ANTIMICROBIAL EFFECT OF THE EXTRACTS OF SELECTED MEDICINAL PLANTS AGAINST SOME MICROORGANISMS ISOLATED FROM POULTRY DROPPINGS

BY

OLUMOHUNLE, Folake Elizabeth
MTECH/SLS/2017/6708

DEPARTMENT OF MICROBIOLOGY,
FEDERAL UNIVERSITY OF TECHNOLOGY,
MINNA, NIGER STATE

JULY, 2021

ABSTRACT

Every culture has relied on herbal plants due to the presence of huge variety of natural constituents present which are used for therapeutic purposes. This study assessed the antimicrobial effects of the leaves of selected medicinal plants against some microorganisms isolated from poultry droppings. The crude leaf extracts were obtained using maceration. The phytochemical composition of the crude extracts was also analysed. Antimicrobial activity of the extracts was tested against the isolated microorganisms using agar well diffusion and agar dilution. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were done using broth macrodillution assay. The microorganisms isolated from poultry droppings were *Staphylococcus aureus, Salmonella* Typhi*, Aspergillus niger* and *Candida albicans*. The aqueous leaf extract of *Azadirachta indica* revealed the maximum antibacterial activity at concentration of 400mg/mL against *Staphylococcus aureus* (21.0±1.0), *Salmonella* Typhi (26.0±1.0mm), as compared to other extracts; hence it was found to be the most active extract. The methanol fraction of *Azadirachta indica* showed the highest antibacterial activity against *Salmonella* Typhi (18.0±1.0) while the aqueous fraction of of *Azadirachta indica* showed the highest antibacterial activity against *Staphylococcus aureus* (16.0±1.0). The aqueous leaf extract of *Azadirachta indica* revealed the maximum antifungal activity at concentration of 400mg/mL against *Candida albicans* as compared to other extracts. The fractions had no activity on *Aspergillus niger*, hence they were resistant to all the four fractions. The MIC of the aqueous extract of *Azadirachta indica* against *S. aureus, S.* Typhi*, C. albicans* was 31.25mg/mL respectively. MBC of 125mg/mL was recorded against *C. albicans* and *S. aureus* respectively while 250 mg/mL was recorded against *S.* Typhi. The aqueous and methanol fractions of *Azadirachta indica* had MIC of 31.25mg/mL against *S. aureus* while the methanol and ethyl acetate fractions had MIC of 62.5 mg/mL against *S.* Typhi. The fractions had MBC of 125 mg/mL for both organisms. The results of this study showed that *Azadirachta indica*, *Allium sativum*, *Moringa oleifera* and *Jatropha curcas* possess antimicrobial potentials and may be useful in treating infections caused by pathogenic isolates.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The consumption of synthetic antimicrobials exhibits some deleterious effects on humans, one of which is the disruption of the natural human microbiota known to play key roles in nutrition, development, metabolism, pathogen resistance and regulation of the human immune responses (Pourmand *et al.,* 2017). Therefore, there is a need for the development of new naturally sourced antimicrobial drugs to reduce the risk associated with the synthetic ones. In recent years, there has been a rapid upsurge in the emergence of multi-drug resistance among pathogenic bacteria due to extensive use of antibiotics (O'Neill, 2016). To this end, the pharmaceutical industry is in a continuous struggle to keep up with the production of new synthetic antibiotics to combat the emerging multi-drug resistant bacterial strains. Medicinal plants thus remain a feasible source of new compound for the drug development process (Klein *et al.,* 2018).

Jatropha curcas Linn. is fast becoming a very useful economic resource in phytomedicine development and development of new lead compounds and is closely related to other important cultivated plants like rubber and castor plants (Abhilash *et al.,* 2011). In Nigeria specifically, the leaf and stem decoction is used singly or in combination with other recipes for the treatment of skin diseases, and stomach disorders (Pandey *et al.,* 2012). The word '*Jatropha*' is derived from Greek words 'Jatros' and 'trope' (food/nutrition) which implies medicinal uses. The genus *Jatropha* belongs to family *Euphorbiaceae* and subfamily Acalyphoideae. It is commonly known as physic nut, purging nut, barbados nut and nutmeg plant in English (Brittaine and Lutaladio, 2010, Erinoso and Aworinde, 2012).

Various parts of *J. curcas* can be used for healthcare management of plants, animals and human being. *J. curcas* is also useful to control soil erosion and improved water infiltration, to reclaim wasteland, phytoremediation of various contaminated soils, livestock barrier and land demarcation, soil carbon sequestration and sustainable environmental development (Warra, 2012).

Moringa oleifera Lam is one of the best known, widely distributed and grown species of a monogeneric family *Moringaceae* (Dubey *et al.,* 2013). The plant is referred to as drumstick tree or the horse radish tree. It is native to the western and sub-Himalayan parts of Northwest India, Pakistan and Afghanistan. It is a drought tolerant plant that thrives best under the tropical climate and tolerates different soil types. The plant is highly valued since almost every part of the tree (leaves, roots, bark, fruit flowers, immature pods and seeds) is used as food with high nutritional value (Moyo *et al.,* 2012). In addition, the plant has been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases (Dubey *et al.,* 2013).

Azadirachta indica (Neem) is a tree of the Meliaceae family found worldwide in semi-tropical and tropical climates. In Nigeria, it is popularly known as *Dogonyaro*. *Azadirachta indica* (Neem) and its leaves used for the treatment of various diseases including eczema, ringworm, acne, anti-inflammatory activities, anti-hyperglycemic and also treat chronic wounds, diabetic foot and gangrene. It also removes toxins from the body, neutralize the free radicals present in body and used as blood purifier. Recently, it was reported as anticancer and used for hepatorenal protective activity and hypolipidemic effects (Saba *et al.,* 2011).

Garlic is the common name of the genus *Allium sativum*. There are other closely related species including the shallot, the onion and others (Muhsin and Hussein 2014). Garlic has

been reported to have prophylactic and therapeutic properties in conditions such as microbial infections, thrombosis, hypertension, hyperglycemia, hyperlipidemia, cancer, and thrombosis. Garlic can provide proper management for bacterial growth ranging from disinfectant, antiseptic, bacteriostatic and even bactericidal characteristics. Besides that, garlic may have the ability to prevent and manage viral, fungal and even helminth infections (Venancio *et al.,* 2017).

Microorganisms (e.g. virus, bacteria, protozoa, and fungi) surround us, they are ubiquitous and everything in the world is governed by them. They are part of our everyday lives. They influence the quality of our soil, food grown on that soil, and how our body reacts to that food. Most environmental microorganisms spend their entire lives as quiet members of their ecological society, but some reach a level of infamy. Pathogens may only represent a very small portion of all microorganisms, but they are often the most visible.

In Nigeria, as in most of the developing countries, poultry farming is one of the widespread livestock enterprises. The chickens are often administered antibiotics for prevention and treatment of diseases (Manyi-Loh *et al.,* 2018).

1.2 Statement of Research Problem

Intensive poultry rearing has simultaneously increased the use of antibiotics in poultry more than in humans. It has also been observed that disposal of poultry products in the environment and poor hygiene have enhanced the ability of bacteria to remain in the environment thereby posing serious threat to human health. The poultry infections mostly occur as a result of intake of contaminated feed and water. The pathogen could be released into the environment via their droppings (Manyi-Loh *et al.,* 2018).

In Nigeria, poultry droppings are extensively used as manure for cultivation of crops and this practice pose danger to public health, when the crops cultivated with the droppings are eaten raw/ not properly cooked. There is need to identify the organisms isolated from poultry droppings and investigate the antimicrobial activities of some selected plant extracts on some of the isolated pathogens (Madaki *et al.,* 2019).

1.3 Justification for the study

The development of new antibiotics required to combat microbial pathogens from poultry droppings has been discovered to be expensive and time-consuming. Hence, the current trend is to acquire antimicrobials from plants.

Jatropha curcas, Azadirachta indica (Neem), *Moringa oleifera* (Moringa) and *Allium sativum* (Garlic) are valuable medicinal plant in Nigeria that are known for their therapeutic potentials. In Nigeria, the application of poultry droppings to land provides nutrients and organic matter for the crop's growth and soil conditioning but this can also pose danger to public health especially when the crops are eaten raw. The handlers are also exposed to microbial infections as they carry out their daily routines. Due to this exposure, human health had been threatened with diseases that had resulted in the use of different conventional and herbal medications.

Hence, this research evaluates the antimicrobial activity of leaf extracts of *Jatropha curcas*, *Moringa oleifera* (Moringa), *Azadirachta indica* (Neem) and *Allium sativum* (Garlic) bulbs as a resort for the treatment of microbial pathogens from poultry droppings.

1.4 Aim and Objectives of the Study

The study was aimed at determining the antimicrobial effect of the extracts of selected medicinal plants against some microorganisms isolated from poultry droppings while the objectives of the study to:

i. identify the bacterial and fungal isolates from the droppings of chickens.

ii. determine the phytochemical components of the crude extracts of *Jatropha curcas, Azadirachta indica*, *Moringa oleifera* and *Allium sativum*.

iii. determine the antimicrobial effects of the crude extracts and fractions of the most active medicinal plants against the test organisms.

iv. determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts and fractions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herbal medicine as an antibiotic agent

Herbal medicines are important part of the culture and traditions of many developing countries that depend on them in treating health care needs. The use of medicinal plants for treating certain diseases and ailments has been on the increase from time immemorial. These plants are believed to be nature's pharmacy to us and only require us to acknowledge their powerful abilities in tackling most diseases (Bereksi *et al.,* 2018). The use of antibiotics is widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes, this has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages. Natural products are typically secondary metabolites, produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely used in the pharmaceutical industry for their remarkable structural diversity and range of pharmacological activities (Bereksi *et al.,* 2018).

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents. Several works have been done to examine the antimicrobial effects of herbal plants extracts, including roots, stem, leaves or flowers (Dash, 2017). Many countries in Africa and other parts of the world have continued to encourage screening programs of plants used in traditional medicine in order to authenticate their antimicrobial activities and possible inclusion in primary health care. Like the other countries of the Maghreb and Africa, the empirical use of medicinal plants

continues to retain great popularity in Algeria. In some rural areas, resorting to natural remedies with “miraculous” plants is preferred to modern medicine (Bereksi *et al.,* 2018).

Since ancient time, people are exploring the plant species in search of new drugs, which has resulted in exploitation of large number of medicinal plants with curative properties to treat various ailments. The importance of medicinal plants becomes more patent now in developing countries (Dash, 2017).

2.2 Incidence of bacteria from poultry droppings

In most developing countries including Nigeria, poultry farming is one of the widespread livestock enterprises. The industry has expanded extensively in commercial levels as well as in household traditional levels in Nigeria. In the recent past, antibiotics have been used widely in poultry production as growth promoters, prophylaxis and therapeutics. The chickens are often administered antibiotics for prevention and treatment of diseases. This suggests that the intensive poultry keeping will simultaneously increase use of antibiotics more than in human use. It can also be observed that disposal of poultry products in the environment, poor hygiene and ability of bacteria to remain in the environment could likely contribute to dissemination of drug resistance variants strains (Manyi-Loh *et al.,* 2018). These bacteria are carried by healthy chickens and are communicable to people through direct contact, exposure to manure, or consumption of undercooked chicken and eggs. Infection is characterized by diarrhea, vomiting, fever, and/or abdominal cramps; small children, elderly persons, and those with weakened immune systems are more susceptible to severe illness. Young birds may be especially prone to shed these organisms in their droppings. This poses a hazard to anyone who comes into contact with the droppings. The public health hazards associated with *Salmonella* and *Campylobacter* are expected to be limited to those who are in contact with the chickens or their droppings or

consume their meat or eggs without thorough cooking. There have been several multi-state outbreaks of human *Salmonella* infections from handling baby chicks. These hazards could be mitigated by avoiding contact with poultry feces, carefully washing hands with soap and water after handling the birds, avoiding hand-to-mouth contact while working with birds and education about food safety. *Salmonella* infections, especially the enteric fever (typhoid and paratyphoid fever), is endemic in many parts of the country (Mike *et al.,* 2015). The wide host range, the large number of convalescent and chronic healthy carriers and environmental sources in the communities increase the reservoir status of *Salmonella* infection and enhance its endemicity, especially in areas with low environmental hygiene. Poultry droppings have been shown to be a potential reservoir for many enteric organisms. In Nigeria, poultry droppings are extensively used as manure for the cultivation of crops. The application of poultry droppings to land provides nutrients for the crop's growth as well as organic matter for soil conditioning and this can pose a danger to public health especially when the crops are eaten raw. The frequency of reports of typhoid and paratyphoid fever in Nigeria. There is a continual need to search for other environmental sources that could be important in the spread of typhoid and paratyphoid fever in Nigeria (Mike *et al.,* 2015).

In addition to foodborne pathogens, bacteria responsible for spoilage may lead to large economic losses. Their growth and metabolic activity during shelf life leading to color, odor, taste, or texture defects are responsible for waste and losses of food products and have therefore, an important impact on the economy of the poultry meat production sector (Chai *et al.,* 2017). Meat and poultry products are some of the sources for transmitting food borne pathogens to humans with 40 % of the clinical cases attributed to the consumption of egg and other poultry products (Mohanta *et al.,* 2016). The incidence of food borne diseases in humans has increased considerably worldwide in the last few years.

Poultry products have been repeatedly implicated in food borne infections (Madaki *et al.,* 2019).

2.3 Sources of contamination

Poultry feeds are contaminated during processing, by handling, mixing of ingredients and exposing the raw materials and finished products to the atmospheric microorganisms. Therefore, high rate of poultry disease and death occur as a result of consumption of contaminated feeds (Nieminen *et al.,* 2012).

The most common vehicles for transmission of food-borne salmonellosis are meat, meat products, eggs, and egg products that are contaminated as a direct result of animal infection or faecal contamination during processing. Prominent microorganisms found in the poultry farms include species of *Escherichia, Enterococcus, Staphylococcus, Proteus, Clostridium, Salmonella, Providencia, Lactobacillus, Aspergillus* and *Candida* have been shown to be of critical importance in tropical countries and elsewhere in the world. Microbial contamination may occur from equipment surfaces, water, and animal microbiota, air and the environment (Madaki *et al.,* 2019).

2.4 Medicinal plants used for the study

2.4.1.1 Jatropha curcas

Jatropha curcas Linn. is fast becoming a very useful economic resource in phytomedicine development and development of new lead compounds. The plant belongs to the family *Euphorbiaceae,* and is closely related to other important cultivated plants like rubber and castor plants. It originated from South America and Africa but has spread to other parts of the world (Saetae & Suntornsuk, 2010; Shamweel & Dar, 2011). The plant has been demonstrated to be useful in the treatment of infectious and non-infectious ailments such as gonorrhoea, dropsy, gout, paralysis, scabies, eczema, dermatitis and rheumatoid

arthritis (Mkoma & Mabiki, 2012). Many parts of this plant such as leaves, stem bark and latex have been reported to exhibit antibacterial activity. Over 35,000 species of the plant exist with various phytochemicals in them being used for medicinal purposes around the world. *Jatropha curcas* has a long history of use in Africa (Ajayi and Awala, 2016). In Nigeria specifically, the leaf and stem decoction is used singly or in combination with other recipes for the treatment of skin diseases, and stomach disorders (Daniyan *et al.,* 2011).

Jatropha curcas is a multipurpose perennial shrub/small tree of 3-6 m height. It may be evergreen or deciduous, depending on climate. It has a short tap root, robust laterals and many fine tertiary roots (Agamuthu *et al.,* 2010). The stem is woody, erect, cylindrical, solid and branched. Branches are stout, green and semi woody. Leaves are palmate and have 5-7 shallow lobes and are arranged in alternate with spiral phyllotaxis. Length and widths of leaves varies from 16-21 and 14-18 cm and are cauline and ramel, ex-stipulate, petiolate. Petioles are 12-19 cm long. Venation is multicostate, reticulate and divergent type (Brittaine & Lutaladio, 2010). *Jatropha curcas* is monoecious and the terminal inflorescences contain unisexual greenish yellow 17-105 male and 2-19 female flowers in loose panicle of cymes. The ratio of male to female flowers ranges from 13:1 to 29:1. The inflorescence is composed by a main florescence and a distinct coflorescence. There are nodes on the upper pedicels of male (staminate) flowers and no node on the upper pedicels of female (pistillate) flowers. The flowers are tiny (about 7 mm), unisexual, regular, petals are oblong and light green in colour and sepals are quinquepartite. Androecium is absent in female flower, present in male flower with ten stamens. Gynoecium is absent in male flowers but present in female flowers and is tricarpellary, syncarpous with trilocular and superior ovary. Flowers are pollinated by moths and bees. Fruits- trilocullar, ellipsoidal, sudrupaceous. The exocarp remains fleshy until the seeds become mature, finally

separating into three cocci. The fruit is 2.5-3.5 cm long to 2-2.5 cm wide. Seeds are black, oblong, 2.5-3 cm long and 1 cm thick, more or less spherical or ellipsoidal. Seed weight (10 seed) ranges from 53-77g which contains 13.06-42.41% oil content (Pandey *et al.,* 2012).

2.4.1.1 Ethnobotany and economic botany

Jatropha curcas is emerging as an interesting multipurpose species within academic, civil society and policy makers. The seed oil can be easily converted into liquid biofuel which meets the American and European standards (Agamuthu *et al.,* 2007). Besides biofuels and healthcare management, *J. curcas* is also useful to control soil erosion and improved water infiltration, to reclaim wasteland, phytoremediation of various contaminated soils, livestock barrier and land demarcation or live fence around agricultural fields, fuel wood and support for vanilla, green manure, soil carbon sequestration and sustainable environmental development. Other economic products obtained from processing various parts of *J. curcas* are glycerol, soap, cosmetics, varnish, dye, molluscicide, pesticide, fertilizer, silver nano-particles (Brittaine and Lutaladio, 2010).

2.4.1.2 Benefits of Jatropha curcas

The seeds of *J. curcas* or the expressed oil have been used medicinally as a purgative and as a remedy against syphilis. The viscid sap (latex) is employed for cleaning teeth, to cure sores on the tongues of babies and for toothache (Warra, 2012). The leaves of *Jatropha curcas* are utilised extensively in West Africa ethnomedical practice in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Warra, 2012). The sap and crushed leaves have also shown anti-parasitic activity. The water extract of the branches also strongly inhibited the HIV induced cytopathic effects with low cytotoxicity (Tiwari *et al.,* 2007). The

roots of *Jatropha curcas* are used in decoction as a mouthwash for bleeding gums, toothache, eczema, ringworm, scabies and to cure dysentery and venereal diseases like gonorrhoea. It is also reported that the root methanol extract of *Jatropha curcas* exhibited anti-diarrhoeal activity in mice through inhibition of prostalglandin biosynthesis and reduction of osmotic pressure (Daniyan *et al.,* 2011).

2.4.1.4 Antimicrobial activities of Jatropha curcas

Medicinal plants like *J. curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. Acetone, chloroform, ethanol and methanol extracts of *J. curcas* root bark has been reported to inhibit the growth of both Gram-positive (*Staphylococcus aureus*) and Gram negative bacteria like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium (Sundari *et al.,* 2011). Various plant parts of *J. curcas* have antifungal activity against *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Bacillus subtillis*, *Phymatotrichopsis omnivore, Candida albicans* amongst other, which are responsible for many diseases in human being and plants (Hu *et al.,* 2011). Latex of *J. curcas* possesses inhibitory property against *Water melon mosaic virus* (Agamuthu *et al.,* 2010).

Root bark extract of *J. curcas* were capable of scavenging hydroxyl in a concentration dependent manner and have a stronger hydroxyl ions scavenging activity of compared with ascorbic acid (Sundari *et al.,* 2011). The petroleum ether and methanol extracts of *J. curcas* roots shows anti-diarrhoeal activity in different species of albino mice (Agamuthu *et al.,* 2010). Pregnancy terminating effect of methanol, petroleum ether and dichloromethane extracts of *J. curcas* fruits in rats have been reported by Tewari and Akhilesh (2015).

Sachdeva *et al.* (2011) had scientifically evaluated wound healing potential of white soft paraffin base ointment containing 5 and 10% (w/w) extract of stem bark of *J. curcas* using

incision and excision wound model in albino rats. Various plant parts of *J. curcas* have been reported to possess insecticidal and larvicidal and anthelmintic activity. Insect pests of stored grains (*Sitophilus zeamais* and *Rhyzorpertha dominica*) are susceptible to seeds and pericarps of *J. curcas* (Silva *et al.,* 2012).

Ethanol extract of leaves of *J. curcas* may be as useful for developing a safe and ecofriendly therapeutic agent to combat the problems of tick *Rhipicephalus* (*Boophilus*) *annulatus* and tick-borne diseases (Sanis *et al.,* 2012). *Jatropha curcas* is a potential source of herbal mosquito control agent (Cantrell *et al.,* 2011). Aqueous extract of leaves of *J. curcas* possesses anthelmintic activity against *Pheritima poshtuma.* The latex of *J. curcas* also showed antibacterial activity against *Staphylococcus aureus*(Ahirrao *et al.,* 2011).

2.4.1.4 Scientific classification

Taxonomic group	Plant
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Subclass	Rosanae
Order	Malpighiales
Family	Euphorbiaceae
Genus	*Jatropha*
Species	*curcas*
Binomial name	*Jatropha curcas*

Source: Govaerts, 2015

2.4.2 *Azadirachta indica*

Azadirachta indica is a tree within the *Meliaceae* family. Neem is also known as ‘arista’ in Sanskrit-A word that suggests ‘ultimate, entire and imperishable’, is also a common medicinal plant in Nigeria popularly known as ‘Dogonyaro’. *Azadirachta indica* (Neem) is a tree in the mahogany family, native to India, Burma, Bangladesh, Sri Lanka, Malaysia

and Pakistan, growing in tropical and semi-tropical regions. It blossoms in spring with the small white flowers (Saba *et al.,* 2011).

2.4.2.1 Description

Neem tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth and requires little water and plenty of sunlight. *Azadirachta indica* is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species (El-Mahmood *et al.,* 2010). The tree grows naturally in areas where the rainfall is in the range of 450 to 1200 mm. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm. Neem grows on altitudes up to 1500 m. It can grow well in wide temperature range of 0°C to 49°C (Uwimbabazi *et al.,* 2015).

2.4.2.2 Antimicrobial activities

Fruit, seeds, oil, leaves, roots, bark and just about each part of the tree is bitter and contain compounds with verified antiviral, anti-inflammatory, anti-ulcer and antifungal, antiplasmiodal, antiseptic, antipyretic and anti-diabetic potentials (Dash, 2017).

Decoction and oil extracted from Neem leaf is reported to have excellent antiseptic and broad spectrum antibacterial activity against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and Streptomycin resistant strains (Gajendrasinh *et al.,* 2012).

However, the potential side effects associated with neem leaf before starting any type of treatment involving the herb. The appropriate dose of neem depends on several factors such as the user's age, health, and several other conditions (Dash, 2017).

The biological activity of Neem has been reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. Neem oil, the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident (Shravan *et al.,* 2011).

Neem and its leaves are used for the treatment of various diseases including eczema, ringworm, acne, anti-inflammatory activities, anti-hyperglycemic and also treat chronic wounds, diabetic foot and gangrene. It also removes toxins from the body; neutralize the free radicals present in body and used as blood purifier. Recently it is reported as anticancer and used for hepatorenal protective activity and hypolipidemic effects (Saba *et al.,* 2011). Neem leaves have also been demonstrated to have vast properties such as immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, anti-tumour, pesticidal, anti-helmintic, sedative, contraceptive antioxidant, antimutagenic and anticarcinogenic which suggest why the plant is very popular in the world (Hoque *et al.,* 2017).

Bark, leaf, root, flower and fruit together cure biliary afflictions, itching, skin ulcers, burning sensations and phthisis. Neem can be used as a vaginal suppository to avoid and control the spread of sexually transmitted infections. Neem is anti-fungal and antibacterial in nature hence the leaf extracts can be used for treating skin infections such as; psoriasis, eczema, scabies and acne. Neem oil can be used as nasal drops for treating sinusitis. Neem can be used to treat food poisoning associated with *Staphylococcus and Salmonella* bacteria. El-Mahmood *et al.* (2010) observed the antibacterial effects of crude extract of neem seed against pathogens involved in eyes and ear infections.

2.4.2.3 Pharmacological activities

Several pharmacological activities and medicinal applications of various parts of Neem are well known. The Chemical components incorporate many biologically energetic compounds that can be extracted from neem like alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones (Girish and Shankara, 2008).

Neem possesses many medically beneficial bioactive phytochemicals such as hydrocarbon, terpenoids, phenolic, alkaloids, and their derivatives. Some of these phytochemicals exhibits acaricidal, antibacterial, gastroprotective, immu-nostimulant and insecticidal properties. The occurrence and the concentration of secondary metabolites are restricted to the plant taxonomy. Basically, these metabolites are not related to primary metabolism, but exhibit several biological activities or defense. Certain secondary metabolites are classified to be volatile compounds (Akin-Osanaiye *et al.,* 2013).

The Chemical constituents of Neem contain many bioactive compounds including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, Azadirachtin, which is a mixture of some isomeric compounds is used to clean wounds, swellings and erases skin problems.

2.4.2.4 Benefits of Azadirachta indica (Neem)

The presence of edunin and nimbidol compounds makes the neem leaves very destructive to fungi. Neem trees can be used for erosion control and deforestation. Neem helps in the detoxification process of the body, which helps to maintain healthy respiratory, digestive and circulatory systems. Neem extracts can be used in making soaps and oils, which when used can help to maintain healthy and glowing skin (Mamman *et al.,* 2013).

Neem helps with swollen and puffy eyes due to its anti-inflammatory properties. Neem oil is antiseptic and antibacterial in nature, hence, it is very useful for treating cavities, dandruff, halitosis, gum disease and controlling hair lice. Individuals suffering from athlete' foot can dip their feet in warm water containing neem oil. Individuals suffering from itchy and dry scalp can wash their hair with boiled neem leaves water but the water has to cool down before being used (Akin-Osanaiye *et al.,* 2013). Neem can act as a blood cleanser thus stimulates antibody protection to boost the immune system. Due to the antibacterial properties of neem, the leaves extract is trusted and recommended constituents of oral hygiene products, mouthwash and toothpaste. Neem water when taken orally is capable of reducing the insulin level of diabetic patients (Mamman *et al.,* 2013). Individuals suffering from hemorrhoids can apply few drops of neem oil on the affected part. Neem tea helps in alleviating and preventing fatigue, aches, sore throat, pains, cough, colds and fever (Akin-Osanaiye *et al.,* 2013).

2.4.2.5 Scientific classification

Taxonomy	Plant
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*indica*
Binomial name	*Azadirachta indica*

Source: Singh *et al.,* (1980)

2.4.3 *Allium sativum* (Garlic)

Garlic (*Allium sativum*) is a bulbous perennial plant with a powerful onion such as aroma and pungent taste that has been used as a flavoring agent, condiment, and for medicinal purposes for over 5,000 years. Garlic benefits have been greatly recognized for many years by numerous health authorities and enthusiasts for its effects on promoting better health. Garlic is also known as Rocambole, ajo, Allium, stinking rose, rustic treacle, nectar of the gods, camphor of the poor, poor man's treacle, and clove garlic (Adaki *et al.,* 2014).

Garlic is native to Central Asia and northeastern Iran, and has long been a common seasoning worldwide, with a history of several thousand years of human consumption and use. It was known to ancient Egyptians, and has been used both as a food flavoring and as a traditional medicine. In Ancient Rome, it was "much used for food among the poor". China produces some 80% of the world's supply of garlic (Meredith and Drucker, 2014).

The recorded use of garlic in the treatment of tumors dates all the way back to 1,550 BC when Egyptians realized the benefits of garlic as a remedy for a variety of diseases, and administered it orally and topically; the modern era, however, begins in the 1950s when demonstrated *in vitro* and *in vivo* that thiosulfinate extracts of garlic inhibited the growth of malignant cells and prevented growth of sarcoma 180 ascites tumor (Adaki *et al.,* 2014).

The use of garlic for medicinal purposes dates to antiquity. The Bible mentions garlic with regard to the Jew's flight from Egypt. Garlic bulbs were found in tombs of the pharaohs, in Crete, and in ancient cultures throughout the world. Indeed, Hippocrates considered garlic to be a vital part of therapeutic armamentarium. The word “garlic” was derived from Old English, garlēac, meaning gar (spear) and leek, as a 'spear-shaped leek' (Block, 2010). *Allium sativum* is a bulbous plant, growing up to 1 metre (3.3 ft) in height. If garlic is planted at the proper time and depth, it can be grown as far north as Alaska. It produces

hermaphrodite flowers and is pollinated by bees, butterflies, moths, and other insects (Rader and McGuinness, 2019).

2.4.3.1 Origin and major types

Allium sativum grows in the wild in areas where it has become naturalized. The "wild garlic", "crow garlic", and "field garlic " of Britain are members of the species *Allium ursinum*, *Allium vineale*, and *Allium oleraceum* respectively (Meredith and Drucker, 2014). In North America, *Allium vineale* (known as "wild garlic" or "crow garlic") and *Allium canadense*, known as "meadow garlic" or "wild garlic" and "wild onion", are common weeds in fields. So-called elephant garlic is actually a wild leek (*Allium ampeloprasum*), and not a true garlic. Single clove garlic (also called pearl or solo garlic) originated in the Yunnan province of China (Meredith and Drucker, 2014).

There are different varieties or subspecies of garlic, most notably hardneck garlic and softneck garlic. The latitude where the garlic is grown affects the choice of type, as garlic can be day-length sensitive. Hardneck garlic is generally grown in cooler climates and produces relatively large cloves, whereas softneck garlic is generally grown closer to the equator and produces small, tightly-packed cloves (Meredith and Drucker, 2014).

2.4.3.2 Cultivation

Garlic is easy to grow and can be grown year- round in mild climates. Sexual propagation of garlic is possible, nearly all of the garlic in cultivation is propagated asexually, by planting individual cloves in the ground. In colder climates, cloves are planted in the autumn, about six weeks before the soil freezes, and harvested in late spring or early summer. The cloves must be planted deep enough to prevent freeze/thaw, which causes mold or white rot (Block, 2010). Garlic plants prefer to grow in a soil with a high organic

material content, but are capable of growing in a wide range of soil conditions and pH levels (Rader and McGuinness, 2019).

2.4.3.3 Contents of garlic

Fresh garlic contains various organosulfur compounds, trace elements and compounds of phenolic and steroidal origin, along with carbohydrates, proteins and fiber. Based on the solubility the contents are divided into two groups. One group is the lipid-soluble allyl sulfur compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS), and the other one is the water-soluble compounds g-glutamyl S-allylcysteine (SAC) group such as SAC and S- allylmercaptocysteine (SAMC) (Adaki *et al.,* 2014).

Diallylsulfide, a powerful garlic component, has been reported to inhibit oxidative stress caused by testosterone and to accelerate testosterone metabolism. It has been postulated that in the early stages of prostate cancer, when sensitivity to testosterone is retained, the predominant effect of *Allium* derivatives is to stimulate testosterone degradation and in the later stages, to interfere with signal transduction. Furthermore, garlic is a seleniferous plant, accumulating selenium from the soil against a concentration gradient. Selenium has many anticancer actions, particularly in control of genes involved in carcinogenesis. In addition to inhibiting primary cancer, Allium derivatives from garlic may further inhibit metastatic processes. In an androgenin-dependent prostate cancer mouse model, the water-soluble Allium derivative, S- allyllmercaptocysteine, inhibited metastases to the lung and adrenal gland by 90% (Rader and McGuinness, 2019).

2.4.3.4 Beneficial effects

Garlic possesses a variety of beneficial pharmacological properties affecting most notably the cardiovascular system (lipid management, decreased blood pressure, platelet inhibition, and decreased fibrinolytic activity), and the immune system as an antineoplastic and

immunostimulant agent and it is also a potent antioxidant. Garlic was shown to be used more than twice as much as any other natural supplement. Garlic is promoted to lower cholesterol and blood pressure, delay the progression of atherosclerosis, prevent heart disease, improve circulation, prevent cancer, and is used topically for tinea infections (Adaki *et al.,* 2014).

Animal studies have shown that garlic can unclog arteries by reducing the size of existing plaque deposits. Garlic also helps prevent breast, colon, and esophageal cancer. Garlic supplements may also be beneficial to patients undergoing chemotherapy, especially those being administered with doxorubicin, by preventing the depletion of glutathione and the increased free radical activity that is associated chemotherapy, which can damage heart, liver, and other vital organs and tissues. Garlic possesses antibiotic, antiviral, and antifungal, antihistamine, anticoagulant, expectorant, antibacterial, anti-parasitic, diaphoretic, diuretic, expectorant, stimulant, and antispasmodic properties (Rader & McGuinness, 2019).

2.4.3.5 Scientific classification

Taxonomy	Plant
Kingdom	Plantae
Division	Magnoliophya
Class	Liliopsida
Order	Liliales
Family	Liliaceae
Subfamily	Liliideae
Genus	Allium
Species	sativum
Binomial name	*Allium sativum*

Source: Kewscience, (2017)

2.4.3.7 Phytoconstituents of allium sativum

Flavones, flavonoids and flavonols are chemical compounds active against microorganisms. Flavones are phenolic structures containing one carbonyl group, they have been found in-vitro to be effective antimicrobial substances against a wide array of microorganisms. More lipophilic flavonoids may also disrupt microbial membranes (Rader & McGuinness, 2019).

Garlic is characterized by a high content of organosulfur. In the bulb, the sulfur is primarily g- glutamyl peptides and allylcysteine sulfoxides. When the bulb is cut, chopped or squeezed, alliin, the main allylcysteine sulfoxide is metabolized to allicin through the action of alliinase. Allicin is a self-reactive constituent and it is converted readily to more stable compounds such as polysulfides (Adaki *et al.,* 2014).

2.4.4 *Moringa oleifera* Lam

2.4.4.1 Geographical information

Moringa oleifera L. (*Moringa*), belongs to family *Moringa*ceae, grows in tropical and subtropical environments. This is one of the best known, widely distributed and grown species of a monogeneric family *Moringa*ceae (Moyo *et al.,* 2012). *Moringa oleifera* is commonly termed the "drumstick tree". Other common names include horseradish tree, ben oil tree, or benzoil tree. The plant is referred to as drumstick tree or the horse radish tree. It is native to the western and sub-Himalayan parts of Northwest India, Pakistan and Afghanistan. The species is now widely cultivated across some African countries, South America and South-east Asia (Hanafy, 2017). This tree also needs a yearly rainfall of between 250 mm and 3000 mm and can survive in temperatures of 25˚C to 40˚C, which makes it suitable for tropical climates. *Moringa* tree grows mainly in semi-arid tropical and subtropical areas. More generally, *Moringa* grows in the wild or is cultivated in Central America and Caribbean, northern countries of South America, Africa, Southeast

Asia and various countries of Oceania. Among the twelve species in the genus *Moringa*, the most commonly cultivated and widespread is *Moringa oleifera*. It is a drought tolerant plant that thrives best under the tropical climate and tolerates different soil types (Hanafy, 2017).

2.4.4.2 Description of Moringa oleifera

Moringa oleifera is a fast-growing, deciduous tree. Its maximum height is 10–12m, while its trunk can reach a diameter of 45 cm. The flowers are approximately 1.0–1.5 cm long and 2.0 cm wide, flowering starts within the first six months after planting (Idris *et al.,* 2016). The fruit is a droopy, three-sided brown capsule, 20–45 cm in size and contains dark brown, spherical seeds of about 1 cm diameter. The seeds have three thin, whitish wings, which are responsible for the smooth distribution of the seed by water and wind (Hanafy, 2017).

2.4.4.3 Nutritional composition of the Moringa oleifera

All parts of *Moringa oleifera* are consumed by humans in different ways. *Moringa* leaves are a source of highly digestible nutrients and can be eaten fresh, cooked or stored as dried powder and have been advocated as suitable for nutritional and therapeutic use in many developing regions of the world (Desoky *et al.,* 2017). *Moringa* aids in improving nutrition and support immune functions of poultry and animal. Seeds are eaten green or dry (Rady *et al.,* 2015). *Moringa* seeds contain a high percentage of sweet oil (30–40% of the seed weight) and contain around 76% polyunsaturated fatty acids which can control cholesterol. The leaves and seeds of *Moringa oleifera* are a source of protein, iron, calcium, ascorbic acid vitamin A and antioxidant compounds such as carotenoids, flavonoids, vitamin E and phenolics (Afzal *et al.,* 2012). The presence of vitamins and minerals benefit in improving the immune system and cure a myriad of diseases (Basra *et al.,* 2011).

Moringa oleifera leaves can be used as a feed supplement, to improve feed efficiency and livestock performance, or as a replacement for conventional crops to obtain more economically sustainable, environmentally friendly and safer production. Better feed digestibility occurs in animals fed *Moringa* leaves, probably due to their nutritional profile especially the neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), gross energy (GE), ether extract (EE) and amino acids (Asaolou *et al.,* 2012). Some parts of the *Moringa* tree contain toxins and other anti-nutritional factors, which limit their utility as a source of food for humans or animals.

2.4.4.4 Antimicrobial activities

Microbial diseases are widespread and there is a need to use antimicrobial agents. It has been shown that *Moringa* is an effective antimicrobial agent. The antibacterial effects of the seeds were determined by screening for the antibacterial compounds moringine, benzyl isothiocyanate and pterygospermin. *Moringa* seed aqueous extracts are reported to have antimicrobial properties that can lead to the inhibition of bacterial growth, thereby averting waterborne diseases. These properties of *M. oleifera* seeds not only have a broad application in preventing diseases but can also potentially enhance the quality of life in rural communities (Mohamed *et al.,* 2018).

Hussain *et al.* (2014) conducted an *in vitro* study to test the effect of two different concentrations of *Moringa oleifera* leaf and seed aqueous extracts in inhibiting the mycelial growth of two soil-borne pathogens: *Fusarium solani* and *Rhizoctonia solani*. Results revealed that both *Moringa* aqueous extracts showed 50% growth inhibition of *F. solani* at the 30% concentration level. The maximum inhibition percentages recorded against *R. solani* were 45% and 50% using *Moringa* seed aqueous extract at 25% and 30% concentrations, respectively. They concluded that *Moringa* seed and leaf aqueous extracts

contain antifungal properties, which resulted in effective growth inhibition of *F. solani* and *R. solani*. *Moringa* aqueous extract concentration influenced the antifungal activity the higher concentration levels displayed an increase in antifungal efficacy. *Moringa* seed aqueous extract reduced the number of fecal coliforms, *Staphylococcus aureus*, in water from rivers and wells.

Antibiotic and antifungal effects against *Fusarium solani*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* result from pterygospermin, which is present in *Moringa* seeds. Many in-vitro studies have highlighted *Moringa*' ability to inhibit food disease pathogens, such as *Salmonella* Typhi, *Salmonella* ParaTyphi, *Escherichia coli*, *Shigella dysenteriae*, *Citrobacter* spp. and *Pseudomonas aeruginosa* (Abalaka *et al.,* 2012).

2.4.4.5 Phytochemicals of the Moringa oleifera

A phytochemical analysis of *Moringa* leaves and seed solvent aqueous extracts found flavonoids, alkaloids, tannins, glycosides, terpenoids and phenolic compounds, etc. *Moringa oleifera* leaves contain some fatty acids, crystalline alkaloids, proteins, niazirin and glycosides, which are thought to be responsible for their antimicrobial activities (Hussain *et al.,* 2014). Phytochemical studies of *Moringa* leaves have indicted unique compounds, including rhamnose (i.e., simple sugar), isothiocyanate and glucosinolates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects (Osman & Abohassan, 2015).

Al-Asmari *et al.* (2015) reported that such ethanol extracts had cancer preventative effects, when they assayed the activity against human promyelocytic leukemia cells (HL-60). *Moringa oleifera* is the best source of a wide spectrum of dietary antioxidants, including flavonoids such as kaempferol and quercetin (Bashir *et al.,* 2016).

Pakade *et al.* (2013) also stated that *Moringa* exceeds some vegetables in their strength as an antioxidant, because its content of total phenolics was almost twice that of the vegetables (broccoli, spinach, peas and cauliflower) and total flavonoids were three times that of the same vegetables. Also, the reducing power of *Moringa* was higher and free radicals remaining were lower compared with these vegetables (Muhammed *et al.,* 2013).

Moringa leaves also contain other important flavonoids such as kaempferol and quercetin, which exhibit higher antioxidant activity than ascorbic acid, they also contain fairly high amounts of ascorbic acid (Yameogo *et al.,* 2011). These antioxidants provide protection to animals against degenerative diseases and infections, which might be associated with the direct trapping of free radicals to avoid DNA damage from excessive oxidation. *Moringa* seeds contain ferulic acid, gallic acid, epicatechin, catechin, vanillin, protocatechuic acid, caffeic acid, cinnamic acid, phytosterol, quercetin, chlorogenic acid and quercetin rhamnoglucoside. The immature pods (fruits) and flowers of *Moringa oleifera* have been characterized by the content of carotenoids (Al-Asmari *et al.,* 2015). Additionally, the presence of flavanoids give the anti-inflammatory, antioxidant and antidiabetic properties and as anti-proliferative and anticancer agent (Gopalakrishnan *et al.,* 2016).

The phytochemical compounds of *Moringa* possess various biological actions, including antidiabetic, hypocholesterolemic, and hypertensive agent and regulate thyroid hormone, central nervous system, digestive system, as well as nutrition and metabolism (Rady *et al.,* 2013). The bark of the tree contains alkaloids, tannins, saponins and some inhibitors. There are two types of alkaloids in *Moringa* root bark, i.e., moringinine and moringine (Asaolou *et al.,* 2012). The bark of *Moringa* tree has toxicity profiles due to its content of two alkaloids and the toxic hypotensive moringinine. The bark of the tree may cause violent uterine contractions that can be fatal. Additionally, changes in clotting factor, changes in serum composition (e.g., total protein, bilirubin, cholesterol), along with

enzyme inhibition, induction, or change in blood or tissue levels of other transferases have been noted after the mice treatment by root bark extract (Rady *et al.,* 2015). *Moringa* is rich in phytochemical compounds that confers on the plant significant medicinal properties that could be valuable for treating certain ailments (Shahzad *et al.,* 2013).

2.4.4.6 Uses of Moringa oleifera

Moringa is a multipurpose tree and, recently, a lot of research has gone into its medicinal use against human pathogens. There is no doubt that crops in third world countries, particularly African countries, are victims to fungal toxins (known as mycotoxins), heavy metals and chemicals from the indiscriminate use of pesticides, which present a threat to ecosystems and future generations (Shahzad *et al.,* 2013). These toxins not only lead to imbalances in ecosystems but also interfere with food chains, resulting in environmental abnormalities. It is, therefore, important to find better alternatives for botanical fungicides, which have minimal environmental impact and no danger to human consumption compared with synthetic pesticides (Zorriehzahra & Adel, 2016). Intensive and continued fungicide usage is associated with development of resistance by fungi to systemic fungicides and the specificity of fungicide formulations, which affect only one pathway in the biosynthesis of fungal pathogens, processes that reduce the efficacy of fungicides. Hence, use of biological agents, such as *Moringa*, in the control of soil-borne fungal pathogens notorious for causing root-rot diseases, might prove to be more effective than fungicides (Amirigbal *et al.,* 2014). Not only are these bio-agents environmentally friendly compared to chemical methods, but they have also been shown (in several in-vitro studies) to effectively inhibit pathogen growth.

Saavedra and Van der Maden, (2015) reported that plants sprayed with *Moringa* leaf ethanol extract were firmer and more resistant to disease and pests. Moreover, *Moringa*

has the potential to be used as a biopesticide. Incorporating leaves into the soil can prevent seedling damping off. Consequently, foliar application of *Moringa* aqueous leaf extract can be regarded as a cheap biostimulant a source of plant growth hormones and minerals for improving the yield and quality of plant crops, especially given the trend toward organic farming (Zorriehzahra and Adel, 2016). Interestingly, in a study of the mycelial growth inhibition of *Aspergillus flavus* isolated from stored maize grains, *M. oleifera* seed powder was the most favorable treatment, compared with Fernazzan D (a chemical fungicidal material) (Saavedra and Van der Maden, 2015).

Moringa is resistant to diseases and pests itself because of its relatively fast vegetative growth, which allows it to regenerate quickly after any disturbance from the most common pests and diseases, including grasshoppers, crickets, caterpillars, termites and fungal disease (Saavedra and van der Maden, 2015). The synergistic effects of *Trichoderma* and *Moringa* can protect plant growth against pathogen infection. Farmers could use this combination to decrease the yield losses caused by disease pathogens and therefore increase their income.

Moringa has been used to control the *Rhizopus* pathogen, which is a major causal organism in food spoilage and losses. Leaves of *M. oleifera* are used as food or animal feed during the dry season or periods of drought, almost all parts of *Moringa oleifera* are used as food (Mohamed *et al.,* 2018).

Anaemic patients are treated with its leaves to increase their iron levels and its roots and bark are used in the treatment of cardiac issues. The efficiency of animal feed concentrates can be improved by supplementation with *M. oleifera* leaves. Despite the great advantages of *Moringa oleifera* when used in animals or poultry, excessive use in large quantities lead

to negative results because it contains some anti-nutritional properties that show their effectiveness with the addition of a larger quantity of the animal (Moyo *et al.,* 2012).

Moringa is one of the most important natural substances that can be used in the purification of drinking water at low cost and low risk to human health and the environment. Dried, ground *Moringa* seeds coagulate debris in water due to their active soluble protein component, which is a natural cationic polyelectrolyte. Initial water hardness of 80.3 g L^{-1} $CaCO_3$ was found to have decreased between 50% and 70% after coagulation and softening with *M. oleifera* (Mohamed *et al.,* 2018).

Moringa seed powder had bacterial removal efficiency of up to 99.5%. In addition, *Moringa* seed powder can reduce heavy metals such as manganese, iron, copper, chromium and zinc in water, for the best results using *Moringa oleifera* seed powder or extract for water treatment, pH, contact time and temperature should be controlled and monitored (Rehman *et al.,* 2014). The leaves can be used in many ways, perhaps most commonly added to clear broth-based soups, such as the Filipino dishes tinola and utan. For long-term use and storage, *Moringa* leaves may be dried and powdered to preserve their nutrients (Muhammed *et al.,* 2013). The powder is commonly added to soups, sauces and smoothies. Owing to its high nutritional density, *Moringa* leaf powder is valued as a dietary supplement and may be used to enrich food products ranging from dairy, such as yogurt and cheese, to baked goods, such as bread and pastries, with acceptable sensory evaluation (Jaafaru *et al.,* 2018).

In Nigeria, the seeds are prized for their bitter flavor; they are commonly added to sauces or eaten as a fried snack. The edible seed oil may be used in condiments or dressings. Ground, debittered *Moringa* seed is suitable as a fortification ingredient to increase the protein, iron and calcium content of wheat flours (Yasmeen *et al.,* 2012). Overall, *Moringa* is an underused plant that could bring a multifaceted approach to addressing

water and nutrition issues in agriculture, particularly in rural sub-Saharan African communities.

2.4.4.7 Scientific classification

Taxonomy	Animal
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae
Genus	*Moringa*
Species	*oleifera*
Binomial name	*Moringa oleifera* Lam

Source: Olson, (2010)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and identification of plant materials

Leaves of *Jatropha curcas* (physic nut), *Azadirachta indica* (Neem), *Moringa oleifera* (moringa) and *Allium sativum* (Garlic bulbs) were collected from Bosso, Bosso Local

Government Area of Niger State. The plants were identified and authenticated at NIPRID, Idu, Abuja. Voucher specimen numbers were assigned: *Jatropha curcas* NIPRD/H/5485, *Azadirachta indica* NIPRD/H/6879, *Moringa oleifera* NIPRD/H/7081, *Allium sativum* NIPRD/H/8313. Samples were deposited in the herbarium department of NIPRD for future references.

3.2 Preparation and extraction of plant materials

3.2.1 Preparation of plant materials

The leaves were washed, air-dried at 25° C, and pounded using a mortar and pestle. The pulverized plant materials were blended to obtain a fine powdered-like texture in order to increase the surface area of the leaves and enhance the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active constituents.

3.2.2 Extraction of plant materials

Pulverized 65 g of *Jatropha curcas* leaves was soaked in 250 mL of methanol, *n*-Hexane, ethyl acetate respectively, 65 g of *Moringa oleifera* leaves was soaked in 250 mL of methanol, *n*-Hexane, ethyl acetate respectively for 72 h, the setup was stirred every 24 h for three days.

Eighty grams (80 g) of *Azadirachta indica* leaves was soaked in 300 mL of distilled water and 80 g of fresh garlic bulb in 300mL of distilled water respectively for 72 h, the setup was stirred every 24 h for three days.

After 72 h, the plant materials were sieved and concentrated by evaporation to dryness at 60°C in a water bath to obtain dry crude extract, the dried crude extracts were stored for subsequent experiments.

The percentage of extract yield was calculated using Equation (3.1) below.

$$\text{Extract yield (wt \%)} = \frac{\text{M E}}{\text{M P P}} \times 100 \quad (3.1)$$

Where M E: mass of extract obtained

M P P: mass of pulverized plant material

3.2.2 Preparation of plant extracts concentrations

Each concentration was dissolved in 10 % dimethylsulphoxide (DMSO). Concentrations of 250 mg/mL, 300 mg/mL, 350 mg/mL and 400 mg/mL were used as the working concentrations for all the plant extracts obtained. The extracts were reconstituted using the formula below:

$$\text{Final Concentration (mg/mL)} = \frac{\text{W E}}{\text{V S}} \times 100 \quad (3.2)$$

Where W E: weight of extracts

V S: volume of solvent (DMSO)

3.3 Media and broth used

Nutrient agar, Nutrient broth, *Shigella-Salmonella* agar, Potato dextrose agar, Potato dextrose broth (PDB) were prepared according to manufacturer's instruction.

3.4 Sub-culturing and identification of bacterial isolates

3.4.1 Sub-culturing of bacterial isolates

Approximately 10 g of chicken droppings was enriched overnight in selenite F broth. Selenite enriched samples were sub cultured using the *Salmonella-Shigella* agar. About 6.3 g of the agar was dissolved in 100 mL of distilled water and heated briefly using a hot plate and allowed to cool. The cooled agar was poured into Petri dishes and allowed to gel. A loopful from the enriched samples was streaked on the solidified agar and incubated for 24 h at 37 °C.

3.4.2 Identification of the bacterial isolates

The bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Ajayi & Awala, (2016). The isolates were subjected to relevant biochemical tests.

3.4.2.1 Biochemical test for bacterial isolates

The bacteria isolates were identified based on the following conventional biochemical tests such as: catalase, oxidase, oxidative fermentation, carbohydrate fermentation, indole test, methylred test, starch hydrolysis, urease as shown in appendix II (Cheesebrough, 2010).

3.5 Sub-culturing and identification of fungal isolates

3.5.1 Sub-culturing of fungi isolates

Approximately 10 g of chicken droppings were enriched overnight in buffered peptone broth. A loopful from the enriched samples was streaked on solidified Potato-Dextrose-Agar (PDA) in order to obtain pure colonies and incubated for 72 h at 25 °C. Distinct colonies were sub cultured on Potato-Dextrose-Agar (PDA) and incubated for 72 h at 25 °C and viewed for growth.

3.5.2 Identification of the fungi isolates

The classification was done by microscopic and macroscopic analyses of the colonies, according to criteria from Rebell *et al.* (1979) and Kane *et al.* (1997) as identified via macroscopic and microscopic observations, chlamydospore production on Potato-Dextrose-Agar (PDA) (Domsch *et al.,* 1980).

3.6 Antimicrobial susceptibility testing of isolates

3.6.1 Preparation of turbidity standard for the inoculum

The aim of this preparation was for standardisation of the test organisms. 0.5 McFarland standards was prepared by dissolving 1 % barium chloride (BaCl) in 1 % sulfuric acid (H_2SO_4) in order for barium sulfate precipitate to be obtained. This was used to standardize the population of bacteria in a liquid suspension (Abalaka *et al.,* 2012).

To prepare 10 mL of 0.5 McFarland standard, 9.95 mL of 1 % H_2SO_4 was transferred to a screw capped test tube followed by 0.05 mL of 1 % barium chloride solution and thoroughly mixed for even distribution. This was used to compare the turbidity of a bacterial suspension. The turbidity of the actively growing culture of the bacteria in the broth was adjusted with nutrient broth to obtain turbidity that is visually comparable with 0.5 McFarland standard (Ochei & Kolhatkar, 2000).

3.6.2 Susceptibility testing for bacterial isolates

The Agar well diffusion method described by Pelczar *et al.* (1993) was used to determine the antimicrobial activity of the extracts. This method was as follows: Identified pathogens were inoculated in nutrient broth tubes separately and incubated at 37 °C for 1 h to attain turbidity of the 0.5 McFarland standard. The turbidity of the actively growing culture of the bacteria in the broth was adjusted with nutrient broth to obtain turbidity that is visually comparable with 0.5 McFarland standard (Ochei and Kolhatkar, 2000). The prepared nutrient agar plates were inoculated with 0.1 mL (1.0×10^4CFU/mL) of standardized test bacteria, using sterile cotton swabs. The plates were allowed to dry for 5-10 minutes at room temperature. Agar wells of 5 mm diameter were made on the seeded agar plate using a sterile cork borer and approximately 0.3 mL volume of each plant extracts with concentrations ranging from 250-400 mg/mL were dispensed into the wells. Ciprofloxacin served as positive control while DMSO served as negative control. The plates were allowed to stand for 1 h for the pre-diffusion of the extract to occur. Plates were incubated overnight at 37 °C. The zones of inhibition around the wells was measured to the nearest

whole millimetre using a meter ruler, and taken as an indication of antimicrobial activity. The experiment was carried out in duplicates.

3.6.3 Antimicrobial activity test for fungi

3.6.3.1 Preparation of fungal inoculum

Fungal strains were freshly subcultured on sterile Potato-Dextrose-Agar (PDA) and incubated at 30 °C for 5 days. The resultant cells and spores were washed into sterile normal saline and the turbidity adjusted to a 0.5 McFarland standard equivalent. This results in a 1 × 10 CFU/mL. The suspension was further diluted in a 1: 10 ratios in Potato-Dextrose Broth to give a turbidity of 5 × 10 CFU/mL.

3.6.3.2 Dilution assays

The agar dilution method was performed as described by Souza *et al*. (2002) with slight modification. Two-fold serial dilution of the plant extracts were done in Potato-Dextrose-Agar (PDA) medium, to obtain concentrations ranging from 1.25 to 640 µg/mL. The plant extracts were diluted 1:10 in plates Potato-Dextrose-Agar (PDA). Positive Control test was carried out using 10 µg/mL of clotrimazole while DMSO was used for negative control.

3.7 Determination of minimum inhibitory concentrations

The Minimum Inhibitory Concentration of the extracts was tested against the organisms using two-fold broth macrodilution assay. Serial dilutions generating decreasing concentrations of the plant extracts was prepared in tubes and was expressed in mg/mL. An equal volume of a known bacterial culture concentration comparable to 0.5McFarland standard was added to each of the tubes containing the varying concentrations of each plant extracts and the tubes containing the combined extract. These tubes were incubated at 37 °C for 24 h (Lourens *et al.,* 2004). All test tubes were properly corked and incubated

at 37 ºC for 24 h, the tubes were observed for absence or presence of visible growth. Each test included controls consisting of the medium with the solvent (DMSO) and medium with bacterial suspension as well as sterility control. After incubation, the tubes were examined for changes in turbidity as an indicator of growth. The first tube showing complete inhibition of visual growth of the pathogens was taken to be the MIC of the extract.

A microplate method, as described by Eloff (1998), was used with slight modifications to determine minimal inhibitory concentration (MIC) values of plant extracts. Plant extracts were serially diluted, ranging from 1/2 up to a 1/100 dilution from the crude extract. In each well, 100 µL of each extract dilution was mixed with 100 µL of the fungal spore suspension (2×10^6 spores mL^{-1} in fresh PDB). The microplates were incubated for 2-3 d at 27 °C with daily monitoring. All experiments were done in triplicate. The MIC of the extracts is the lowest concentration of the plant extract that prevent the growth of microorganism is the Minimum Inhibitory Concentration.

3.7.1 Minimum bactericidal concentrations and minimum fungicidal concentrations

Minimum Bactericidal Concentration (MBC) is the lowest concentration of the extract that kills 99.9 % of a given strain of bacteria. 5 mL of each culture broth of the MIC was transferred onto the agar plates that is extract free and was incubated for 24 h at 37 °C. All experiments were conducted in triplicate. If no growth is seen, then the extract was said to be bactericidal at that concentration.

Minimum Fungicidal Concentrations (MFC) was determined using the content from the broth dilution of MIC test. The surface of sterile Sabouraud Dextrose Agar (SDA) medium (20 mL) in petri dishes was uniformly smeared with sterile cotton swabs moistened with

the contents of the test tubes from MIC test. The plates were then incubated at 25 ^{o}C for 48 h. The minimum fungicidal concentration (MFC) was recorded as lowest concentration of the extract that showed no growth on the Sabouraud Dextrose Agar (SDA).

3.8 Phytochemical analysis of plant extracts for active components

The qualitative phytochemical investigation of the extract was carried out for the detection of various active components in the extracts of each plant (*Jatropha curcas* and *Moringa oleifera, Azadirachta indica*, garlic bulb) according to the methods described by Pramila & Prerana (2014) for the detection of active components like alkaloids, tannins, flavonoids, saponins, glycosides, terpenoids, steroids, phenols in each as shown in appendix I.

3.9 Data analysis

Results were expressed as the mean values ± standard deviations (S.D.) by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Duncan's test was performed to test the significance of differences between means obtained among the treatments at the 5 % level of significance using IBM SPSS Statistics (Version 21). Differences was considered significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Total yield and percentage yield of crude extracts of the medicinal plants

The total yield (measured in gram) and the percentage recovery yield (in %) of the methonolic, *n*-Hexane, ethyl acetate and aqueous extract of *Jatropha curcas*, *Moringa oleifera, Azadirachta indica* and *Allium sativum* was shown in Table 4.1. Methanolic extract of *Jatropha curcas* had the highest yield (24.9 g of 65 g) and percentage yield (60%), followed by aqueous extract of *Azadirachta indica* (with 39 g of 80 g and 48.70 % respectively) while the *n*-Hexane extract of *Moringa oleifera* had the least total and percentage yield (with 10.80 g of 65 g and 16.60 % respectively).

Table 4.1 Total and Percentage (%) Yield of Crude Extracts of Various Plants

Plant Material	Weight of Pulverized Samples (g)	Volume of Solvent	Percentage Yield (%)	Total Yield (g)
Methanolic solvent				
Jatropha curcas	65.00	250.00	24.90	16.20

Moringa oleifera	65.00	250.00	60.00	39.00
Azadirachta indica	80.00	NA	NA	NA
Allium sativum	80.00	NA	NA	NA
***n*-Hexane**				
Jatropha curcas	65.00	250.00	11.50	7.50
Moringa oleifera	65.00	250.00	16.60	10.80
Azadirachta indica	80.00	NA	NA	NA
Allium sativum	8000	NA	NA	NA
Ethyl acetate				
Jatropha curcas	65.00	250.00	20.30	13.10
Moringa oleifera	65.00	250.00	27.20	17.70
Azadirachta indica	80.00	NA	NA	NA
Allium sativum	80.00	NA	NA	NA
Aqueous				
Jatropha curcas	65.00	NA	NA	NA
Moringa oleifera	65.00	NA	NA	NA
Azadirachta indica	80.00	250.00	48.70	39.00
Allium sativum	80.00	250.00	39.70	27.80

NA: not applicable

4.1.2 Total yield of fractions of aqueous leaf extract of *azadirachta indica*

Table 4.2 reveals the total yield of the fractions of aqueous leaf extract of *Azadirachta indica*. Ethyl acetate fraction had that the highest yield (6.02 g) followed by aqueous fraction (1.58 g), followed by *n*-Hexane fraction (1.45 g) while methanolic fraction (1.22 g) had the least total yield.

table 4.2: Total yield of fractions of aqueous leaf extract of *azadirachta indica*

Plant materials	*Azadirachta indica*
Weight of crude extract (g)	10.00
n-Hexane fraction (g)	1.45
Ethyl acetate fraction (g)	6.02
methanol fraction (g)	1.22
Aqueous fraction (g)	1.58

4.1.3 Biochemical characteristics and identities of bacterial isolates

The biochemical characteristics and identities of the test isolates are revealed in Table 4.3. The test isolates A and B were positive for citrate utilization test, catalase test, Mannitol fermentation test, and triple sugar iron test (with isolate A negative for H_2S production and isolate B positive for H_2S production) while they were negative for indole test, methyl red, and gelatin hydrolysis test. Isolates A and B were suspected to be *Staphylococcus aureus* and *Salmonella* Typhi.

Table 4.3: Biochemical Characteristics and Identities of Bacterial Isolates

IS	GSR	SH	CA	OX	IN	MR	VP	CI	UR	MO	TSI	GH	LA	H_2S	CO	MA	Suspected Organisms
A	+	Cocci	+	-	-	-	+	+	+	-	+`	-	+	-	+	+	*Staphylococcus aureus*
B	-	Rods	+	+	-	-	-	+	-	+	+	-	-	+	-	+	*Salmonella* Typhi

IS: isolates, SH: shape, GSR: Gram staining reaction, OX: oxidase test, IN: idole test, MR: methyl red test, VP: Voges proskauer test, CI: citrate utilization test, UR: urease test, MO: motility test, TSI: triple sugar iron test, GH: gelatin hydrolysis test, LA: lactose fermentation test, H_2S: hydrogen sulphide production test, CO: coagulase test, MA: mannitol fermentation test, +: positive, -: negative.

4.1.4 Characteristics and identities of fungal isolates

Table 4.4 shows the characteristics and identities of fungal isolates. Two fungal were isolated from samples of poultry droppings. Upon macroscopic observations, isolate A and B had Black fluffy appearance and white, cream to yellow respectively while on microscopic observations, they both had branched septate hyphae. Isolate B produced chlamydiophores on cornmeal agar fortified with Tween 80 polysorbate. Isolate A and B were suspected to be *Aspergillus niger* and *Candida albicans* respectively.

Table 4.4: Characteristics and identities of fungal isolates

Isolates	Appearance	Hyphae Formation	Spore Formation	Suspected Organisms
A	Black,fluffy appearance	Branched septate hyphae	Conidiophore	*Aspergillus niger*
B	White, cream to yellow	Branched septate hyphae	Clamydiophore	*Candida albicans*

4.1.5 Qualitative phytochemical constituents of the crude plant extracts.

The qualitative phytochemical components of the various crude plant extracts are presented in Table 4.5. Anthraquinnones were absent in all the crude extracts while alkaloids and flavanoids were present in all the crude extracts except in aqueous extract of *Allium sativum*. Glycosides, saponins and tannins were also present in the crude extracts except in the methanolic, *n*-Hexane and ethyl acetate extracts of *Moringa oleifera*. Phenols were only present crude extracts of *Jathropha curcas* and aqueous extract of *Azadirachta indica* while reducing sugars were present only in aqueous extracts of *Azadirachta indica* and *Allium sativum*.

Table 4.5 Phytochemical constituents of the various crude plant extracts

Plant samples	Solvents	Phytoconstituents							
		Alkaloids	Glycosides	Phenols	Flavonoids	Saponins	Tannins	Reducing sugars	Anthraquinones
Jatropha curcas	ME	+	+	+	+	+	+	-	-
	n-H	+	+	+	+	+	+	-	-
	EA	+	+	+	+	+	+	-	-
Moringa oleifera	ME	+	-	-	+	-	-	-	-
	n-H	+	-	-	+	-	-	-	-
	EA	+	-	-	+	-	-	-	-
Azadirachta indica	AQ	+	+	+	+	+	+	+	-
Allium sativum	AQ	-	+	-	-	+	+	+	-

AQ: aqueous, n-H: *n*-Hexane, ME: methanol, EA: ethyl acetate, -: absent, +: present

4.1.6 Antibacterial activities of aqueous extracts of *Azadirachta indica* and *Allium sativum* on test isolates

The antibacterial activities of aqueous extracts of *Azadirachta indica* and *Allium sativum* on test isolates were presented in Table 4.6. There was significant difference at P>0.05 in the zones of inhibition produced by the aqueous extracts *Azadirachta indica* and *Allium sativum* against *Staphylococcus aureus* and *Salmonella* Typhi respectively. At concentrations of 250mg/mL, 300mg/mL, 350mg/mL and 400 mg/mL, aqueous extract of *Azadirachta indica* produced zones of inhibition of 21.00 ± 1.00 mm, 24.00 ± 1.00 mm, 25.00 ± 2.00 mm and 26.00 ± 1.00 mm against *Salmonella* Typhi and 10.50 ± 0.50 mm, 16.00 ± 1.00 mm, 20.50 ± 0.50 mm and 21.00 ± 4.00 mm against *Staphylococcus aureus*. Aqueous extract of *Allium sativum* produced zones of inhibition of 0.00 ± 0.00 mm, 8.50 ± 0.50 mm, 9.00 ± 3.00 mm and 10.00 ± 1.00 mm against *Salmonella* Typhi and 0.00 ± 0.00 mm, 0.00 ± 0.00 mm 5.00 ± 0.00 mm and 6.50 ± 0.50 mm against *Staphylococcus aureus*. The standard drug (8 µg/mL ciprofloxacin) produced zones of inhibition of 48.50 ± 1.50 mm and 42.50 ± 2.50 mm against *Salmonella* Typhi and *Staphylococcus aureus* while dimethyl sulphoxide was inactive against the isolates.

Table 4.6: Antibacterial activities of aqueous extracts of *Azadirachta indica* and *Allium sativum*

Concentrations of extracts/ STD (mg/mL)	Zones of Inhibition in Diameter (mm)			
	Azadirachta indica		*Allium sativum*	
	ST	SA	ST	SA
250	$21.00^{b}\pm1.00$	$10.50^{b}\pm0.50$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
300	$24.00^{bc}\pm1.00$	$16.00^{bc}\pm1.00$	$8.50^{ab}\pm0.50$	$0.00^{a}\pm0.00$
350	$25.00^{bc}\pm2.00$	$20.50^{c}\pm0.50$	$9.00^{b}\pm3.00$	$5.00^{b}\pm0.00$
400	$26.00^{c}\pm1.00$	$21.00^{c}\pm4.00$	$10.00^{b}\pm1.00$	$6.50^{b}\pm0.50$
CPX (8 µg/mL)	$48.50^{d}\pm1.50$	$42.50^{d}\pm2.50$	$48.50^{c}\pm1.50$	$44.00^{c}\pm1.00$
DMSO	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$

Mean values with same letter(s) in the same column do not differ significantly at $p>0.05$. CPX: Ciprofloxacin, DMSO: dimethyl Sulphoxide, SA: *Staphylococcus aureus*, ST: *Salmonella* Typhi, mg/mL: milligram per millimeter, STD: standard drug, µg/mL: microliter per milliliter.

4.1.7 Antifungal activity of aqueous extracts of *Azadirachta indica* and *Allium sativum* on test isolates

The antifungal activity of aqueous extracts of *Azadirachta indica* and *Allium sativum* on test isolates is shown in Table 4.7. Aqueous extract *Azadirachta indica* inhibited the growth of *Candida albicans* concentrations of 250mg/mL, 300mg/mL, 350mg/mL and 400 mg/mL while *Aspergillus niger* was resistant to it. Aqueous extract of *Allium sativum* only inhibited the growth of C*andida albicans* at 400 mg/mL while *Aspergillus niger* was resistant. The standard drug (10 µg/mL of clotrimazole) inhibited the growth of both isolates while dimethyl sulphur oxide was inactive.

Table 4.7: Antifungal activity of aqueous extracts *of Azadirachta indica* and *Allium sativum* on test isolates

Concentrations of extracts /STD (mg/mL)	*Azadirachta indica*		*Allium sativum*	
	CA	**AN**	**CA**	**AN**
250	+	-	-	-
300	+	-	-	-
350	+	-	-	-
400	+	-	+	-
Clotrimazole (10 µg/mL)	+	+	+	+
DMSO	-	-	-	-

DMSO: dimethyl Sulphoxide, CA: *Candida albicans*, AN: *Aspergillus niger*,STD: standard drug, -: no activity, +: activity, mg/mL: milligram per millimeter, µg/mL: microliter per milliliter.

4.1.8 Antibacterial activity of extracts of *Jatropha curcas* on test isolates

Table 4.8 shows the antibacterial activity of extracts of *Jatropha curcas* on test isolates. At concentrations of 250mg/mL, 300mg/mL, 350mg/mL and 400 mg/mL, the *n*-Hexane, methanolic, and ethyl acetate extracts of *Jatropha curcas* were significantly different at $P > 0.05$ except for ethyl acetate that were not significantly different at $P < 0.05$ from each other against *Salmonella* Typhi. The *n*-Hexane extract produced zones of inhibition of 0.00± 0.00 mm, 9.50 ± 0.50 mm, 16.00 ± 0.00 mm and 19.00 ± 2.00 mmagainst *Salmonella* Typhi and 0.00 ± 0.00 mm, 13.00 ± 1.00 mm, 16.00 ± 2.00 mm and 21.50 ± 1.50 mm against *Staphylococcus aureus*. Methanolic extract produced zones of inhibition of 0.00 ± 0.00 mm, 3.50 ± 0.50 mm, 6.00 ± 1.00 mm and 7.00 ± 2.00 mm against *Salomonella* Typhi and 0.00 ± 0.00 mm, 5.50 ± 0.50 mm, 6.00 ± 0.00 mm and 7.00 ± 0.00 mm against *Staphylococcus aureus*. Ethyl acetate extract produced

zones of inhibition of 4.00 ± 2.00 mm, 6.50 ± 1.50 mm, 7.00 ± 4.00 mm and 10.50 ± 5.00 mm against *Salmonella* Typhi and 11.00 ± 1.00 mm, 13.50 ± 0.50 mm, 15.00 ± 1.00 mm and 17.00 ± 3.00 mm against *Staphylococcus aureus*. The standard drug (8 µg/mL of ciprofloxacin) produced zones of inhibition of 48.50 ± 1.50 mm and 43.00 ± 2.00 mm against *Salmonella* Typhi and *Staphylococcus aureus* while dimethyl sulphur oxide was inactive.

Table 4.8: Antibacterial Activity of *Jatropha curcas* Leaf Extract

Conc. of extracts/STD (mg/mL)	Zones of Inhibition in Diameter (mm)					
	***n*-Hexane**		**Methanol**		**Ethyl Acetate**	
	ST	**SA**	**ST**	**SA**	**ST**	**SA**
250	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$4.00^{a}\pm2.00$	$11.00^{b}\pm1.00$
300	$9.50^{b}\pm0.50$	$13.00^{b}\pm1.00$	$3.50^{ab}\pm0.50$	$5.50^{a}\pm0.50$	$6.50^{a}\pm1.50$	$13.50^{bc}\pm0.50$
350	$16.00^{c}\pm0.00$	$16.00^{b}\pm2.00$	$6.00^{b}\pm1.00$	$6.0^{a}\pm0.00$	$7.00^{a}\pm4.00$	$15.00^{bc}\pm1.00$
400	$19.00^{c}\pm2.00$	$21.50^{c}\pm1.50$	$7.00^{b}\pm2.00$	$7.0^{b}\pm0.00$	$10.00^{a}\pm5.00$	$17.00^{c}\pm3.00$
CPX(8µg/mL)	$48.50^{d}\pm1.50$	$43.00^{d}\pm2.00$	$49.00^{c}\pm1.00$	$43.0^{c}\pm2.00$	$50.00^{b}\pm0.00$	$43.00^{d}\pm2.00$
DMSO	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.0^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$

Mean values with same letter(s) in the same column do not differ significantly at P>0.05. CPX: ciprofloxacin, DMSO: dimethyl Sulphoxide; SA= *Staphylococcus aureus*; ST= *Salmonella* Typhi, Conc.: concentration, mg/mL: milligram per millimeter, STD: standard drug, µg/mL: microliter per milliliter.

4.1.9 Antifungal activity of extracts of *Jatropha curcas*on test isolates

The antifungal activity of the extracts of *Jatropha curcas* on test isolates is presented in Table 4.9. At concentrations of 250, 300, 350 and 400 mg/mL, *n*-Hexane extract of *J. curcas* inhibited the growth of *Candida albicans* and *Aspergillus niger* while ethyl acetate was inactive against growth of both isolates. Methanolic extract of *J. curcas* was only active against the growth of *Candida albicans* at concentrations of 350 and 400 mg/mL. The standard drug (10 µg/mL of clotrimazole) was active against the growth of all test isolates while dimethyl sulphuroxide was inactive.

Table 4.9: Antifungal activity of *Jatropha caucus* leaf extract

Conc. of extracts/ (mg/mL)	**STD**	***n*-Hexane**		**Methanol**		**Ethyl Acetate**	
		CA	**AN**	**CA**	**AN**	**CA**	**AN**
250		+	+	-	-	-	-
300		+	+	-	-	-	-
350		+	+	+	-	-	-
400		+	+	+	-	-	-
Clotrimazole (10 µg/mL)		+	+	+	+	+	+
DMSO		-	-	-	-	-	-

DMSO: dimethyl Sulphoxide, CA:*Candida albicans*, AN: *Aspergillus niger*, STD: standard drug, -: no activity, +: activity, mg/mL: milligram per millimeter, µg/mL: microliter per milliliter, Conc: concentration.

4.1.10 Antibacterial activity of extracts of *Moringa oleifera* on test isolates

Table 4.10 shows the antibacterial activity of extracts of *Moringa oleifera* on test isolates. There were significant different at $P > 0.05$ in the zones of inhibition produced by the extracts of *M. oleifera*. At concentrations of 250, 350 and 400 mg/mL, *n*-Hexane extract of *M. oleifera* produced zones of inhibition of 0.00 ± 0.00 mm, 5.00 ± 1.00 mm,

6.00 ± 3.00 mm and 7.00 ± 0.00 mm against *Salmonella* Typhi and 0.00 ± 0.00 mm, 7.00 ± 0.00 mm, 8.50 ± 1.50 mm and 9.00 ± 1.00 mm against *Staphylococcus aureus*. Methanolic extract of *M. oleifera* produced zones of inhibition of 0.00 ± 0.00 mm, 0.00 ± 0.00 mm, 7.00 ± 1.00 mm and 11.50 ± 6.50 mm against *Salmonella* Typhi and 0.00 ± 0.00 mm, 4.00 ± 0.00 mm, 5.50 ± 0.50 mm and 7.50 ± 1.50 mm against *Staphylococcus aureus* while ethyl acetate extract produced zones of inhibition of 0.00 ± 0.00 mm, 5.00 ± 1.00 mm, 6.00 ± 0.00 mm and 7.00 ± 0.20 mm against *Salmonella* Typhi and 0.00 ± 0.00 mm, 0.00 ± 0.00 mm, 5.00 ± 0.00 mm and 7.00 ± 1.00 mm. The standard drug (8 µg/mL of ciprofloxacin) produced zones of inhibition of 48.00 ± 2.00 mm and 43.00 ± 0.00 mm against *Salmonella* Typhi and *Staphylococcus aureus* while dimethyl sulphuroxide was inactive.

Table 4.10: Antibacterial Activity of *Moringa oleifera* Leaf Extract

Conc. of extracts/STD (mg/mL)	*n*-Hexane		Methanol		Ethyl acetate	
	ST	SA	ST	SA	ST	SA
250	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
300	$5.00^{ab}\pm1.00$	$7.00^{b}\pm0.00$	$0.00^{a}\pm0.00$	$4.00^{b}\pm0.00$	$5.00^{b}\pm1.00$	$0.00^{a}\pm0.00$
350	$6.00^{b}\pm3.00$	$8.50^{b}\pm1.50$	$7.00^{ab}\pm1.00$	$5.50^{b}\pm0.50$	$6.00^{b}\pm0.00$	$5.00^{b}\pm0.00$
400	$7.00^{b}\pm0.00$	$9.00^{b}\pm1.00$	$11.50^{b}\pm6.50$	$7.50^{b}\pm1.50$	$7.00^{b}\pm0.20$	$7.00^{b}\pm1.00$
CPX (8µg/mL)	$48.00^{c}\pm2.00$	$43.00^{c}\pm2.00$	$49.00^{c}\pm1.00$	$43.00^{c}\pm2.20$	$50.00^{c}\pm0.00$	$43.00^{c}\pm2.00$
DMSO	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$

Mean values with same letter(s) in the same column do not differ significantly at P>0.05.

CPX: ciprofloxacin, DMSO: dimethyl Sulphoxide, SA: *Staphylococcus aureus*, ST: *Salmonella* Typhi, Conc: concentration, mg/mL: milligram per milliliter, STD: standard drug, µg/mL: microliter per milliliter, mm: millimeter.

4.1.11 Antifungal activity of *Moringa oleifera* leaf extract

The antifungal activity of the extracts of *Moringa oleifera* on test isolate is shown in Table 4.11. At concentrations of 250 mg/mL, 300 mg/mL, 350 mg/mL and 400 mg/mL, *n*-Hexane extract of *M. oleifera* was only active against the growth of *Aspergillus niger* while methanolic and ethyl acetate extracts of *M. oleifera* were inactive against the test isolates at concentrations of 250 mg/mL, 300 mg/mL, 350 mg/mL and 400 mg/mL. The standard drug (10 µg/mL of clotrimazole) inhibited the growth of both test isolates while dimethyl sulphuroxide was inactive.

Table 4.11: Antifungal Activity of *Moringa oleifera* Leaf Extract

Concentrations of extracts/STD (mg/mL)	n-Hexane		Methanol		Ethyl Acetate	
	CA	AN	CA	AN	CA	AN
250	-	-	-	-	-	-
300	-	+	-	-	-	-
350	-	+	-	-	-	-
400	+	+	+	-	-	-
Clotrimazole (10 µg/mL)	+	+	+	+	+	+
DMSO	-	-	-	-	-	-

DMSO: dimethyl Sulphoxide, CA: *Candida albicans*, AN: *Aspergillus niger*, STD: standard drug, -: no activity, +: activity, mg/mL: milligram per millimeter, µg/mL: microliter per milliliter.

4.1.12 Antibacterial activity of fractions of aqueous extract of *Azadirachta indica*

The antibacterial activity of the fractions of aqueous extract of *Azadirachta indica* on test isolates is presented in Table 4.12. There was significant difference at $P > 0.05$ in the zones of inhibition produced by the fractions of *Azadirachta indica*. Methanolic fractions of *Azadirachta indica*when compared with positive control (Ciprofloxacin),

Negative control (DMSO) was also used. Among the two (2) tested organisms, both were susceptible to the fractions of *Azadirachta indica* aqueous leaf extract. Aqueous (ST 12.0mm, SA 16.0mm), methanol (ST 18.0mm, SA 15mm), Ethyl Acetate, (ST-16mm, SA 0.0mm) and n-Hexane (ST 0.0mm, SA 10.5mm) fractions respectively. All the test organisms were found to be susceptible to the positive control.

Table 4.12: Antibacterial activity of fractions of aqueous extracts of *Azadirachta indica* at 400 mg/mL

Concentrations of Fractions/STD (mg/mL)	Zones of Inhibition in Diameter (mm)	
	ST	SA
Ethyl Acetate	$16.00^{c} \pm 1.00$	$0.00^{a} \pm 0.00$
Methanol	$18.00^{c} \pm 1.00$	$15.50^{c} \pm 0.50$
Aqueous	$12.00^{b} \pm 0.00$	$16.00^{c} \pm 1.00$
n-Hexane	$0.00^{a} \pm 0.00$	$10.50^{b} \pm 0.50$
CPX (8 µg/mL)	$48.50^{d} \pm 1.50$	$44.00^{d} \pm 1.00$
DMSO	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$

Mean values with same letter(s) in the same column do not differ significantly at P>0.05.

Keys: CPX: ciprofloxacin, DMSO: dimethyl Sulphoxide, SA: *Staphylococcus aureus*; ST: *Salmonella* Typhi, mg/mL: milligram per milliliter, STD: standard drug, µg/mL: microliter per milliliter, mm: millimeter.

4.1.13 Antifungal activity of fractions of aqueous extract of *Azadirachta indica* at 400 mg/mL

Table 4.13 shows the antifungal activity of aqueous fractions of A. indica on test isolates. The ethyl acetate methanolic, aqueous and n-hexane fractions of *Azadirachta indica* were inactive against the fungal isolates (*Candida albicans* and *Aspergillus niger*) while the standard drug (10 µg/mL of clotrimazole) was active against the fungal isolates. dimethyl sulphur oxide was inactive against the test isolates.

Table 4.13: Antifungal activity of fractions of aqueous extract of *Azadirachta indica* at 400 mg/mL

Fractions	*Candida albicans*	*Aspergillus niger*
Ethyl Acetate	-	-
Methanol	-	-
Aqueous	-	-
n-Hexane	-	-
Clotrimazole (10 µg/mL)	+	+
DMSO	-	-

DMSO: dimethyl Sulphoxide, STD: standard drug, -: no activity, +: activity, mg/mL: milligram per millimeter, µg/mL: microliter per milliliter.

4.1.14 Minimum inhibitory and bactericidal concentrations of aqueous extract of *Azadirachta indica*

Table 4.14 reveals the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) aqueous extract of *A*. *Indica*. *Salmonella* Typhi and *Staphylococcus aureus* had an MIC of 31.25 mg/mL for each respectively whereas the MBC for *Salmonella* Typhi and *Staphylococcus aureus* were 250 mg/mL and 125 mg/mL respectively.

Table 4.14: Minimum inhibitory and bactericidal concentrations of aqueous extract of *Azadirachta indica*

Concentrations (mg/mL)	Isolates	
	***Salmonella* Typhi**	***Staphylococcus aureus***
250.00	+**	+
125.00	+	+**
62.50	+	+
31.25	+*	+*
15.63	-	-
7.81	-	-
3.91	-	-
1.95	-	-

+**: MBC, +*: MIC, +: activity, -: inactivity, mg/mL: milligram per millimetre.

4.1.15 Minimum inhibitory and fungicidal concentrations of aqueous extract of *Azadirachta indica*

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of aqueous extract *Azadirachta indica* is presented in table 4.15. The extract had an MIC and MBC of 31.25mg/mL and 125mg/mL against *Candida albicans*.

Table 4.15: Minimum inhibitory and fungicidal concentrations of aqueous extract of *Azadirachta indica*

Concentrations (mg/mL)	*Candida albicans*
250.00	+
125.00	+**
62.50	+
31.25	+*
15.63	-
7.81	-
3.91	-
1.95	-

+**: MBC, +*: MIC, +: activity, -: inactivity, mg/mL: milligram per millimetre.

4.1.16 Minimum inhibitory and bactericidal concentrations of ethyl acetate extract of *Jatropha curcas*

Table 4.16 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethyl acetate extract of *Jatropha curcas.* *Staphylococcus aureus* had MIC of 62.50 mg/mL and an MBC of 250 mg/mL.

Table 4.16: Minimum Inhibitory and Bactericidal Concentrations of Ethyl Acetate Extract of *Jatropha curcas*

Concentrations (mg/mL)	*Staphylococcus aureus*
250.00	+**
125.00	+
62.50	+*
31.25	-
15.63	-
7.81	-
3.91	-
1.95	-

+**: MBC, +*: MIC, +: activity, -: inactivity, mg/mL: milligram per millimetre.

4.1.17 Minimum inhibitory and bactericidal concentrations of *n*-hexane extract of *Jatropha curcas*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract of *Jatropha curcas* is shown in table 4.17. The MIC of *Salmonella* Typhi and *Staphylococcus aureus* was 62.50 mg/mL while the MBC was 250 mg/mL respectively.

Table 4.17: Minimum inhibitory and bactericidal concentrations of *n*-Hexane extract of *Jatropha curcas*

Concentrations (mg/mL)	**Isolates**	
	***Salmonella* Typhi**	***Staphylococcus aureus***
250.00	+**	+**
125.00	+	+
62.50	+*	+*
31.25	-	-
15.63	-	-
7.81	-	-
3.91	-	-
1.95	-	-

+**: MBC, +*: MIC, +: activity, -: inactivity, mg/mL: milligram per millimetre.

4.1.18 Minimum inhibitory and bactericidal concentrations of fractions of *Azadirachta indica*

Table 4.18 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fractions of *A*. *Indica*. Methanolic and aqueous fractions of *A*. *indica* both had an MIC of 31.25 mg/mL respectively for *Staphylococcus aureus* while methanolic fraction of *A*. *indica* had an MBC of 125 mg/mL. The methanolic and ethyl acetate fractions also had an MIC of 62.50 mg/mL and 31.25 mg/mL against *Salmonella* Typhi while ethyl acetate fraction had an MBC 125 mg/mL.

Table 4.18: Minimum inhibitory and bactericidal concentrations of fractions of *Azadirachta indica*

Conc. (mg/mL)	Isolates / Fractions			
	***Salmonella* Typhi**		***Staphylococcus aureus***	
	M-fraction	**EA-fraction**	**M-fraction**	**A-fraction**
250.00	+	+	+	+
125.00	+	+**	+**	+
62.50	+*	+	+	+
31.25	-	+*	+*	+*
15.63	-	-	-	-
7.81	-	-	-	-
3.91	-	-	-	-
1.95	-	-	-	-

+**: MBC, +*: MIC, +: activity, -: inactivity, mg/mL: milligram per millimetre, Conc: concentration, M-fraction: methanolic fraction, EA-fraction: ethyl acetate fraction, A-fraction: aqueous fraction.

4.2 Discussion

In the present study, the percentage recovery yield of the methanolic, ethyl acetate, n-hexane and aqueous extracts of *Moringa oleifera*, *Azadirachta indica*, *Allium sativum* and *Jathropha curcas* revealed that the methanolic extract of *Moringa oleifera* had the best percentage recovery yield (60 g) when compared with other solvents and plants used while n-hexane extract of *J. curcas* had the least percentage recovery yield. The disparity in the recovery yield observed in most plant extract may be due to the difference in the polarity of solvent used as well as the method of extraction (cold maceration). This result is in agreement with the findings of Samir *et al*. (2017) who reported that high yield of plant extracts could be attributed to polarity and method of extraction which in turn affects the solubility of extractable bioactive secondary metabolites in plants.

Four organisms (2 bacterial and 2 fungal isolates) used in this study were isolated from the poultry droppings and were morphologically, microscopically and biochemically suspected to be *Salmonella* Typhi, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. This result corroborates with the findings of Ochei & Kolhatkat (2010) that isolated similar organisms.

The qualitative components of crude extracts in this study showed that methanolic, *n-hexane* and ethyl acetate extracts of *Jathropha curcas* were observed to contain strong metabolites such as alkaloids, glycosides, phenols, flavonoids, saponins and tannins while alkaloids and flavonoids, were observed in *Moringa oleifera*. Aqueous extract of *Azadirachta indica* had the presence of alkaloids, glycosides, phenols, flavonoids, saponins, tannins, and reducing sugars while glycosides, saponins tannins and reducing sugars were found to be present in the extract of aqueous *Allium sativum*. The presence

of these metabolites could be responsible for the activity of the extracts. This result is in agreement with the findings of (Poonam *et al.,* 2013) who also identified similar metabolites.

In the present study, the zones of inhibition of the aqueous extract of *Azadirachta indica* were significantly different from each other, and the activity observed was concentration dependent. The highest zone of inhibition (26.00 ± 1.00 mm) was at 400 mg/mL against *Salmonella* Typhi while 250 mg/mL had the least zone of inhibition (10.50 ± 0.50 mm) against *Staphylococcus aureus*. The aqueous extract of *Allium sativum* had minimal activity against the bacterial isolates (*Salmonella* Typhi and *Staphylococcus aureus*). The activity was concentration dependent with the highest zone of inhibition (10.00 ± 1.00 mm) at 400 mg/mL against *Salmonella* Typhi while at 250 mg/mL it was inactive. The extract of *A. indica* was active against *Candida albicans* at concentrations of 250, 300, 350 and 400 mg/mL and in active against *Aspergillus niger* while Allium sativum was inactive against *Candida albicans* and *Aspergillus niger*. The extract of *A. indica* had a better activity compared to *A. sativum*, and the observed activity could be due to the presence of active secondary metabolites in higher concentration that singly or synergistically inhibited the growth of the isolates. This result corroborates with the findings of Jayasree *et al*. (2014) who reported that activity of extracts depends on the presence of active biomolecules in high concentration required enough to inhibit the growth of microorganisms.

The present study also established that the n-hexane and ethyl acetate extracts of *Jathropha curcas* had a concentration dependent activity against the isolates with the highest zone of inhibition (21.50 ± 1.50 mm) at 400 mg/mL against *Staphylococcus aureus* while the methanolic extract of *J. curcas* had minimal activity against the isolates. The minimal activity observed in methanolic extract of *J. curcas* as compared

with the n-hexane and ethyl acetate counterpart could be due to the method of extraction and low concentration of the bioactive molecules in the extract. This result is in agreement with the report of Srinivasan *et al*. (2001) and Poonam *et al*. (2013) who reported that the activity of plant extracts may be affected by solubility of bioactive molecules hence the concentration of the bioactive molecules required for inhibition of the growth of microbial isolates. The n-hexane extract of *J*. curcas was active against the growth of *Candida albicans* and *Aspergillus niger* used in this study. The methanolic extract inhibited only the growth of *Candida albicans* at 350 mg/mL and 400 mg/mL while ethyl acetate was inactive at all the concentrations against *Candida albicans* and *Aspergillus niger*. The inactivity observed in the methanolic extract (at 350 mg/mL and 400 mg/mL) of *J*. curcas and ethyl acetate extract of *J*. curcas might be that the active biomolecules in the extracts were in low concentration to inhibit the growth of the isolates. The differences in polarity of the solvents used in the extraction could also be responsible. This result is in agreement with the findings of Aiyelaagbe *et al*. (2007) who found out that ethyl acetate extract of *J*. curcas had activity against *Candida albicans*.

In the present study, n-hexane, methanolic and ethyl acetate extracts of *Moringa oleifera* had minimal activity on the bacterial isolates. The activity was although concentration dependent against the isolates. This could be as a result of the secondary metabolites in the extract not in sufficient concentration to inhibit the growth of the bacterial isolates. The antifungal activity of the extracts of *Moringa oleifera* in this study revealed that methanolic extract was mildly active against *Aspergillus niger* only. This result is in agreement with the findings of Jonathan *et al*. (2012) who reported appreciable activity of *Moringa oleifera* against *Aspergillus flavus*.

In the present study, the fractions of aqueous extract of *A*. *indica* revealed that methanolic fraction of aqueous *A*. *indica* had the best activity while n-hexane fraction of the same plant extract was inactive against the isolates. The observed inactivity in the n-hexane fraction of *A*. *indica* might be due to the high volatility of the fractionating solvent and active bioactive molecules in low concentration to inhibit the growth of the bacterial isolates.

In the present study, the methanolic, aqueous, n-hexane and ethyl acetate fractions of aqueous extract of *A*. *indica* were all inactive against *Aspergillus niger*. This may be due to the low concentration of bioactive molecules in the fractions. This result is in agreement with the findings of (Samir *et al.,* 2017).

In the present study, the minimum inhibitory concentration (MIC) of aqueous extract of *A*. *indica* was at 31.25 mg/mL for both the bacterial and fungal isolates used. The MIC for the ethyl acetate extract of *Jathropha curcas* was at 62.50 mg/mL for both bacterial and fungal isolates used. The MIC for the methanolic fraction of aqueous extract of *Azadirachta indica* was at 31.25 mg/mL and 62.50 mg/mL against *Staphylococcus aureus* and *Salmonella* Typhi respectively while the aqueous and ethyl acetate fractions was 31.25 mg/mL each against *Staphylococcus aureus* and *Salmonella* Typhi respectively. This implies that extracts and /or fractions with low MIC values have promising and good antimicrobial potency. This result corroborates with the findings of Hoque *et al*. (2017).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this study, it can be concluded that, some pathogenic organisms which include *Staphylococcus aureus, Salmonella* Typhi*, Aspergillus niger* and *Candida albicans* were isolated from poultry droppings, identified and characterized.

The qualitative components of the extracts were observed to contain strong metabolites such as alkaloids, glycosides, phenols, flavonoids, saponins and reducing sugars were also found to be present in the extracts.

The crude extracts of *Jatropha curcas, Azadirachta indica* (Neem), *Moringa oleifera* (Moringa) and *Allium sativum* (Garlic), has potential antimicrobial activities against some pathogens of poultry. The plants extracts have large quantity of bioactive component, which could be responsible for the damage of bacterial cell membrane, it also plays a vital role in the plants defense mechanisms and antimicrobial activity.

The plants used *Jatropha curcas, Azadirachta indica* (Neem), *Moringa oleifera* (Moringa) and *Allium sativum* (Garlic), have shown considerable activities against these pathogenic organisms isolated from chicken droppings, with *Azadirachta indica* (Neem) being the most active.

5.2 Recommendations

From this study, it can be recommended that:

1. The use of these plants extracts could be a promising naturally occurring antibacterial agent with potential applications in the pharmaceutical industry for controlling multidrug resistant microorganisms.
2. They are economical in use and there would be no case of drug resistance, as it is usually recorded in standard antibiotics.

REFERENCES

Abalaka, M. E., Daniyan, S. Y., Oyeleke, S. B., & Adeyemo, S. O. (2012). The antibacterial evaluation of *Moringa oleifera* leaf extracts on selected bacterial pathogens. *Journal Microbiological Research,* 2,1-4.

Abhilash, P. C., Srivastava, P., Jamil, S., & Singh, N. (2011). Revisited *Jatropha curcas* as an oil plant of multiple benefits: critical research needs and prospects for the future. *Environmental Science and Pollution Research,* 18(1), 127-131.

Adaki, S., Adaki, R., Shah, K. & Karagir A., (2014). Garlic: Review of literature. *Indian Journal on Cancer,* 51,577-81.

Adline, J., & Devi, J. (2014). A study on phytochemical screening and antibacterial activity of *Moringa oleifera*. *International Journal of Resources Application,* 2, 169–176.

Afzal, I., Hussain, B., Basra, S. M. A. & Rehman, H. (2012). Priming with MLE reduces imbibitional chilling injury in spring maize. *Seed Science and Technology,* 40, 271–276.

Agamuthu, P., Abioye, O. P.& Aziz, A. A. (2010). Phytoremediation of soil contaminated with used lubricating oil using *Jatropha curcas*. *Journal of Hazard Material*, 179, 891-894.

Ahirrao, R. A., Patel, M. R., Sayyed, H., Patil, J. K., Suryawanshi H. P.& Tadavi, S.A. (2011). *In vitro* anthelmintic property of various herbal plants extracts against *Pheritima posthuma*. *Pharmacology online,* 2, 542-547.

Aiyelaagbe, O. O., Adeniyi, B. A., Fatunsin, O. F., & Arimah, B. D. (2007). *In vitro* antimicrobial activity and phytochemical analysis of *J. curcas* roots. *International Journal of Pharmacology*, 3, 106-110.

Ajayi, O. E., & Awala, S. I. (2016). *I*n-*vitro* Anti-bacterial Effects of *Jatropha curcas* on *Salmonella* Typhi and *Salmonella* Typhimurium Isolated from Presumptive Typhoid Fever Patients in Akure Metropolis, *Nigeria European Journal of Medicinal Plants,* 12(2), 23-26.

Akin-Osanaiye, B. C., Nok, A. J., Ibrahim S., Inuwa, H. M., Onyike, E., Amlabu, E. & Haruna, E. (2013), Antimalarial Effect of Neem Leaf and Neem Stem Bark Extracts on *Plasmodium Berghei* Infected in the Pathology and Treatment of Malaria, *International Journal of Research in Biochemistry and Biophysics*, 3(1), 7-8.

Al-Asmari, A. K., Albalawi, S. M., Athar, M. T., Khan, A. Q., Al-Shahrani, H., & Islam, M. (2015). *Moringa oleifera* as an anti-cancer agent against breast and colorectal cancer cell lines. *Public Library of Science ONE*, 10(8),1371-1373.

Amirigbal, M., Nadeemakbar, A., Abbas, R., Khan, H., & Maqsood, Q. (2014). Response of Canola to foliar application of Moring (*Moringa oleifera* L.) and Brassica (*Brassica napus* L.) water extracts. *International Journal of Agricultural Crop Science,*14, 1431–1433.

Asaolou, V., Binuomote, R., Akinlade, J., Aderinola, O., & Oyelami, O. (2012). Intake and growth performance of West African dwarf goats fed *Moringa oleifera*, *Gliricidia Sepium* and *Leucaena leucocephala* dried leaves as supplements to cassava peels. *Journal of Biology and Agricultural Healthcare,* 2, 76–88.

Bashir, K. A., Waziri, A. F., & Musa, D. D. (2016). *Moringa oleifera*, a potential miracle tree; a review. *Journal Pharmaceutical Biology Science,* 11, 25-30.

Basra, S. M. A., Iftikhar, M. N., & Afzal, I. (2011). Potential of *Moringa* (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *International Journal of Agricultural Biology*, 13, 1006-1010.

Bereksi M. S., Hassaïne H., Bekhechi, C., & Abdelouahid, D. E. (2018). Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. *A Multifaceted Journal in the field of Natural Products and Pharmacognosy,* 10(3), 507-512.

Block, E. (2010). Garlic and Other Alliums: The Lore and the Science. Royal Society of Chemistry, 190-199.

Brittaine, R. & Lutaladio N. (2010). *Jatropha*: A Smallholder Bioenergy Crop: The Potential for Pro-Poor Development. Food and Agriculture Organization of the United Nations, Rome, Italy, 8, 96.

Bulmer, G. S. (1978). Medical Mycology. 1st Edition, Scope Publication, UK, 70(2), 397-405.

Cantrell, C. L., Ali, A., Duke, S. O. & Khan, I. (2011). Identification of mosquito biting deterrent constituents from the Indian folk remedy plant *Jatropha curcas*. *Journal of Medical Entomology,* 48, 836-845.

Chai, S. J., Cole, D., Nisler, A., & Mahon, B. E. (2017). Poultry: The most common food in outbreaks with known pathogens, United States, 1998–2012. *Epidemiological Infection,*145, 316–325.

Cheesebrough, M. (2010). *Biochemical tests to identify bacteria. In*: *District laboratory practice in tropical countries*. Cambridge, UK: Cambridge University Press, (part 2) 71-76.

Daniyan, S. Y., Abalaka, M. E, Elemba, O. M., & Aransiola, S. A. (2011). In vitro antimicrobial activity and phytochemical screening of *Jatropha curcas* seed extract. *International Research Journal of Pharmacy,* 2(7), 60-64

Dash, S. P., Dixit, S. & Sahoo, S. (2017). Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta Indica*: An Important Medicinal Plant. *Biochemistry & Analytical Biochemistry,* 6, 2.

Desoky, E. M., Merwad, A. M. & Elrys, A. S. (2017). Response of pea plants to natural bio-stimulants under soil salinity stress. *American Journal Plant Physiology,* 12, 28-37.

Domsch, K. H., Gams, W., & Anderson, T. H. (1980) Compendium of Soil Fungi. Academic Press, New York, 11-20.

Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A multipurpose tree-*Moringa oleifera. International Journal of Pharmaceutical and Chemical Sciences*, 2(1), 415-423.

El-Mahmood, A. M., Ogbonna, O. B., & Raji, M. (2010). The antibacterial activity of *Azadarichta indica* (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. *Journal of Medicinal Plants Research,* 4(14), 1414-1421.

Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology*. 60, 1-8.

Erinoso, S. M. & Aworinde, D. O. (2012). Ethnobotanical survey of some medicinal plants used in traditional health care in Abeokuta areas of Ogun State, Nigeria. *African Journal Pharmacy and Pharmacology*, 6, 1352-1362.

Gajendrasinh, P. R., Bhavika, M. K., Rohit, S., Hetal, A. & Prajapati, P. K. (2012). *In vitro* Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*). *International Journal of Pharmaceutical & Biological Archives*, 3(3), 582-586.

Girish, K., & Shankara, B. S. (2008). *Electronic Journal of Biology*. 4(3), 102-111.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science Human Wellness*, 5, 49–56.

Govaerts, R. (2015). World Checklist of *Euphorbiaceae*. Richmond, UK: Royal Botanic Gardens, Kew. http://apps.kew.org/wcsp/.

Hanafy, R. (2017). Using *Moringa oleifera* leaf extract as a bio-fertilizer for drought stress mitigation of *Glycine max* L. plants. *Egyptian Journal of Botany*, 57,281–292.

Hassan, S. W., Umar, R. A., Ebbo, A. A. & Matazu, I. K. (2005). Phytochemical, antibacterial and toxicity studies of *Parkinsonia aculeate* L. (Fabaceae). *Nigerian Journal of Biochemistry and Molecular Biology*, 20(2), 89-97.

Hoque, M. M. D., Bari, M. L., Inatsu, Y., Juneja, V. K., & Kawamoto, S. (2017). Antibacterial activity of Guava (*Psidiumguajava* L.) and Neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease.* 4(4), 481-488.

Hu, P., Wang, A. S., Engledow, A. S., Hollister, E. B. & Rothlisberger, K. L. (2011). Inhibition of the germination and growth of *Phymatotrichopsis omnivora* (cotton root rot) by oilseed meals and isothiocyanates. *Applied Soil Ecology,* 49, 68-75.

Hussain, S., Malik, F., & Mahmood, S. (2014). Review: An exposition of medicinal preponderance of *Moringa oleifera* (Lank). *Pakistan Journal Pharmaceutical Science,* 27, 397-403.

Idris, M. A., Jami, M. S., Hammed, A. M., & Jamal, P. (2016). *Moringa oleifera* seed extract: A review on its environmental applications. *International Journal of Applied Environmental Science,* 11, 1469-1486.

Irobi, O. N., Moo-Young, M., Anderson, W. A., & Daramola, S. O. (1994). Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphorbiaceae). *International Journal of Pharmacology,* 34,87-90.

Jaafaru, M. S., Nordin N., Shaari, K., Rosli R., & Abdull Razis, A. F. (2018). Isothiocyanate from *Moringa oleifera* seeds mitigates hydrogen peroxide-induced cytotoxicity and preserved morphological features of human neuronal cells. *PLoS ONE*, *13*.

Jayasree, T., Anuradha, B., & Praveena, (2014). Antimicrobial activity of methanolic extracts of *Azadirachta indica*, *Rosmarinus officinalis* and *Lagenaria siceraria* leaves on some important pathogenic organisms. *Journal of Chemical and Pharmaceutical Research.* 6(4), 766-770

Jonathan, S. G., Olawuyi, O. J., Aina, D. A., Odeniyi, S. O., Adediji, I. O. & Ikhedia, A. (2012). Comparative studies on antifungal, anti-oxidant and phytochemical

potential of *Momordica charantia* and *Moringa oleifera*. *New York Science Journal*:5(12)

Kane, J., Summerbell, R., Sigler, L., & Krajden, S.L. (1997). Laboratory Handbook of Dermatophytes: A Clinical Guide and Laboratory Handbook of Dermatophytes and Other Filamentous Fungi from Skin, Hair, and Nails. Belmont: Star Publishing Company. 1-50.

Kewscience (2017). *Allium sativum* L".Plants of the World Online; Royal Botanic Gardens, Kew, England. Retrieved May 26, 2017.

Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant S., Gandra, S., Levin, S.A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academic Science*, (1),72-95.

Lourens, A. C. U., Reddy, D., Baser, K. H. C., Viljoen, A. M., & Van Vuuren, S. F. (2004). *In vitro* biological activity and essential oil composition of four indigenous South African *Helicchrysum* species. *Journal of ethnopharmacology*, 9, 253 – 258.

Madaki, E. C., Anthony, J. D., & Auwalu, U. (2019). Isolation of Enteric Bacteria from Various Sources in Selected Poultry Farms in Kaduna State. *Bioprocess Engineering*, (3), 1-5.

Mamman, P. H., Mshelia, W. P., Subatrus, S. C. & Sambo, K.W. (2013) Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli, Salmonella* spp and *Staphylococcus aureus*. *International Journal of Medicine and Medical Sciences*, 5(1), 14-15.

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*, 23(4), 795.

Mboto, C. I., Eja, M. E., Adegoke, A.A., Iwatt, G. D., Asikong, B. E., Takon, I., Udo, S. M., & Akeh, M. (2009). Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia Kola*, *Vernonia amygdalina* and honey on some medically important microorganisms. *African Journal of Microbiological Resources,* 3(9), 557-559.

Meredith, T. J. & Drucker, A. (2014)."Growing Garlic from True Seed". Blogspot: Garlic Analecta.

Mike, U. O., Henry C. O., & Theodore I. M. (2015) Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. *International Journal of Infectious Diseases,* 9, 86-89.

Mkoma, S. L., & Mabiki, F. P. (2012). *Jatropha* as energy potential biofuel in Tanzania. *International Journal of Environmental* Science, 2(3),1553-1564.

Mohamed, E. A. M. A., Ahmed, S. E., El-Sayed, M. D., Hala, M. N. T., Ahmed, S. M. E., Shaaban, S. E., & Ayman, A. S. (2018). *Review* on the effect of forage *Moringa oleifera* L. (Moringa) on animal health and nutrition and its beneficial applications in soil, plants and water purification. *Agriculture*, 8, 145.

Mohanta, M. K., Islam, M. S., Saha, A. K., Khatun, S., & Khatun, Z. (2016). Antibiogram Profiles of Bacterial Isolates from Poultry Feeds. *Imperial Journal of Interdisciplinary Research,* 2(5),41-46.

Mormile, M. R., Oakley, B. B., Morales, C. A., Line, J., Berrang, M. E., Meinersmann, R. J., Tillman, G. E., Wise, M. G., Siragusa, G. R., Hiett, K. L., & Seal, B. S. (2013). The poultry-associated microbiome: Network analysis and farm-to-fork characterizations. *Public Library of Science ONE* 8, 190.

Moyo, B., Masika, P. J., & Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology*, 11(11), 2797-2802.

Muhammed, R., Olurokooba, M., Akinyaju, J., & Kambai, E. (2013). Evaluation of different concentrations and frequency of foliar application of *Moringa* extract on growth and yield of onions. *Agroresearch,* 13, 196–205.

Muhsin, D. A. & Hussein, F. M. (2014). The Antibacterial Effect of Ginger and Garlic Extracts on Some Pathogenic Bacteria Isolated from Patients with Otitis Media. *International Research Journal of Medical Sciences*, 2(5), 1-5.

Nieminen, T. T., Koskinen, K., Laine P., Hultman, J., Sade, E., Paulin, L., Paloranta, A., Johansson, P., Björkroth, J., & Auvinen, P. (2012). Comparison of microbial communities in marinated and unmarinated broiler meat by metagenomics. *International Journal of Food Microbiology,* 157, 142–149.

O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations. In: Review of antimicrobial resistance. London: HM Government and Welcome trust.

Ochei, J., & Kolhatkar, A. (2000). Medical Laboratory Science, Theory and Practical. *Bacteriology antimicrobial susceptibility test.* New Delhi: Tata McGram-Hill Publishing company limited. 801-804.

Olufunsho, A., Ibrahim A. O., Saidi O., Jaime A. Teixeira S., & Vincent O. O. (2012). Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology,* 139, 330–336.

Osman, H., & Abuhassan, A. A. (2015). *Moringa: The Strategic Tree for the Third Century*; King Abdulaziz University Publishing Center: Jeddah, Saudi Arabia; ISBN 7-746-06-9960.

Padmini, E, Valarmathi, A & Rani, U. M. (2011). Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*. *Asian Journal of Experimental Biological Science*, 4,772-778.

Pakade, V., Cukrowska, E., & Chimuka, L. (2013). Comparison of antioxidant activity of *Moringa oleifera* and selected vegetables in South Africa. *South African Journal of Science,* 109, 3–4.

Pandey, A. (2012). *Moringa oleifera* Lam. (Sahijan) A plant with a plethora of diverse therapeutic benefits: An updated retrospection. *Medicinal Aromatic Plants,* 1,101.

Pandey, V. C., Singh, K., Singh, J. S., Kumar, A., Singh, B., & Singh, R. P. (2012). *Jatropha curcas*: A potential biofuel plant for sustainable environmental development. *Renewable and Sustainable Energy Reviews,* 16(5), 2870-2883.

Pelczar, M. J., Chan, E. C. S. & Krieg, N. R. (1993). Microbiology: concepts and applications (pp. 180) McGraw Publication., New York.

Poonam, P., Himani, B., & Shivangi, M. (2013). *Azadirachta indica* (NEEM): antibacterial effects against *Escherichia coli* and *Salmonella, Guru Drone Journal of Pharmacy and Research,* 1(1), 18-21.

Pourmand, A., Mazer-Amirshahi, M., Jasani, G., & May, L. (2017). Emerging trends in antibiotic resistance: implications for emergency medicine. *American Journal Emergency Medicine,* 35(8),1172–1176.

Pramila, K., & Prerana, A. (2014). Antimicrobial activity and phytochemical analysis of *Calotropis gigantea* root, latex extracts. *International Organisation of Scientific Research Journal of Pharmacy,* 4 (6), 7-11.

Praveen, P. K., Debnath, C., Shekhar, S., Dalai, N., & Ganguly, S. (2016). A review: Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazards. *Veterinary World,* 9, 6–11.

Rader, H., & McGuinness, J. (2019). Growing Garlic in Alaska" Cooperative Extension Service. University of Alaska Fairbanks.

Rady, M. M., Bhavya, V. C., & Howladar, S. M. (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Science and Horticulture*, 162, 63–70.

Rebell, G., & Taplin, D. (1979) Dermatophytes, Their Recognition and Identification. Coral Gables, Florida: University of Miami Press; 1-50.

Rehman, H., Nawaz, M. Q., Basra, S. M. A., Afzal, I., Yasmeen, A., & Hassan, F. U. (2014). Seed priming influence on early crop growth, phenological development and yield performance of linola (*Linum usitatissimum* L.) *Integrated Agriculture,* 13, 990-996.

Rouger, A., Remenant, B., Prévost, H., & Zagorec, M. (2017). A method to isolate bacterial communities and characterize ecosystems from food products: Validation and utilization as a reproducible chicken meat model. *International Journal of Food Microbiology,* 247, 38-47.

Saavedra, G. Y. R., & Van der Maden, E. C. L. (2015). Opportunities for Development of the Moringa Sector in Bangladesh: Desk-Based Review of the *Moringa* Value Chains in Developing Countries and End-Markets in Europe; Centre for Development Innovation: Wageningen, WD, USA, 13, 176-185.

Saba, I., Muneeba, B., & Hira, Y. (2011). In-Vitro Antibacterial Activity of Two Medicinal Plants Neem (*Azadirachta indica*) and Peppermint. *International Research Journal of Pharmaceuticals,* 1,9-14.

Sachdeva, K., Garg P., Singhal, M. & Srivastava, B. (2011). Wound healing potential of extract of *Jatropha curcas* L. (Stem bark) in rats. *Pharmacognocsy Journal*, 3, 67-72.

Saetae, D., & Suntornsuk, W. (2010). Antifungal activities of ethanolic extract from *Jatropha curcas* seed cake. *Journal of Microbiology Biotechnology,* 20(2), 319–324.

Samir, F., Amal, D., Hafedh, H., Kais, M., Neji, G., & Adel, K. (2017). solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Food science and technology Campinas,* 37(3), 483- 492.

Sanis, J, Ravindran, R., Ramankutty, S. A., Gopalan, A. K. K., Nair, S. N., Kavillimakkil, A. K.,& Ghosh, S. (2012). *Jatropha curcas* (Linn) leaf extract–a possible alternative for population control of Rhipicephalus (Boophilus) annulatus. *Asian Pacific Journal of Tropical Disease*, 2(3), 225-229.

Shahzad, U., Khan, M.A., Jaskani, M. J., Khan, I. A., & Korban, S. S. (2013). Genetic diversity and population structure of *Moringa oleifera*. *Conservation Genetics,*14,1161-1172.

Shamweel, A., & Dar, M. I. (2011). Antibiotics and preventing their misuse. *Physical Academics,* 5(3),24-28.

Sharma, D. K.& Pandey, A. K. (2009). Use of *Jatropha curcas* hull biomass for bioactive compost production. *Biomass Bioenergy,* 33, 159-162.

Shravan, K. D., Ramakrishna, R., Santhosh, K. M., & Kannappan, N. (2011). *In vitro* Antibacterial Activity and Phytochemical Screening of Crude Extracts from *Jatropha curcas* Linn. *Journal of Applied Pharmaceutical Science,* 01(04),100-105.

Shuford J. A, Steckelberg, J. M. & Patel, R. (2005). Effects of fresh garlic extract on *Candida albicans* biofilms, *Antimicrobial Agents as Chemotherapy,* 49 (1), 473.

Silva, G. N., Faroni, L. R. A., Sousa, A. H. & Freitas, R. S. (2012). Bioactivity of *Jatropha curcas* L. to insect pests of stored products. *Journal of Stored Production Research,* 48, 111-113.

Singh, U. P., Singh, H. B., & Singh, R. B (1980). The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil borne pathogens*, Mycologia,* 7, 1077-1093.

Sneath, P. H. A., Mair, N. S., Sharpe, M. E., & Holt, J. G. (1986). *Bergey's Manual of Systematic Bacteriology.* Williams and Wilkins, Philadelphia, (2), 398.

Souza, L. K. H., Oliveira, C. A. D., Ferri, P. H., Santos, S. C. (2002). Antifungal properties of Brazilian cerrado plants. *Brazilian Journal of Microbiology,* 33(3), 247-249.

Spelman, K., Burns, J., Nichols, D., Winters, N., Ottersberg, S., & Tenborg, M. (2006). Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. *Alternative Medicine Review;* 11, 128-150.

Srinivasan, D., Perumalsamy, L., Nathan, S. & Sures, T. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology*, 94, 217–222.

Sundari, J., Selvaraj, R. & Prasad, N. R. (2011). Antimicrobial and antioxidant potential of root bark extracts from *Jatropha curcas* (Linn). *Journal of Pharmacy Research,* 4, 3743-3746.

Tewari, S. K. & Akhilesh, K. (2015). Origin, Distribution, Ethnobotany and Pharmacology of *Jatropha curcas*. *Research Journal of Medicinal Plants,*9,48-59.

Thomas, R., Sah N.K., & Sharma, P. B. (2008). Therapeutic biology of *Jatropha curcas*, A mini review. *Current Pharmaceutical Biotechnology,* 9, 315-324.

Tiwari, A. K., Kumar, A., & Raheman, H., (2007). Biodiesel production from Jatropha oil (*Jatropha curcas*) with high free fatty acids: An optimized process. Biomass *Bioenergy.* 31, 569-575.

Uwimbabazi, F., Uwimana, J., & Rutanga, J. P. (2015). Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli Journal of Medicinal Plants Studies,* 3(4), 85-91

Venâncio, P. C., Figueroba, S. R., Nani, B. D., Ferreira, L. E. N., Muniz, B. V., Del Fiol, F. D. S., & Groppo, F. C. (2017). Antimicrobial activity of two garlic species (*Allium sativum* and *A. tuberosum*) against *Staphylococci* infection. in vivo study in rats. *Advanced pharmaceutical bulletin,* 7(1), 115.

Vinoth, B., Manivasagaperumal R., & Balamurugan S. (2012). Phytochemical analysis and antibacterial activity of *Moringa oleifera* lam, India. *International Journal of research in biological sciences,* 2(3), 98-102.

Warra, A. A., (2012). Cosmetic potentials of physic nut (*Jatropha curcas* Linn.) seed oil: A review. *American Journal of Scientific Industrial Research,* 3, 358-366.

Yameogo, C., Bengaly, M., Savadogo, A., Nikiema, P., & Traore, S. (2011). Determination of chemical composition and nutritional values *Moringa oleifera* leaves. *Pakistan Journal on Nutrition, 10*, 264–268.

Yasmeen, A., Basra, S.M.A., Ahmad, R., & Wahid, A. (2012). Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. *Chile Journal of Agriculture Resources*, 2, 92-97.

Yesmin, M. N., Uddin, N. S., Sanzida, M., & Muhammad A. A. (2008). Antioxidant and Antibacterial Activities of *Calotropis procera*. *America*n-*Eurasian Journal of Agriculture and Environmental Science,* 4 (5),550-553.

Zorriehzahra J., & Adel M., (2016). Beneficial Impacts of Thymol Essential Oil on Health and Production of Animals, Fish and Poultry: A review. *Journal Essential Oil Resources*, (4), 228-241.

Appendices

Appendix I

Phytochemical Analysis of Plant Extracts for Active Components

3.8.1 Alkaloids

Approximately 1 cm^3 of 1% HCl was added to 3 cm^3 of the extract in a test tube. The mixture will then be heated for 20 minutes, cooled and filtered. About 2 drops of Mayer's reagent was added to 1 cm^3 of the extract. A creamy precipitate was an indication of the presence of alkaloids.

3.8.2 Tannins

Approximately 1 cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of the extract. A dirty white precipitate shows the presence of tannins.

3.8.3 Flavonoids

About 1 cm^3 of 10% NaOH was added to 3 cm^3 of each extract. Yellow coloration is indication of the presence of flavonoids.

3.8.4 Saponins

Approximately 2mLs of each extract was measured in test tube and 3mLs of distilled water was added and shaken vigorously. Brick red precipitate or frothing persistence shows the presence of saponins

3.8.5 Glycosides

About 0.5g of each extract was dissolved in 2 mL of glacial acetic acid and mixed well. To this, few drops of ferric chloride and concentrated sulphuric acid was added and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

3.8.6 Terpenoids

Approximately 4mg of each extract was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Concentrated solution of sulphuric acid was added slowly and red violet color was observed for the presence of terpenoid.

3.8.7 Steroids

About 4 mg of each extract was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Concentrated solution of sulphuric acid was added slowly and green bluish color was observed for presence of steroids.

3.8.8 Phenols

Approximately 1mL of each plant extract was mixed with 4 drops of ethanol 100 % (v/v) and 3 drops of 1 % ferric chloride solution in test tube. Formation of green or red-brown indicates the presence of phenols.

3.8.9 Carbohydrates

About 0.5 mL of each extract was mixed with 5 mL of Benedict's reagent and boiled for 5 min. Formation of bluish green colour indicates the presence of carbohydrate solution when boiled for few minutes.

Appendix II

3.4.1 Biochemical identification of test isolates

3.4.1.1 Gram staining

A smear of the bacterial isolates was made on a clean grease free slide with the aid of a droplet of sterile water. The slides were passed through flame twice to heat fix them and then allowed to air dry. The slides were placed on a staining rack and flooded with crystal violet (primary stain) and allowed to stand for 60 seconds. Afterwards, the slides were rinsed with water and drained, then flooded with Grams iodine and allowed to stand for 30 seconds. The slides were again rinsed with water after which 95 % alcohol was used to decolourise the primary stain. The alcohol was rinsed with water and the slides were flooded with safranin (a counter stain) and allowed to stand for 60 seconds before rinsing with water. The slides were viewed under a light microscope using oil immersion objective lens.

3.4.1.2 Catalase test

A drop of 3 % hydrogen peroxide was made on clean grease free slides, then, a smear of the 24 h culture of bacterial isolates were made on the slides with the aid of a sterile wire loop and it was observed for bubble formation which indicated the reaction was positive.

3.4.1.3 Citrate utilization test

Sterile Simon citrate medium were kept in slanted position and allowed to solidify. A straight, sterile wire was used to streak the slopes and stabbed the bottom of the slopes with the bacterial isolates and then incubated for 48 hours at 37 ºC. Bright blue

colouration indicated a positive citrate reaction while no change in colour indicated a negative citrate reaction.

3.4.1.4 Coagulase test

A drop of human plasma was placed on clean grease free slides, with the aid of a sterile wire loop, a colony of the bacterial isolates were picked and smeared on the slides. The formation of a clump by the bacterial isolates indicated a positive result for coagulase.

3.4.1.5 Starch hydrolysis

Nutrient agar (4 g) was added to 0.3 g of soluble starch in a conical flask. One hundred milliliter (100 ml) of distilled water was added to the mixture. It was pre-heated and then sterilized by autoclaving at 121 ^{o}C for 15 minutes. The medium was allowed to cool to 40 ^{o}C and aseptically poured into sterile Petri dishes and allowed to solidify. Each test bacterial isolate was inoculated by streaking, while a plate was left un-inoculated (to serve as control) and incubated at 37 ^{o}C for 24 hours. After incubation, the plates were flooded with Gram's iodine and observed for colour change. A clear zone shown around the colonies of the test organism confirmed a positive result, while blue-black colouration with Gram's iodine indicated a negative result.

3.4.1.6 Methyl red test

Sterile peptone broth was dispensed into sterile test tubes and allowed to cool. The test tubes containing the broth medium were inoculated with bacterial isolates and incubated at 37 ^{o}C for 48 hours. At the end of the incubation period, four drops of methyl red indicator were added and gently mixed. Positive test was indicated by bright or brick red colour while those that were negative were indicated by yellow colour.

3.4.1.7 Indole test

Sterile peptone broth was dispensed into sterile test tubes and allowed to cool. The test tubes containing the broth medium were inoculated with bacterial isolates and incubated at 37 ^{o}C for 48 hours. At the end of the incubation period, four drops of Kovac's reagent were added and gently mixed. Positive test was indicated by a brown ring while those that were negative were indicated by absence of the brown ring.

3.4.1.8 Sugar fermentation

Seven point five grams (7.5 g) of Peptone water was prepared and 0.1 g of phenol red was added. The medium was divided into 5 beakers and 1 g of each sugar (glucose, lactose, maltose, sucrose, and fructose) was added to the respective beakers containing the medium. This was then dispensed into test tubes and Durham tubes were introduced in an inverted position into each test tube, they were sealed and autoclaved at 121 °C for 15 minutes. After autoclaving, the media were allowed to cool and bacterial isolates were inoculated into each sugar medium in the test tube and then incubated afterwards at 37 °C for 48 hours. At the end of incubation, the tubes were observed for gas production (via the Durham tubes) and by change in colour from red to yellow indicated positive test while a change from red to any colour different from that of the control indicated a negative test.

3.4.9 Oxidase test

A drop of oxidase reagent was added on a filter paper, colonies from 24 hour cultures were picked and smeared on the spot where the reagent was dropped on the filter paper and examined for 10 seconds. Oxidase positive isolates developed a bluish-purple colouration while oxidase negative isolates remained colourless.

3.4.10 Urease test

One point four grams (1.4 g) of urea agar base was dispensed into a 250 ml conical flask and then autoclaved at 121 °C for 15 minutes and allowed to cool, 40 % of urease salt (4 g in 10 ml of autoclaved water) was also prepared and added to the cooled urea agar base. The resulting mixture was swirled together gently and then dispensed into sterile test tubes and kept in a slanted position until it solidified. Bacterial isolates were inoculated on to the slant at 37 °C for 24 hours and then observed. Positive result showed a colour change from light red or pink while a negative result showed no colour change.