).

2.6.2.3 Indole production

Indole broth was prepared in test tubes and inoculated with the pure culture isolate one was left un-inoculated to serve as control. The test tubes were incubated at 37^{o}C for 24 hours. After 24 hours, 10 drops of Kovac's reagent were added into the inoculated test tubes and observed. The presence of indole was detected by the immediate formation of a red layer at the top of the broth (Atlas *et al,* 1995).

2.6.2.4 Citrate utilization

The pure culture isolate was inoculated into slants of Simmon's Citrate agar and incubated at 37^{o}C for 24 hours (one was left un-inoculated to serve as control). After incubation, the slants were observed. A royal blue color indicated a positive test while a green color indicated a negative test (Atlas *et al,* 1995).

2.6.2.5 Carbohydrate fermentation (Sugar fermentation)

The sugar broths for Glucose, Maltose and Fructose were prepared in test tubes. The test microorganism was inoculated into the sugars. The test tubes were incubated at 37^{o}C for 24- 48 hours after incubation, the tubes were observed for result. Yellow color indicated positive acid production, while a red color indicated a negative acid production (methyl red being the indicator used). The Durham tubes were also examined for gas production (Atlas *et al,* 1995).

2.6.2.6 Methyl Red test

Methyl Red –Voges-Proskauerbroth was prepared and placed in test tubes and the test organism was inoculated into the broth and incubated at 37^{o}C for 24-48 hours. After 48 hours, 5 drops of methyl red were added to the broth containing the already inoculated organism. Change of the broth medium to red within 2-3 minutes indicated a positive test while yellow color indicated a negative test (Atlas *et al,*1995).

2.6.2.7 Voges Proskauer

Methyl Red –Voges-Proskauerbroth was prepared and placed in test tubes. The test microorganism was inoculated into the broth and incubated at 37^{o}C for 24-48 hours. After 48 hours, 0.6mL of 5% alpha-naphthol, followed by 0.2 mL of 40% KOH were added. The tube was shaken gently and then left undisturbed for 10-15 minutes. Development of a red color after 15 minutes indicates a positive result while a copper like or yellow color indicated a negative result (Atlas *et al,* 1995)

2.6.2.8 Starch hydrolysis

Lugol's iodine was poured around the plate containing pure culture that had been incubated at 37^{o}C for 24hrs. Presence of clear blue-black tone surrounding

colonies was positive for their ability to digest the starch and therefore indicates presence of starch.

2.7.0 **Confirmation of microbial isolate by Molecular Techniques**

In addition to the cultural, morphological and biochemical tests described above, the isolated organisms were further characterized using molecular methods described as follows.

2.7.1 Extraction of microbial DNA

DNA of isolated microbial pure culture was extracted using the Quick-DNA^{TM} DNA extraction kit (Zymo Research, USA). Briefly, a loop-full of the microbial culture was re-suspended in 200µl of DNA elution buffer. Then, 20 µl of proteinase K was added to the mixture. The mixture was thoroughly mixed using a vortex and subsequently incubated at 55^{o}C for 10 minutes using a water bath. A 420µl of Genomic Binding Buffer was then added to the digested sample and mixed thoroughly using a vortex. The mixture was then transferred to a Zymo-Spin^{TM} IIC-XL Column in a collection tube and centrifuged for 1 min at $\geq$ 12,000xg. The collection tube and its content were discarded. Next, 400 µlof DNA Pre-Wash Buffer was added to the column in a new collection tube and centrifuged for 1 min. at $\geq$ 12,000xg. The collection tube and its content were discarded. Next, 700 µl g-DNA wash buffer was added to the column in a new collection tube and centrifuged for 1 min. at $\geq$ 12,000xg. 200 µl g-DNA wash buffer was added to the column in a new collection tube and centrifuged for 1 minute at $\geq$ 12,000xg. The collection tube and its content were discarded. Finally, the column was transferred to a clean micro centrifuge tube and 100 µl of DNA Elution Buffer was added to the column followed by 5 mins. of incubation at room temperature, centrifugation for 1 min. at

≥ 12,000rpm. Extracted DNA was stored at 4^0C until required (Zymo Research, 2016).

2.7.2 Amplification of genetic material (16s RNA)

The 16S RNA sub-unit was amplified using polymerase chain reaction (PCR). The primers and amplification conditions (Table 5) used to amplify this region of the microbial genome was done according to a slightly modified method of Amutha and Kokila (2014). Briefly, the PuReTaq™ Ready-to-Go™ PCR beads (GEHealthcare, UK, Ltd) were reconstituted with 15µl master mix consisting of 1µl of Forward primer- 27F (5' AGAGTTTGA TCCTGGCTCAG 3'), 1µl of Reverse primer- 1492R (5' TACCTTGTTACGACTT 3') and 13µl Nuclease-free water (Ambion®, USA). 5µl of the DNA template was dispensed into the PCR tube containing master-mix. The amplification was carried out in a thermal cycler (Eppendorf™ Mastercycler Pro, USA) for 40 cycles using the following reaction conditions, denaturation of DNA at 94°C for 1 minute, annealing at 56°C for 30 seconds and extension at 72°C for 1 minute. 4µl of amplified PCR product was mixed with 2µl of gel loading buffer. 1.5% agarose gel was casted. The samples were loaded along with 5µl of 1kb DNA ladder as a molecular marker. The gel was run and examined on UV trans illuminator to visualize the bands. Amplicons were stored at -4°C for sequencing and bioinformatics analysis (Amutha and Kokila, 2014).

Table 4: Amplification of 16sRNA sub-unit of the microbial genome

	Primary PCR		
Gene	Primers	Components	Reaction Condition
16s RNA	27F: 5' AGAGTTTGA TCCTGGCTCAG 3'	ddH_2O (13µL)	94°C/1mins
		$MgCl_2$ (1.5mM)	56^oC/30 secs
	1492R: 5' TACCTTGTTACGACTT 3'	DNA Template (5µL)	
			72^oC/1min
			40 cycles
			72^oC/ 5mins.

2.7.3 Plant extracts preparation

16.40g and 45.20g of *Moringaolifera*was both weighed and added to 200ml of ethanol and methanol respectively in a laboratory with soxhlet apparatus; 0.32g and 15.10g was further obtained after extraction. Also, 31.2gand 40.10g of Ginger (*Zinigiber officinale)* was weighed and added to ethanol and methanol respectively using same apparatus for extraction; 0.21g and 4.90g was obtained after extraction separately. The plant extracts were reconstituted DMSO to obtain final concentration.

2.7.4 **Chemical analysis of plant extract by Fourier transform infrared spectroscopy (FTIR)**

An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. Using the method of Sasidharan *et al,* (2011), the samples were grinded with potassium bromide (KBR) and a specially purified poly-L-Lysine fatty-acyl-chain (PLL-FAC) was used to eliminate the dispersion effect of big crystals. The pulverized blend was flattened in a manual form to a transparent pellet via which the beam of the spectrometer can pass. Hence, a fresh page was opened on the system, which showed the functional group of the compounds in the sample. Fourier transformed infrared ray (FTIR) otherwise referred to, as Spectroscopy or IR is a chemically specific analytical technique used in the identification of chemical compounds and their substituent groups either organic or inorganic compounds. FTIR adopts a spectroscopy technique that is vibrational, absorbing infrared light and interacting with the molecules present in the sample leading to an increased vibrational energy. During this process, absorption take place when the infrared radiation interrelates with particle that experience change in

dipole or when the inward bound infrared photon has adequate energy to permit the alteration of the molecule to a different or higher vibrational energy state.Plant extracts in this study, were properly packaged and sent the Department of Chemical Sciences, Redeemers University, Ede, Osun State, where they were analyzed using the Fourier transform infrared spectroscopy (FTIR).

The FTIR machine has several parts which are;

A **monochromater**: Splits radiation (the source) into its diverse wavelengths and can be either salt prism or a grating with excellently set apart fixed lines).

A **source**: Produces light through the spectrum of concern.

A **slit**: Chooses the gathering of wavelengths that sparkle via the samples at any assumed time.

A **beam splitter**: Splits the incident beam into two i.e. half goes to the sample while the other half goes to a reference. The *sample* captivates light according to its chemical possessions.

A **detector***:* Sets on view an electrical signal, which is usually referred straight to an analog recorder. Assembles the radiation that permits via the sample, and in double-beam process, relates its energy to that accepted over the reference. A connection amidst the monochromater and the recorder also permits the recording of energy as a purpose of frequency or wavelength, reliant on how the recorder is standardized or calibrated.

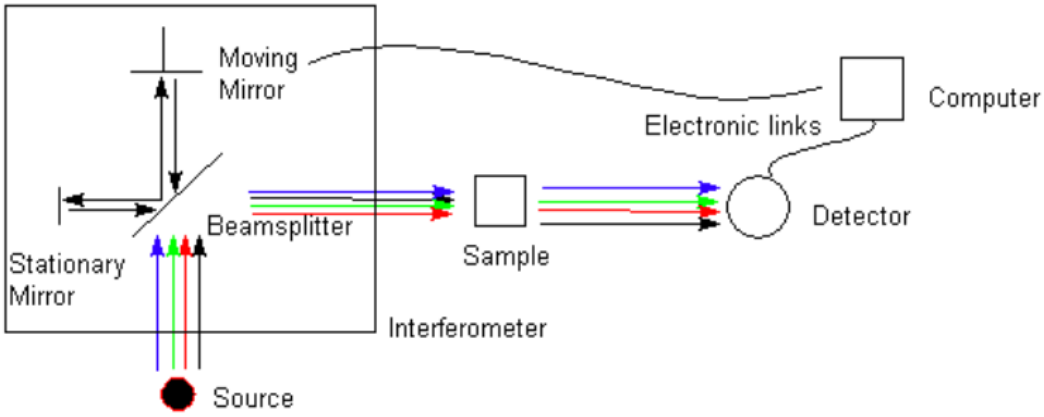

Figure 12: The Fourier transformed infrared ray (FTIR) Instrumentation

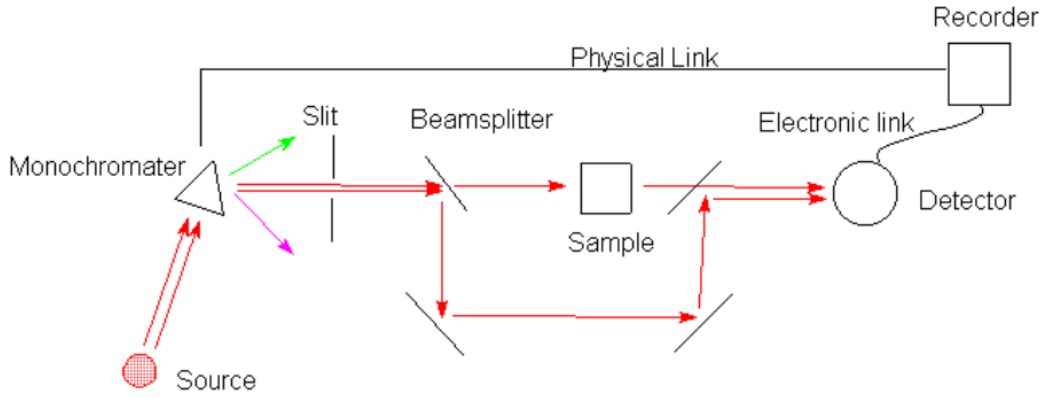

Figure 13: The Mechanism of action of Fourier transformed infrared ray (FTIR) (FC. UP, 2016).

2.8. Determination of anti-microbial activities of plant extract

The method of Amadioha and Obi (1999) was used to determine the effect of extract on bacterial (rot) growth. Microbial content pre-enriched in nutrient broth was spread by lawning technique unto the Muller-Hinton agar plates using sterile swab uniformly spread to the. Using a sterile 6mm cork borer, five (5) wells were aseptically burrowed into a solidified seeded Muller-Hinton agar (MHA) plate and then, 0.1ml from each solution was dispensed into the wells as appropriate using a sterile injecting needles.The plate was incubated for 24hrs at 37°C to observe zone of inhibition. The antibiotics, Penicillin, Erythromycin, Ciprofloxacin, Amoxicillin and Streptomycin disks were used as positive controls while methanol and ethanol were used as negative controls. The choice of these antibiotics as control was based on prior reports of earlier studies. The percent growth inhibition was expressed using the following formula:

Growth inhibition in percentage=dc-dt/dc × 100,

Where **dc**=average diameter of control

dt =average of bacterial colony with treatment.

Interpretation of zone of inhibition was done in accordance to modified protocols of Delahaye *et al.* (2009) and Joshua and Takudzwa (2013).

CHAPTER THREE

RESULTS

3.1 Identification and frequency of occurrence of isolates.

3.1.1 Biochemical Test Results

As shown on Table 6, a total of 8 bacterial isolates (pure culture) were obtained and subjected to various biochemical tests to aid in their identification. The results show the identities of the organisms isolated from healthy, soft and dry rot yam samples. The organisms belong to species of the following genera; *Streptococcus, Staphylococcus, Aeromonas, Enterococcus* and *Micrococcus*. Further observation revealed that 4 of the isolates, representing 50% of the total recovered from healthy yam, while 3 (37.5%) and 1 (12.5%) were isolated from soft and dry rot yam samples respectively (Table 7). As shown on table 7, *Staphylococcus aureus* was the most highly occurring organism (37.5%), occurring both in healthy and dry rotted yam samples, followed by *Streptococcus* spp (25%). The following organisms were found in the soft rotted yam samples, namely, *Aeromonas* spp., (1; 12.5%); *Enterococcus* spp., (1; 12.5%) and *Micrococcus* spp(1;12.5%).

3.2 Confirmation of microbial isolate by Molecular Techniques

3.2.1 Amplification of genetic material (16s RNA)

Figure 14 represents positive amplification of the 16s rRNA sub-unit resolved in 2% agarose gel. The first well (M) represent the 100bp molecular ladder while wells 2-9 represents resolved amplified DNA from 8 pure cultures of bacteria isolated from yam (healthy, soft-rot and dry-rot) samples. The results show a 1200bp band on

Table 6**:** Biochemical tests results of pure culture isolates

	BIOCHEMICAL TESTS															
TEST ORGANISM	**Gram Staining**		**Sugar Fermentation**			**Starch test**	**Citrate test**	**MR test**	**VP test**	**Motility test**	**Indole test**	**Urease test**	**Catalase Test**	**Oxidase test**	**Hemolysis test**	**Suspected organism**
	Rnx (+/-)	R/C	M	S	G											
HEALTHY YAM SAMPLE																
ISOLATE A	+	**C**	+	+	+	+	+	+	**-**	+	+	+	-	+		*Streptococcus spp*
ISOLATE B	+	**C**	+	+	+	+	+	+	-	+	+	-	+	+		***Staphylococcus aureus***
ISOLATE C	+	**C**	+	+	+	+	+	+	+	+	+	-	+	+		***Staphylococcus aureus***
ISOLATE D	+	**C**	+	+	+	+	+	+	-	+	+	-	-	+		***Streptococcus spp***
SOFT-ROT YAM SAMPLE																
ISOLATE E	+	**R**	+	+	+	+	+	+	-	+	+	-	-	+		***Aeromonas spp***
ISOLATE F	+	**C**	+	+	+	+	+	+	-	+	+	-	-	-	+	***Enterococcus spp***
ISOLATE G	+	**C**	+	+	+	+	+	+	-	+	+	-	+	+		***Micrococcus spp***
DRY-ROT YAM SAMPLE																

ISOLATE H	+	C	-	+	-	-	+	-	-	-	+	-	+	+	***Staphylococcus aureus***

Rnx: Reaction, **R/C**: Rod/Cocci, **M**: Mannitol, **S**: Sucrose, **G**: Glucose

thereby confirming the presence of the distinctness of the isolates whose DNA were amplified.

3.3 **Chemical analysis of plant extract by Fourier Transform Infrared Spectroscopy**

The FTIR spectrum analyses of methanoic and ethanoic extracts of Moringa are represented in Figs. 16 and 17 respectively. As shown on Fig. 16, the band at 3400cm^{-1}indicates the OH broadband, which is associated with alcohol (ethanol, methanol) that was used for extraction of the *Moringa oleifera* leaves. Bands at 2918cm^{-1}, 2848cm^{-1}and 2360cm^{-1}indicate the presence of asymmetric and symmetric –C-H bond resulting from fatty acids found in the plant cell wall (Table 8). In addition, bands at 1724cm^{-1}, 1656cm^{-1} and 1612cm^{-1}, represent aliphatic C=O bonds due to the presence of aliphatic ketones or esters. Also, the bands at 1656.91cm^{-1} and 1612.54cm^{-1} is indicative of a C=C bond. Furthermore, the band at 1464cm^{-1}indicates –N-O symmetric stretch from aliphatic nitrogen containing organic compound (Table 8). Finally, bands at 1170cm^{-1}, 1109cm^{-1}and 1047cm^{-1}are indicative of Ar-O bond in aromatic rings (Table 9). Fig. 20 shows the FTIR spectrum analyses of water extracts of Moringa the band at 3393cm^{-1}. These results indicate that moringa extraction was most successful using ethanol compared to methanol and water under similar laboratory conditions.

The FTIR spectrum analyses of methanoic and ethanoic extracts of Ginger are represented in Figs 18 and 19. The bands at 3422cm^{-1}, 3421.83cm^{-1}, 3392.90cm^{-1} and 3393cm^{-1}indicate the OH broadband, which is associated with alcohol (ethanol, methanol) that was used for extraction of the Ginger. In addition, bands at 2926cm^{-1} and 2855cm^{-1} represents OH stretch H bonded carboxylic Acid. It can also indicate a C-H stretch of alkanes. Furthermore, the band at 1713cm^{-1} indicates a C=O stretch due to aliphatic ketones and esters. The bands at 1605cm^{-1} and 1516cm^{-1} are indicative of C=C bonds. Also, the band

at 1456cm^{-1}indicates –N-O symmetric stretch from aliphatic nitrogen containing organic compound (Table 8). Likewise, bands at 1271cm^{-1}, 1236cm^{-1} and 1209cm^{-1}, indicates C-O-C stretch of ether. The bands at 1153cm^{-1}and 1122cm^{-1}indicate a C-O-C stretch of ether (Table 9). Finally, the band at 1604cm^{-1} indicates a -C=C- stretching vibration from protein in the plant (ginger) cell wall (Table 9). Fig. 20 shows the FTIR spectrum analyses of water extracts of Ginger the band at 3402cm^{-1}. These results indicate that Ginger extraction was most successful using ethanol compared to methanol and water under similar laboratory conditions.

3.4 Antimicrobial activities of plant extracts

The antibacterial effect of *Moringa* extracts (Methanol, Ethanol and water) and ginger extracts (Methanol, Ethanol and water) on the isolates from the various yam samples are shown on Table 10. The Table shows a comparison of these effects with negative (sterile distilled H_2O) and positive controls. The results show a variation in the effects of the different extracts depending on the particular organism being tested. The highest zones of inhibition were recorded when Ginger extracts were applied to *Streptococcus* spp isolated from healthy yam (25.5 mm), similar effect was observed when Ginger extracts were applied to *Staphylococcus aureus* isolates also from healthy yam. Moringa extracts were also effective at similar concentrations but not as effective.

On the other hand, less effectiveness of Moringa and ginger extract on isolates from diseased yam was observed. Application of Moringa and ginger extracts were not half as effective as they were on isolates from un-diseased/ healthy yam tissues. Whereas, the effects of the positive controls, that is, the antibiotics was fairly uniform regardless of whether the isolates were from healthy or diseased yam tissues.

Using the Clinical Laboratory Standards Institute (CLSI) performance standards for antimicrobial testing (Song, 2004) the zones of inhibition recorded in the present study were further analyzed as follows. Methanoic extracts of *Moringa*were generally not effective against all but one of the 8 bacteria isolates (Isolate 3; *Staphylococcus aureus*), where antibacterial activity was low. Methanoic extracts of ginger had low antibacterial activity on 1 of the 8 bacteria isolates (Isolate 1; *Streptococcus* spp) while in 3 isolates (Isolate 3; *Staphylococcus aureus*; Isolate 6; *Enterococcus* spp; Isolate 8; *Staphylococcus aureus*) intermediate antibacterial activity were observed. Also, 2 of the 8 isolates (Isolate 2; *Staphylococcus aureus* and Isolate 4; *Streptococcus* spp) were susceptible and 1 isolate (Isolate 5; *Aeromonas*spp) was completely resistant to the methanoic extract of Ginger (Table 11).

For ethanoic extract of ginger, five of the eight (Isolate 1; *Streptococcus* spp*;*Isolate 2; *Staphylococcus aureus;*Isolate 3; *Staphylococcus aureus;*Isolate 4; *Streptococcus* spp) bacteria isolates were susceptible while two isolates (Isolate 5; *Aeromonas*spp*;*Isolate 8; *Staphylococcus aureus*) were intermediately inhibited. Only one isolate (Isolate 7; *Micrococcus* spp) was completely resistant to the ethanoic extract of ginger. Also, the ethanoic extract of *Moringa* had low antibacterial effect on four of the eight (Isolate 4; *Streptococcus* spp*;*Isolate 5; *Aeromonas* spp*;*Isolate 6; *Enterococcus* spp*;*Isolate 7; *Micrococcus*spp) bacteria isolates while in the antibacterial effect in two isolates (Isolate 1; *Streptococcus* spp*;*Isolate 2; *Staphylococcus aureus*) were intermediate. Furthermore, one isolate (Isolate 3; *Staphylococcus aureus*) was susceptible to the ethanoic extract of *Moringa* while one isolate (Isolate 8; *Staphylococcus aureus*) was resistant (Table 12).

Results of the comparison of antibacterial activities of known antibiotics with plant extracts of *Moringa* and *Ginger* on the various isolates show that the *ciprofloxacin* was more

effective than all the tested extracts including other positive controls (Fig. 22). The level of effectiveness of Ciprofloxacin on isolate 1 (*Streptococcus* spp) is followed by erythromycin, streptomycin, ethanol, methanol extracts of ginger and methanol extracts of Moringa in decreasing order of effectiveness. Ethanol extracts of Moringa had no effect on this particular isolate (Fig. 22). Moreover, in Fig 23: Ginger and Moringaextracts show similar effect of zone of inhibition with some of the positive control such as ciprofloxacin, erythromycin, streptomycinexcept *Moringa* methanol extract that has no inhibitory effect on isolate 2 (Fig.23). The influence of plants extracts and other drugs were broadly effective on isolate 3 (Fig.24). Also, in Fig: 25 its shows *Moringa* methanol not susceptible to the organism (isolate3) moreover, *ginger* and *Moringa* extracts of ethanol has favorable inhibitory effect than streptomycin and erythromycin. Looking at (Fig: 26), *Moringa* methanol extract shows no inhibitory effect on isolate 4, moreover, *erythromycin* shows the most effective followed by ciprofloxacin, streptomycin and (*Moringa and ginger*) ethanol extracts in order of decreasing potency. On the other hand, in (Figs 27) there was no inhibitory effect of *Moringa* methanol on the pathogens (isolate 5). *Ginger* and *Moringa* ethanol extracts show less effectiveness than ciprofloxacin, streptomycin and erythromycin in order of decreasing potency. Fig 27 and 28 show ethanoic extract of ginger, and *methanoic* extracts of *ginger* and *Moringa* having no inhibitory effect on the pathogens while other positive controls were susceptible to the isolates.

Table 7: Distribution of microbial isolates by sample

S/N	Sample category (Symptoms)	Number of distinct isolates	Suspected organisms
1	HEALTHY YAM	4	*S. aureus* (2; 25%) *Streptococcus* sp(2; 25%)
2	DRY ROT	1	*S. aureus* (1; 12.5%)
3	SOFT ROT	3	*Aeromonas* sp.,(1; 12.5%) *Enterococcus* sp., (1; 12.5%) *Micrococcus* sp.(1; 12.5%)

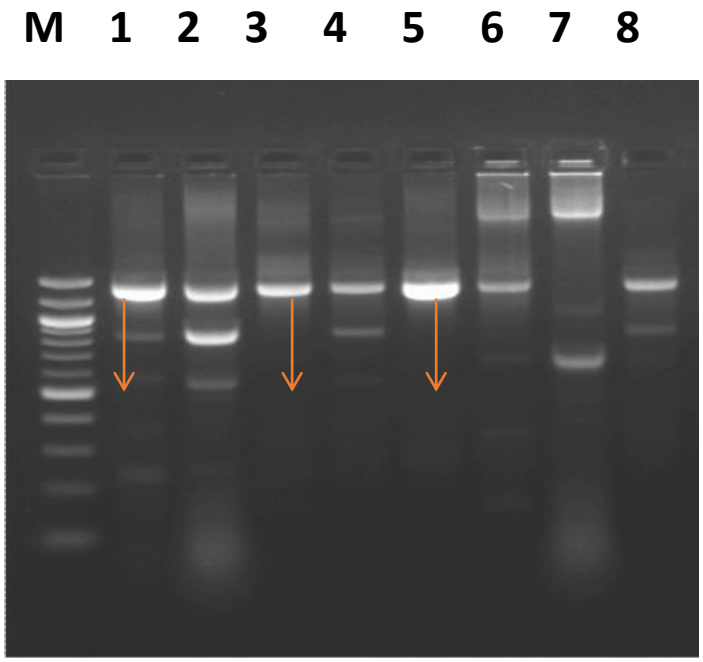

Figure 14: Gel electrophoresis of resolved 16sRNA PCR amplicon of isolated bacterial isolates. Thepresence of a 1200bp band on lane 1, 2, 3,4, 5, 6, and 8 is indicative of a successful amplification of *Streptococcus spp, staphylococcus aureus, staphylococcus aureus, streptococcus, Aeromonas spp, Enterococcus spp, Micrococcus spp, and staphylococcus aureus* the order of listing.M represents the DNA ladder or Marker.

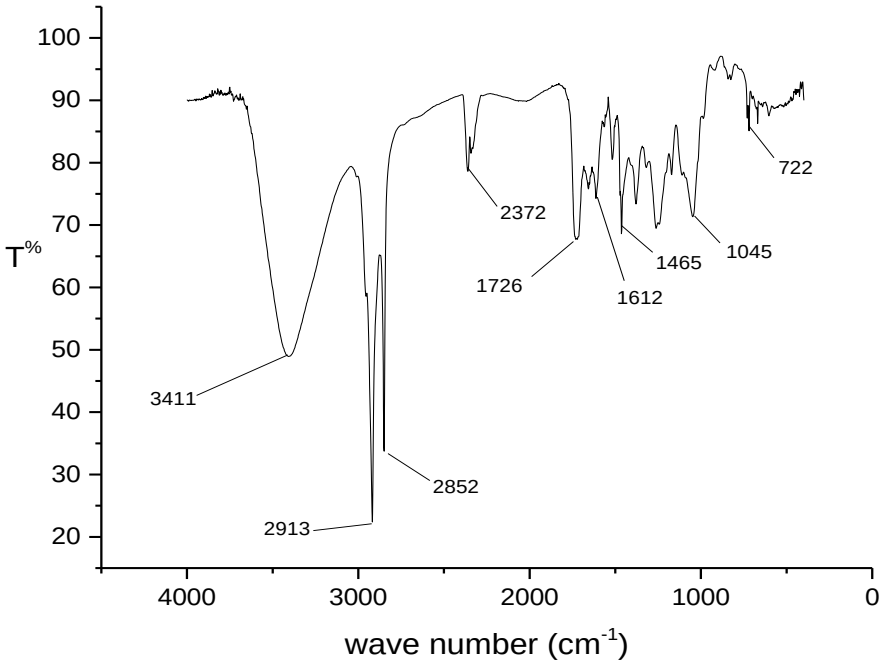

Fig 15: FTIR of Moringa Methanoic extract

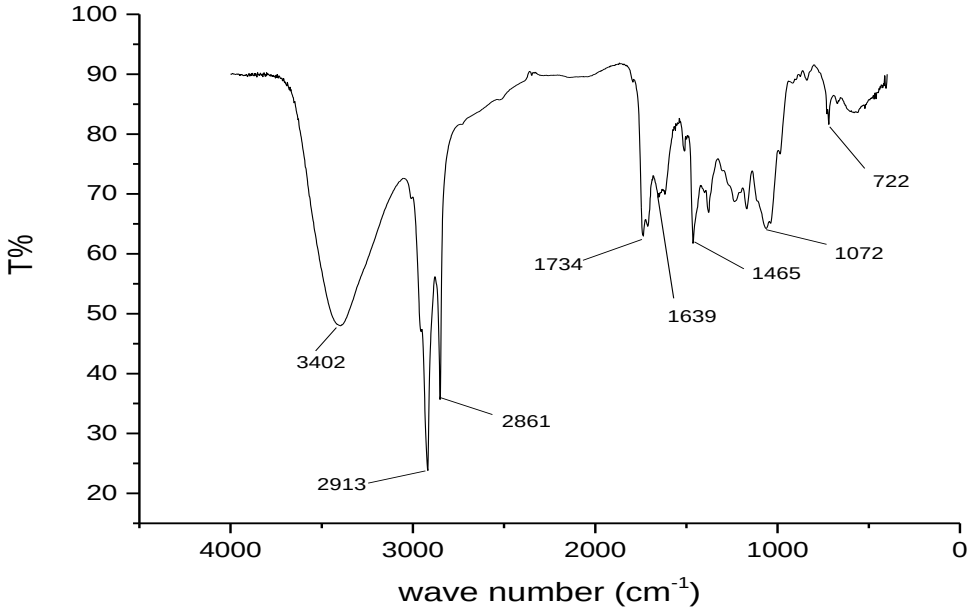

Fig 16: FTIR of Moringa Ethanoic extract

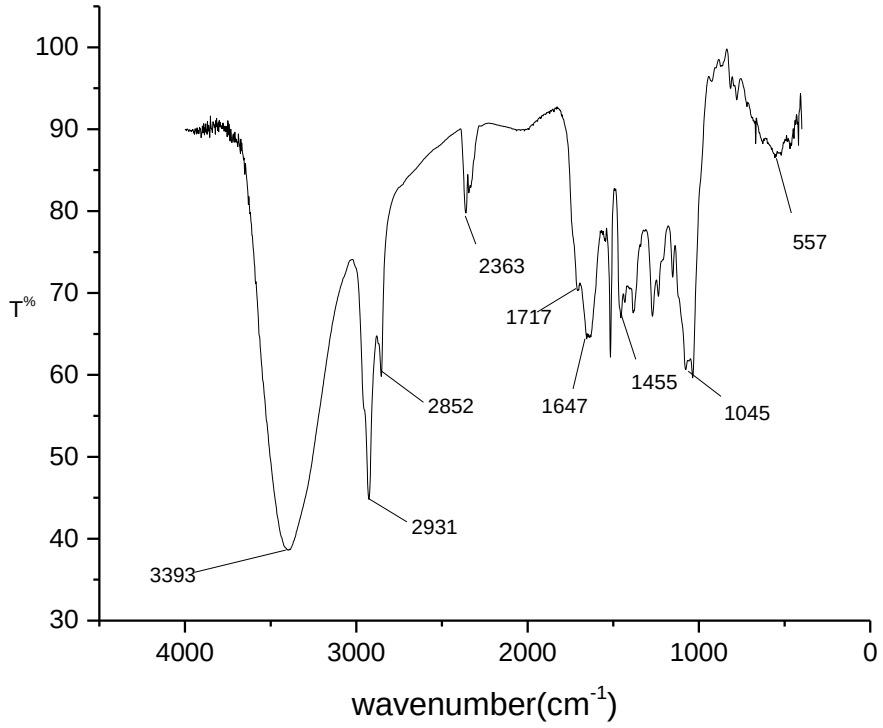

Fig 17: FTIR of Ginger Methanoic extract

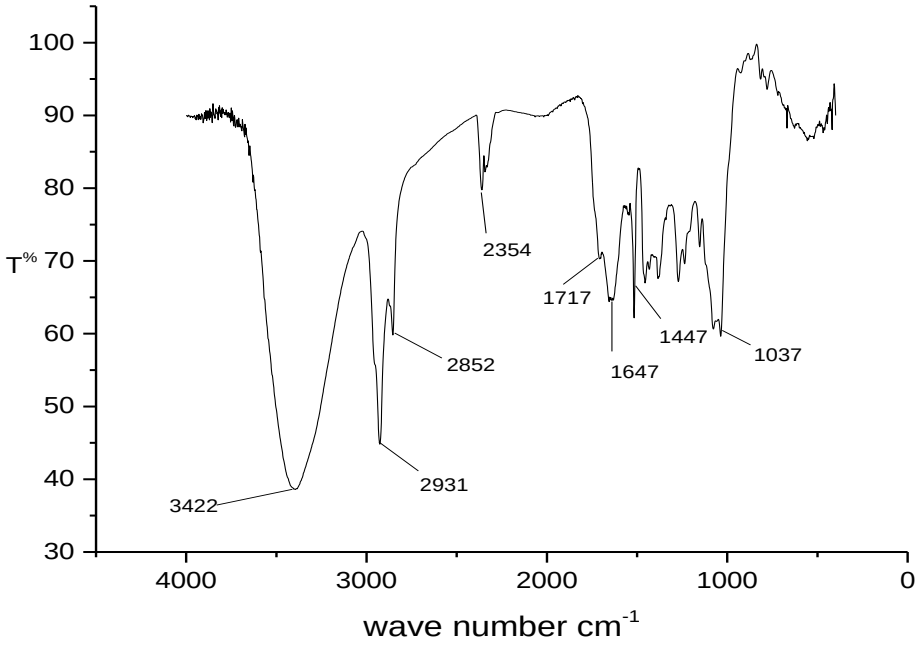

Fig 18: FTIR of Ginger Ethanoic extract

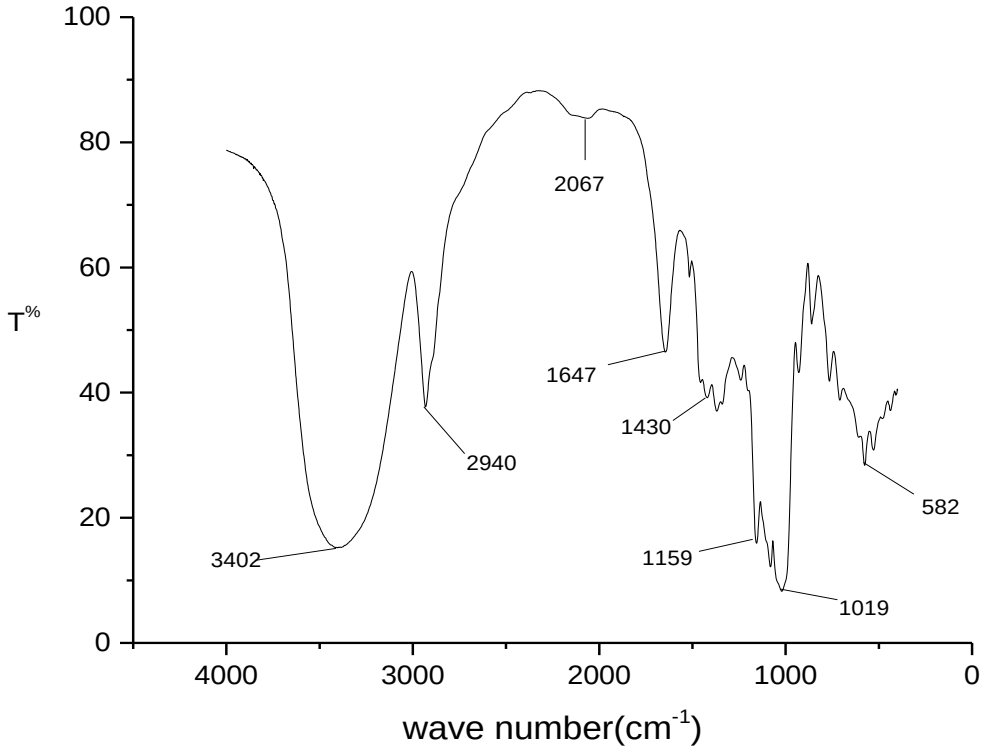

Fig 19: FTIR of Ginger water extract

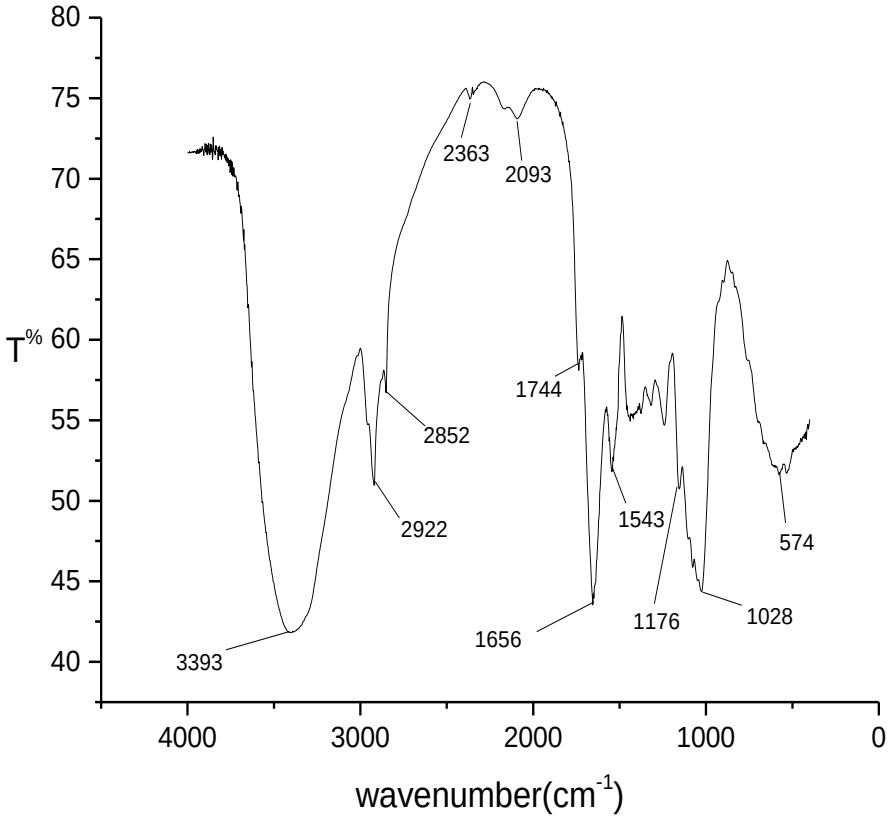

Fig 20: FTIR of Moringawater extract

Table 8: Result of the (FTIR) of the Plant Extracts

Extract	Wave Band Cm^{1}	Description
Methanoic extract *Moringa*	3400.62	OH is indicated associated with ethanol
Methanoic extract *ginger*	3421.83	OH is indicated associated with ethanol
Ethanoic extract *Moringa*	3406.4	OH is indicated associated with ethanol
Ethanoic extract *ginger*	3392.9	OH is indicated associated with ethanol

Table 9: Results of (UV-Visible of Plant Extracts)

Extract	Maximum wave length (mm)	Description
Methanoic extract *Moringa*	48.97	O-H
Methanoic extract *ginger*	48.061	O-H
Ethanol extract *Moringa*	42.928	O-H
Ethanoic extract *ginger*	38.596	O-H

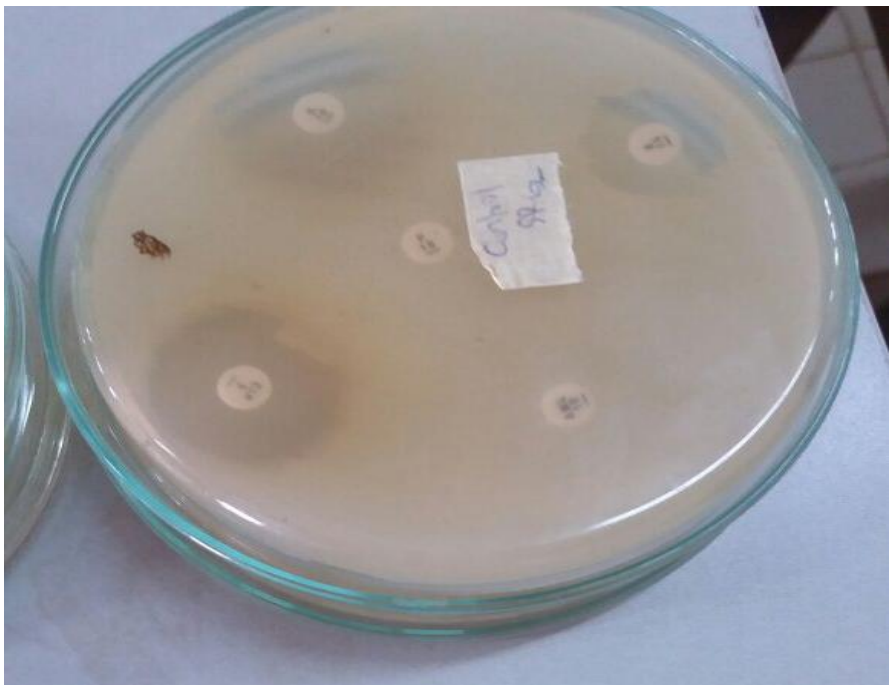

Figure 21: Activities some known antibiotics (positive controls) on *Enterococcus* sp. isolated from soft rot in yam

Table 10: Antibacterial activities of plant extracts on isolated bacteria from yam rot

S/N	ORGANISM	CONTROL (+ve)						CONTROL (-VE)	DRUG EXTRACT OF MeoH/DILUTION (con mm/ml)							DRUG EXTRACT OF EoH/DILUTION (con mm/ml)							
									GINGER			MORINGER				GINGER				MORINGER			
		CIPRO	ERYTHRO	STREPTO	PENICILIN	AMOXYLIN	ETHANOL	METHANOL	10^{-1}	10^{-4}	10^{-7}	10^{-10}	10^{-1}	10^{-4}	10^{-7}	10^{-10}	10^{-1}	10^{-4}	10^{-7}	10^{-10}	10^{-1}	10^{-4}	10^{-7}	10^{-10}
	ISOLATES FROM HEALTHY YAM																						
1	*Streptococcus* spp (Iso 1)	27.5	17.5	14.5	-	-	-	-	-	7	-	-	-	1	-	5	-	15.5	18.0	-	-	-	-	15.0
2	*Staphylococcus aureus* (Iso 2)	23.0	17.5	22.5	-	-	-	-	-	3.5	-	17	-	3.5	-	-	-	21.5	-	-	-	14.5	-	15.0
3	*Staphylococcus aureus*(Iso 3)	22.5	13.0	23.0	-	-	-	-	12.5	-	-	-	9.0	-	-	-	15.0	15.5	17.5	-	-	14.0	18.0	-
4	*Streptococcus* spp(Iso 4)	28.5	12.5	24.5	-	-	-	-	-	17	-	-	-	-	-	-	-	25.5	10.0	-	-	7.0	-	-
	ISOLATES FROM SOFT ROT YAM																						
5	*Aeromonas* spp(Iso 5)	26.5	30.5	16.5	-	-	-	-	5.5	-	-	-	3.0	-	-	-	2.5	15.0	-	-	10.0	4.5	-	-
6	*Enterococcus* spp(Iso 6)	28.0	18.0	23.5	-	-	-	-	14.5	3.5	-	-	3.0	-	-	-	15.0	20.5	-	-	9.5	3.5	-	-

7	***Micrococcus* spp(Iso 7)**	**24.5**	**17.0**	**28.5**	**-**	**-**	**-**	**-**	**-**	**-**	**3.5**	**-**	**5.0**	**2.5**	**4.5**	**2.5**	**-**	**-**	**-**	**5.5**	**-**	**4.5**	**-**	**8.0**
	ISOLATES FROM DRY ROT YAM																								
8	***Staphylococcus aureus* (Iso 8)**	**27.5**	**22.0**	**20.0**	**-**	**-**	**-**	**-**	**-**	**11**	**-**	**-**	**-**	**-**	**-**	**-**	**15.0**	**-**	**4.0**	**-**	**-**	**-**	**4.0**	**-**

Table 11: Antibacterial effect of Methanoic extracts of Ginger and Moringer on bacteria isolates.

METHANOIC EXTRACT						
	GINGER			MORINGER		
Isolates	Size (mm)	Antibacterial Effect	Interpretation	Size (mm)	Antibacterial Effect	Interpretation
Streptococcus spp	7	+	Low	0	-	Resistant
Staphylococcus aureus	17	+++	Susceptible	0	-	Resistant
Staphylococcus aureus	12.5	++	Intermediate	9	+	Low
***Streptococcus* spp**	17	+++	Susceptible	0	-	Resistant
***Aeromonas* spp**	0	-	Resistant	0	-	Resistant
***Enterococcus* spp**	14	++	Intermediate	0	-	Resistant
***Micrococcus* spp**	0	-	Resistant	0	-	Resistant
Staphylococcus aureus	11	++	Intermediate	0	-	Resistant

- **Means no antimicrobial activity; + means presence of antimicrobial activity**

Table 12: Antibacterial effect of Ethanoic extracts of Ginger and Moringer on bacteria isolates.

ETHANOIC EXTRACT						
	GINGER			MORINGER		
Isolates	Size (mm)	Antibacterial Effect	Interpretation	Size (mm)	Antibacterial Effect	Interpretation
Streptococcus spp	18	+++	Susceptible	15	++	Intermediate
Staphylococcus aureus	21.5	+++	Susceptible	15	++	Intermediate
Staphylococcus aureus	17.5	+++	Susceptible	18	+++	Susceptible
***Streptococcus* spp**	25.5	+++	Susceptible	7	+	Low
***Aeromonas* spp**	15	++	Intermediate	10	+	Low
Enterococcus spp	20.5	+++	Susceptible	9.5	+	Low
***Micrococcus* spp**	0	-	Resistant	8	+	Low
Staphylococcus aureus	15	++	Intermediate	0	-	Resistant

- **Means no antimicrobial activity; + means presence of antimicrobial activity**

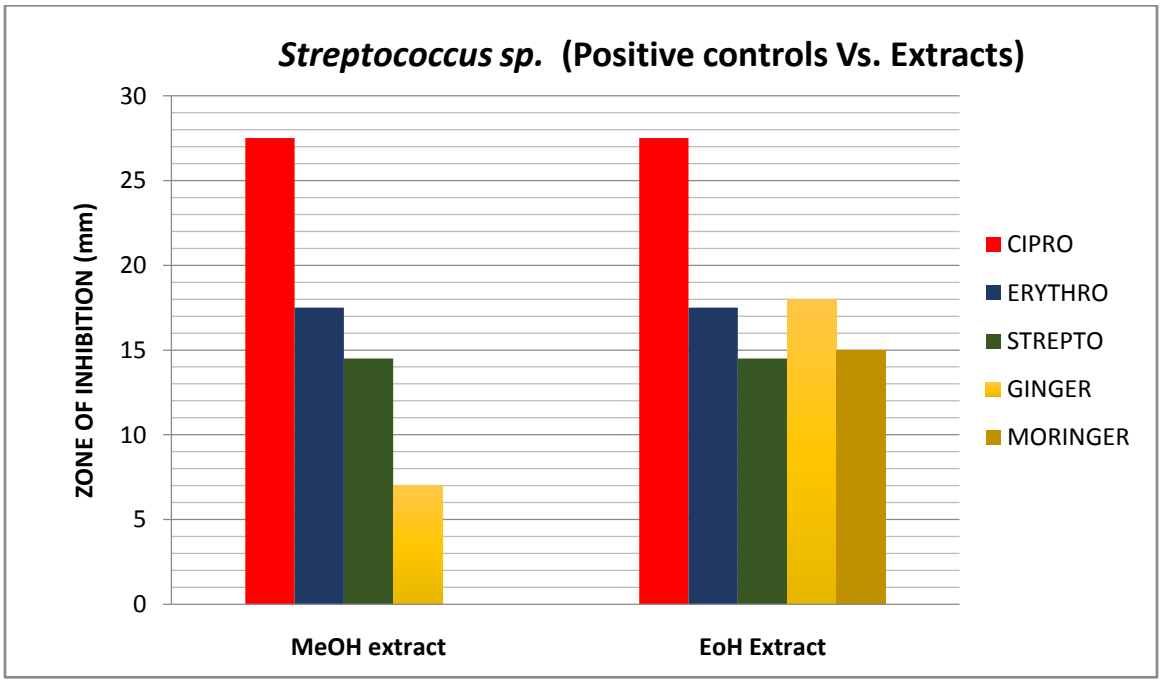

Figure 22: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Streptococcus* spp)

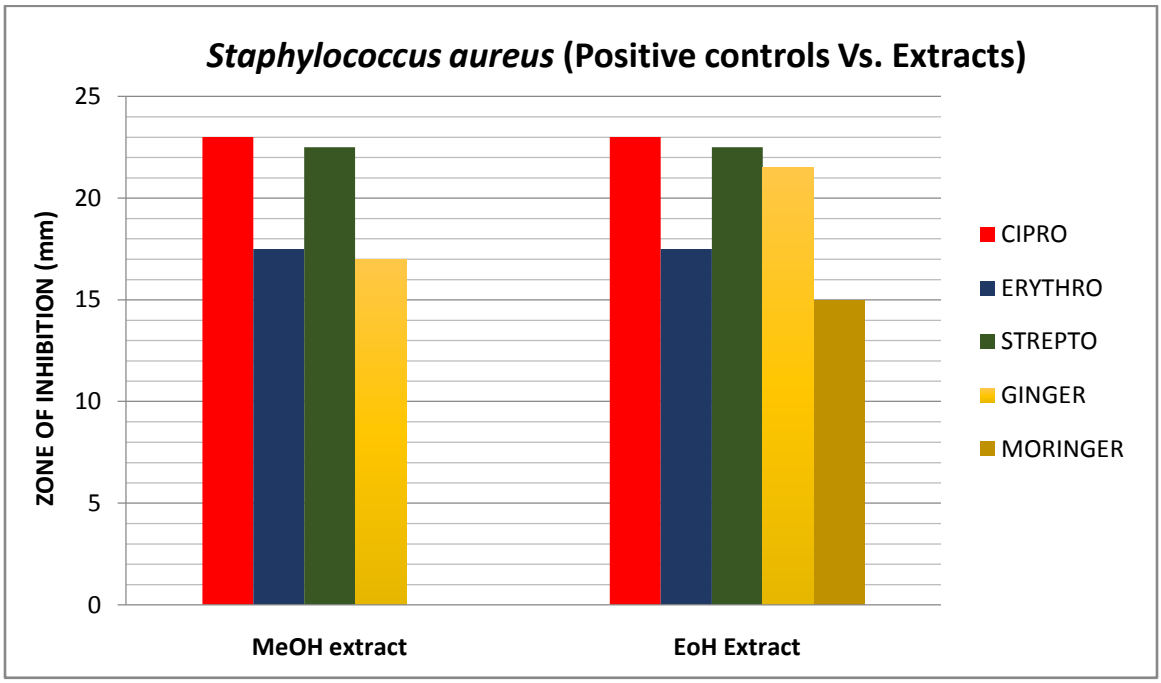

Figure 23: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Staphylococcus aureus*)

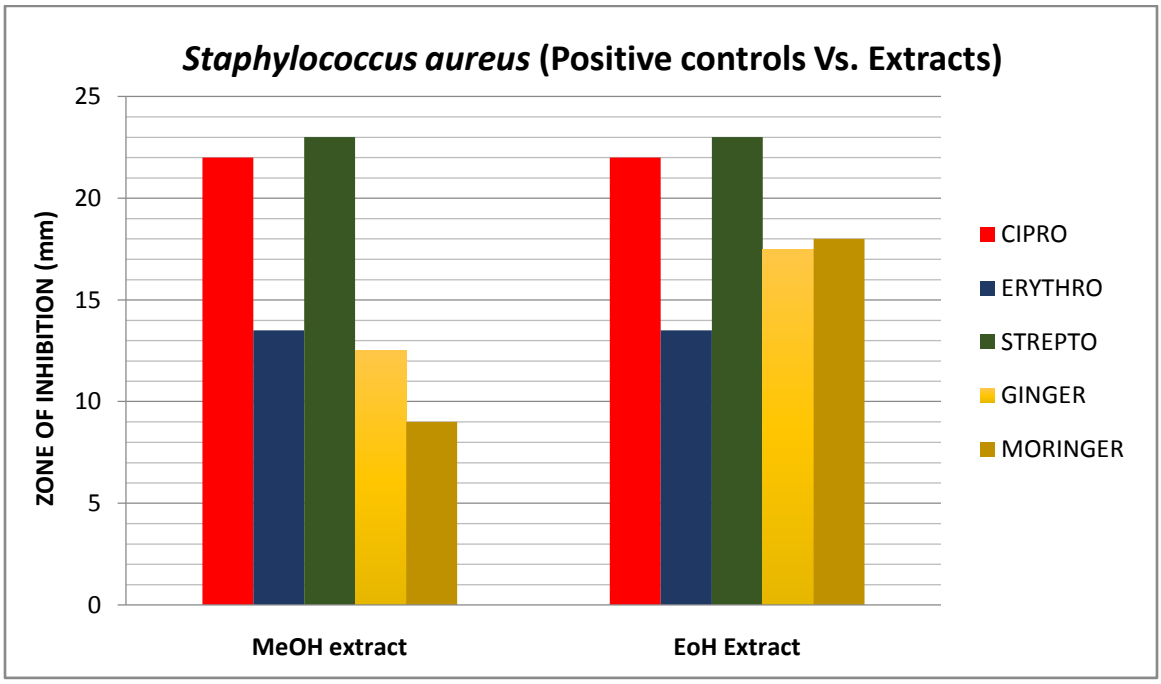

Figure 24: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Staphylococcus aureus*)

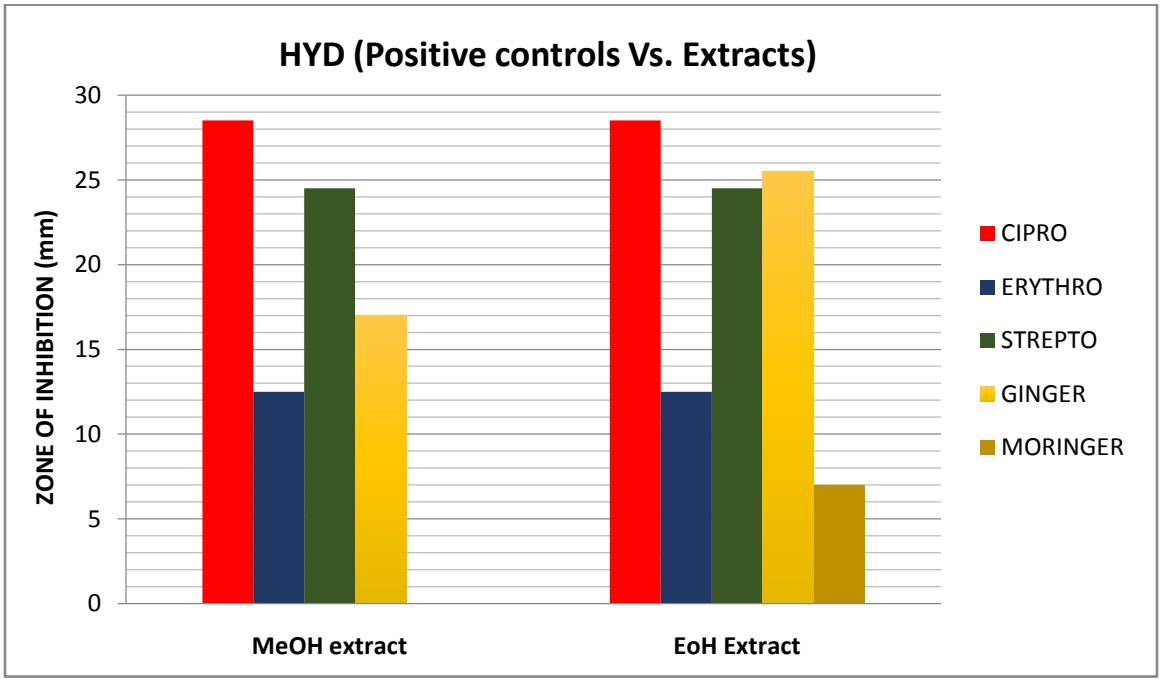

Figure 25: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Streptococcus* spp)

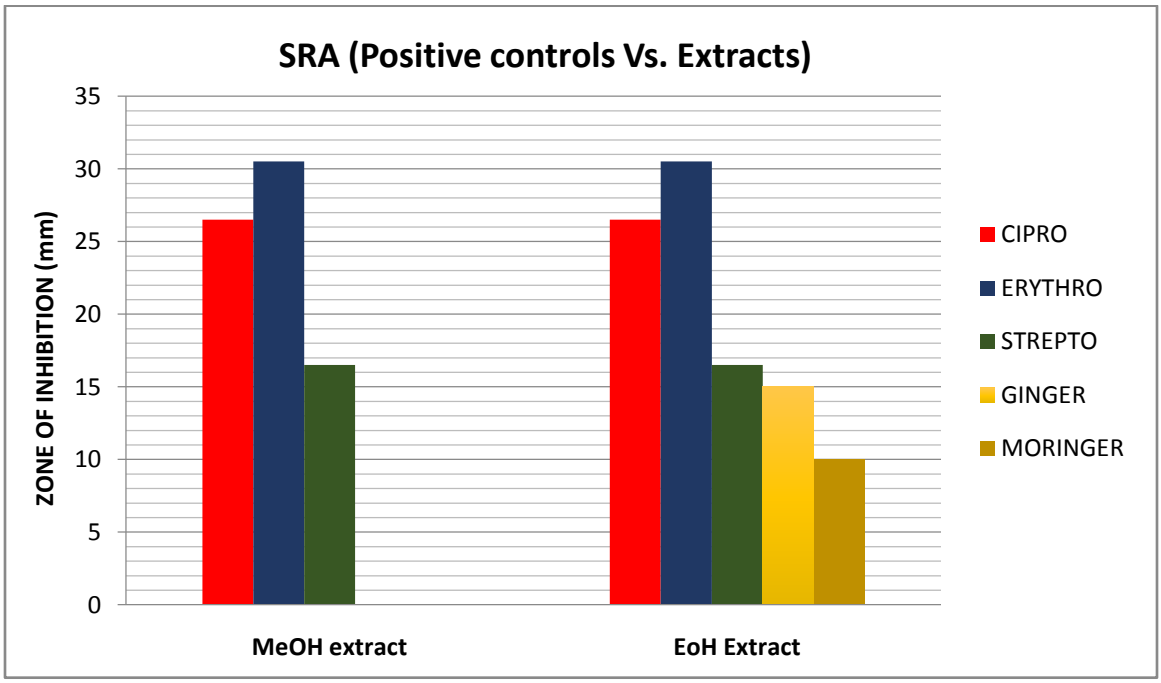

Figure 26: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Aeromonas* spp)

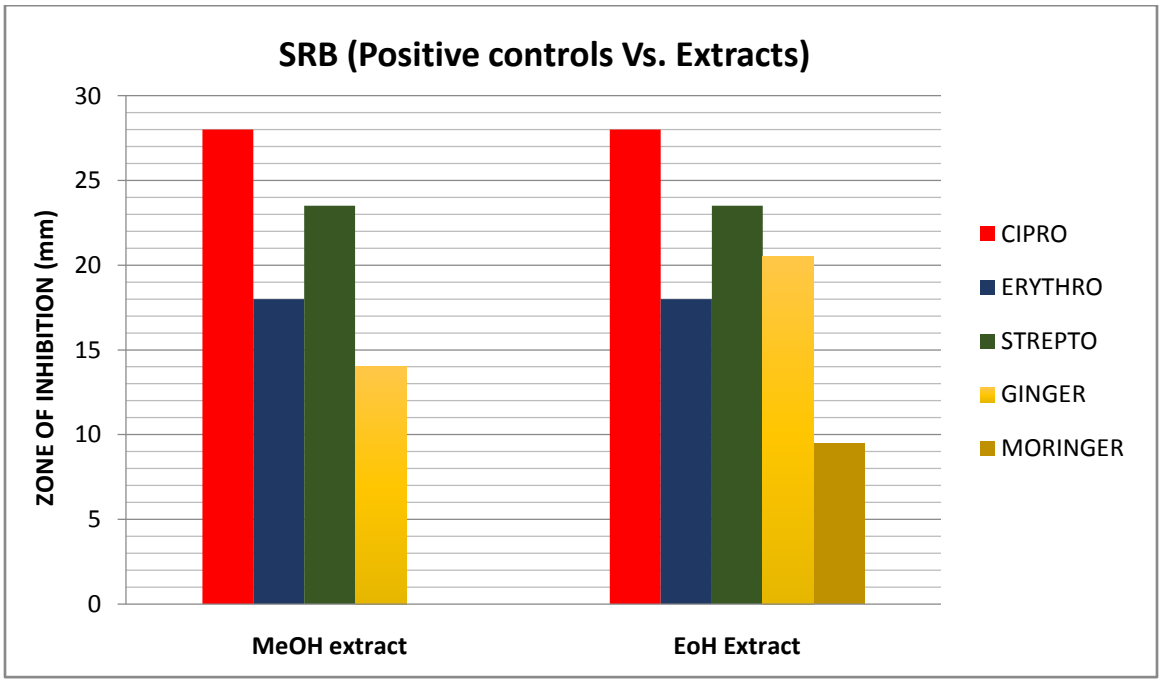
SRB (Positive controls Vs. Extracts)
ZONE OF INHIBITION (mm)
30
25
20
15
10
5
0
MeOH extract
EoH Extract
CIPRO
ERYTHRO
STREPTO
GINGER
MORINGER

Figure 27: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Enterococcus* spp)

Figure 28: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Micrococcus* spp)

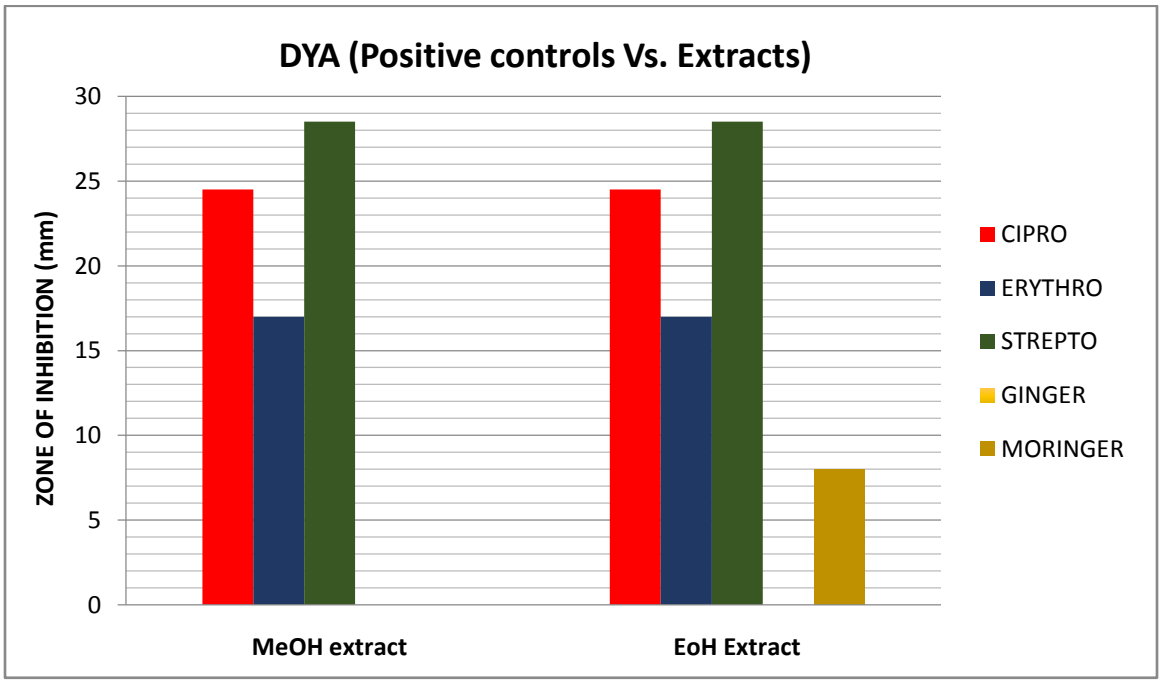

Figure 29: Comparison of antibacterial activities in known antibiotics and plant extracts on (*Staphylococcus aureus*)

CHAPTER FOUR

DISCUSSIONS AND CONCLUSION

The bacterial species isolated from healthy and diseased (rotted) yam tubers sampled for post- harvest infection in the present study fit the profile of organisms typically encountered on human hands (Ayoade and Ardern, 2017), this result confirms earlier report that pathogenic organisms responsible for yam rot are as a result of their ubiquitous nature and the relative ease by which handlers introduce them during harvesting and transport from the field to the storage barn (Ogundana *et al,*1970). The isolated organisms are as follows: *Staphylococcus aureus (3 isolates), Streptococcus* sp. *(2 isolates), Micrococcus* sp., *Aeromonas sp.,* and *Enterococcussp*.

Also, yam tuber may have been infested by pathogens derived from disease foliage, roots or mother tubers during harvesting (Osagie, 1992). A wide range of microorganisms has been isolated from post-harvest rots of yam although relatively few of these have been shown to be appreciably pathogenic (Ogundana 1992; Adeniji, 1970; Osagie, 1992). The results from the present study confirm earlier reports from previous studies, for example, Oloruntoba and Sridhar (2015) reported the isolation of the following species of bacteria from rotted yam:*Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp.,*Micrococcus* sp., *Escherichia coli,* Proteus sp., and *Aeromonas* sp.

Typically, prominent spoilage bacteria cum plant parasitic bacteria such as *Erwinia sp*., *Pectobacterium* sp., *Corynebacterium* sp. (IITA, 2007) and *Flavobacterium* sp. (Oloruntoba and Sridhar, 2015), have been associated with post-harvest diseases of yam. However, in the present study, these were not found. This discovery opens up avenue for further work in the area of pathogenicity and virulence of opportunistic bacterial infections in plants by organisms that typically will not infect the host unless there are some pre-disposing factors. In the present case, our results lead us to suspect that the pathogenic infection by the isolated organisms from rotted yam is opportunistic and the process of the initiation of virulence should be better studied in the future.

Furthermore, the fact that most of the pathogens isolated from the present study are enterotoxigenic is quite worrisome. For example, the presence of *Aeromonas* sp. in most of the sample portions, according to ICMSF (1996), can cause illness ranging from mild diarrhea to a life-threatening, cholera-like disease and although, *Aeromonas* sp. has not been evidently linked to an outbreak of gastrointestinal illness, all species have been isolated and epidemiologically linked to this disease (Oloruntoba and Sridhar, 2015). In addition to spoilage activities, the presence of *Staphylococcus aureus, Streptococcus* sp.,and *Micrococcus* sp., poses significant health hazards, as these bacteria are known pathogens of humans (Akusu, 2016). For instance, food poisoning caused by *Staphylococcus* species is one of the most common causes of foodborne illness due to the widespread occurrence of *S. aureus* and the ability of many strains to produce enterotoxins (Akusu, 2016). It is therefore important to find safe ways of preventing the presence and activities of these spoilage pathogens.

The efficacy of plant extracts to serve as antimicrobial agents is well documented. For instance, the antimicrobial activities of plant species such as *Adhatoda zeylanica*, Medic25,

Trianthema decandra L. (Geethalakshmi *et al.,* 2010) *Argemone mexicana* L. (Rahman *et al.*,2011), *Tinospora cordifolia*, *Cassia fistula* (Upadhyay *et al.,* 2011), and *Mangifera indica*(Joshua and Takudzwa, 2013) have all been studied with positive results obtained. Sen *et al.,* (2012) also reported the antimicrobial efficiency of *Melia azedarach* L.; a medicinal plants (leaf) extracts (using Methanol, Ethanol, Petroleum ether and water as solvents), against eight bacteria pathogens *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.* The report further indicated a significant antibacterial activity by the alcoholic extract of *M. azedarach* against the organisms.

The phytoconstitutuents of *Zinigiber officinale* (ginger) have longed been known and its antibacterial activity is well documented (Roy *et al.,* 2006) and results over the years has been favorable even against known multi-drug resistant pathogens (Okiki *et al.,* 2015; Riaz *et al.,* 2015; Islam *et al.,* 2014; Karuppiah and Rajaram, 2012). The antimicrobial activity *Moringa olifera* extracts against numerous pathogenic bacteria has also been adequately studied and results has been favorable even against known multi-drug resistant pathogens (Moyo *et al.,* 2012; Bukar *et al.,* 2010; Saroj *et al.,* 1995). Reports also show that various parts of this plant (roots and leaves) have broad-spectrum antimicrobial activities against various pathogenic bacteria (Bukar *et al.,* 2010).

The current study investigated the antimicrobial efficacy of two plants *Moringer olifera*and *Zinigiber officinale* extracts (Ethanol, Methanol and Water) against 8 isolated bacteria etiologies of yam rot, which were *Staphylococcus aureus (3), Streptococcus* sp. *(2), Micrococcus* sp., *Aeromonas* sp.,and *Enterococcus*spp. Results obtained showed that ethanoic extracts of Ginger and Moringer were generally more effective against these isolates than Methonoic extracts. This is somewhat similar to reports made by Sen *et al.* (2012). Furthermore, there was

no antibacterial activity of water extracts of ginger and moringer against all 8 bacteria isolates. This is somewhat different from reports by other researchers (Sen *et al*, (2012). However, a possible reason for this is that most antimicrobial agents in plants are organic compounds and as such, not easily dissolvable in water, thus preventing complete extraction.

In conclusion, this work discloses the presence of known bacteria etiologies of post-harvest yam rot and the presence of antibacterial compounds in Moringa and Ginger which were successfully applied in minimizing the growth of these organisms implicated in yam rots. This agrees with earlier reports, which have used the same plant extracts to control diseases in potato and yam (Okigbo and Ikediugwu 2000; Okigbo and Emogbene, 2004). Also, it is important to state that extracts contained antibacterial, which conferred the antimicrobial properties on these plants. Thus, extracts of these plants can serve as alternative ways of reducing and controlling rot by farmers especially those caused by multi-drug resistant bacteria.

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