PHYTOCHEMICAL AND MICROBIAL ANALYSIS OF AFRICA BUSH MANGO (*Irvingia gabonensis*) LEAVES, BARK AND STEM EXTRACT AGAINST SELECTED MICROORGANISMS

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DECEMBER, 2021

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BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN SCIENCE LABORATORY TECHNOLOGY

DECEMBER 2021

CERTIFICATION

This is to certify that this project work was carried out by **A'GANIY MASTURAH ARAMIDE 18-06-0228, ADEBISI SHUKURAT OMOTOYOSI 19-06-0030 and UCHE PREVAILLER CHINEDU ND19-06-0027** in the Department of Science Laboratory Technology, School of Science, Abraham Adesanya Polytechnic, Ijebu-Igbo, Ogun State, under my supervision.

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DEDICATION

We whole-heartily dedicate this research work to Almighty God who has kept us alive and has given us wisdom, knowledge and understanding to complete this project. This book is also dedicated to our families for their love, care, moral, and financial support they showered on us.

ACKNOWLEDGEMENTS

Our sincere appreciation to Almighty God who has seen us through the completion of this project, bestowing us with His care, love, wisdom, knowledge and encouragement. We ascribe all glory and honour to His name.

We also say a big thank you to my amiable and honorable supervisor Dr. S. T. Akindele who gave her best support for this wonderful project and has also be of help to us during the course of this research work.

We really appreciate the school management for the foresight in putting this project in place Mrs. B. A. Oluwabiyi (HOD), Mr. O. O. Otuewu and every other lecturer in the Department of Science Laboratory Technology, School of Science, Abraham Adesanya Polytechnic, Ijebu-Igbo, Ogun State.

We will like to thank our colleagues and others who has supported us by correcting our mistakes and everyone close to us for their support.

We acknowledge greatly the staunch technical support provided during my bench work by Mr. Oke Olalekan Olanrewaju, Central Research Laboratory, Ibadan Polytechnic. We shall ever remain grateful for your readiness to impart knowledge, support and a keen interest in our work. Also, the effort and assistance provided by Mr. Opeyemi appreciated.

ABSTRACT

Plants have been known since ancient times and therefore scientists have found them to be a better choice in such for bioactive compounds. The present work was designed to determine the phytochemical constituents and the antimicrobial activity of *Irvingia gabonensis* (leaves, bark and stem) extracts against *S.aureus, E. coli and S. typhi*. The samples was collected from Ago-Iwoye in Ijebu-North local government of Ogun State and solvent extraction was done using maceration with aqueous and ethanol and kept inside refrigerator for further analyses. The phytochemical analysis was carried out and its antimicrobial activity was examined against *Staphylococus aureus, Escherichia coli and Samollena typhi*. The phytochemical screening of ethanolic and aqueous extract of *Irvingia gabonensis* (leaves, bark and stem) shows that all the samples contain Steroids, Saponins, Flavonoids, Phenols, Tannins and Alkaloids at varying proportion. It has been revealed that all the tested plant extracts possesses antimicrobial properties against *S. aureus and E. coli. E. coli* was more susceptible to various extracts; aqueous and ethanolic extracts. *Irvingia gabonensis* (leaves, bark and stem) exhibited the highest antimicrobial activity at a minimum concentration against *S. aureus*. The results provide justification for the use of this plants in folk medicine to treat various infectious diseases.

Keywords: Phytochemical, antimicrobial, extract, maceration and *Staphylococus aureus*.

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List of Abbreviations

ABM.L	African Bush Mango Leaves
ABM.B	African Bush Mango Bark
ABM.S	African Bush Mango Stem
NZ	No Zones of Inhibition
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plants have been known since ancient times and therefore scientists have found them to be a better choice in such for bioactive compounds (Khan, et al., 2011). Plants have been used since time immemorial to treat most of the diseases affecting human kind. The introduction of synthetic drugs, however, changed the trend and attracted many to turn to use them on the expense of botanical drugs; a trend which according to researchers is changing and many people are using medicinal herbs. According Ngule (2013), about 80% of the individuals from developing countries use traditionally known plants as medicine. The world health organization (WHO), recommends medicinal plants to be the best source of a variety of drugs.

Botanical medicine is the oldest known type of medicine. The use of plants as source of medicine is as old as the origin of man himself. Medicinal plants have been used widely over all the cultures as a source of drugs for treatment of various ailments affecting human beings and animals (Sigh & Singh, 2010). The medicinal values of plants are attributed to pharmacologically active compounds that have no direct impact on the plants main processes but research has proved these compounds to have great medicinal values. These compounds that the plant uses to protect itself against predators are called secondary metabolites or phytochemicals.

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction (Hosahally, et al., 2012; Adu, et al., 2011).

Irvingia gabonensis (Africa Bush Mango) is an ethno-medicinal plant that has been used for traditional therapeutic purposes (Ainge & Brown, 2016). Various parts of *I. gabonensis* have found use in the treatment of a variety of ailments, for example, in the treatment of diarrhoea, gastrointestinal, liver conditions, yellow fever, relieve body pains, sterility, hernia, urethral discharge, as an antidote for poisoning and for reduction of breastfeeding period. It has also been found to fight obesity, reduce body fat, lower body cholesterol and control appetite. The seed has been known to reduce blood glucose levels in subjects with obesity while the bark has been reported to have analgesic effects. The leaf and root were documented to have inhibitory properties against microorganisms (Nworie et al., 2016).

1.2 Statement of the Problem

The increase in resistance to many commercially produced synthetic antimicrobial agents by microorganisms has been increasing with time (Ramesh & Okigbo, 2015). There has been a need to increase alternative antimicrobial agents leading interests of evaluating

extracts from plants known to have medicinal value for the manufacturing of herbal antimicrobial agents by Pharmaceuticals Company. This has led to the search of new raw materials that can be used in developing new antimicrobial agents that can combat the increasing resistance by the pathogenic microbes. Extracts obtained from medicinal plants have been used over some time in the manufacturing of herbal antimicrobial agents by pharmaceutical companies. However, over time some of the pathogenic microorganisms have been gaining resistance against some of the commercially produced herbal antimicrobial agents. This has led to the need for improving the antimicrobial capability of both commercially produced herbal and synthetic/conventional antimicrobial agents. Evaluation of the combined effect of herbal extracts with commercially produced antimicrobial agents and formulation of herbal extracts concoctions as a means of developing new and improved antimicrobial agent. Hence the need of searching for new antimicrobial agents Is imperative.

1.3 Justification

The use of herbal plant extracts with medicinal value has increasingly been advocated and incorporated in the production of antimicrobial agents. This is due to the need of increasing the base of already available antimicrobial agents that are commercially produced. Plants with medicinal value have been used to make homemade concoctions that have not been scientifically validated to have antimicrobial activity. Furthermore, different parts of the same medicinal plants have been used in making antimicrobial agents giving contrasting

antimicrobial activity against pathogenic microorganisms. In this study, the phytochemical constituents and antimicrobial activity of extracts from the leaves, bark and stem of *Irvingia gabonensis* was tested for antimicrobial activity against pathogenic microorganisms.

1.4 Aim and Objectives

The main objective of this study is to determine the phytochemical constituents and the antimicrobial activity of *Irvingia gabonensis* (Africa bush mango) leaves, bark and stem, extracts against *S.aureus*, *E. coli* and *S. typhi*.

The specific objectives are to:

a. Determine the phytochemical constituents of *Irvingia gabonensis* (Africa bush mango) leaves, bark and stem extracts.

b. To determine antimicrobial activity of *Irvingia gabonensis*(Africa bush mango) leaves, bark and stem extracts against *S.aureus, E. coli and S. typhi.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal Plants

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anticancer activity, antioxidant activity, hepato protective activity, *haemolytic* activity, *larvicidal* activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction (Hosahally, et al., 2012).

Infectious diseases have been consistently found to be among the leading causes of threat to global health. The World Health Organization (WHO) in 2013 reported that infectious diseases accounted for 61.7% (5.9 million) of the 9.6 million deaths in the sub-Saharan African region. Plants with medicinal value have found application in healthcare from the olden years. Globally, there are evidence-based studies to verify the efficacy of medicinal plants, and some of these shreds of evidence have provided insights into the synthesis of plant-based compounds with therapeutics application (Dhama et al., 2014). The annual global market value of medicinal plant products has exceeded $100 billion (Singh et al., 2013). The "traditional or herbal medicines" are those originating from plant sources and are generally regarded as safe (GRAS) at the concoction dosage, based on their historical usage in various cultures (Uzodimma, 2013). Thus, plants remain the most abundant natural primary source of active drugs and are invaluable in the ethno medical

treatment of diverse ailments (Olasehinde et al., 2012). Medicinal plants are generally sources of various phytochemicals, some of which are usually responsible for their biological activities.

Traditional medicine as defined by the World Health Organization is the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. However, traditional, complementary medicine in Nigeria continues to thrive as it is commonly practised in other African nations as well as in Asia. Application of ethnomedicinal knowledge in the fields of biosciences for investigation of novel bioactive compounds as well as the polypharmacological formulation of plant extracts for use in primary healthcare has been the central interest in research (Adeeyo et al., 2018).

Phytochemical screening of the medicinal plants is usually done against broad spectrum of microorganisms to ascertain their antimicrobial activities, based on the active constituents of the plants that are primarily secondary metabolites. The present occurrence of antimicrobial drug resistance by most bacteria has posed an enormous problem (Kpadonou et al., 2019), and triggered the need for continuous research for better and safe therapeutic agents. Most of the plants that found application in ethnomedicine have been documented based on their promising activities against multiple disease-causing microorganisms (Ogbole et al., 2018). Research efforts are expediting for better functional understanding of medicinal plants, and this has provided a model for about 25–50% of the

marketed drugs (Segun et al., 2019). Antimicrobial activities of useful plants vary; the majority act in synergy (Oladosu et al., 2015), reducing the side effect of synthetic drugs (El-Ghani, 2016), while others act as quorum quenchers (Moussaoui & Alaoui, 2016).

2.1.1 *Irvingia gabonensis*

Irvingia gabonensis is known as African Bush Mango (in English). Its other common names include bread tree, African wild mango, wild mango, and bush mango, and its local names include Apon (in Yoruba, Southwest Nigeria), Ogbono (in Igbo, Southeast Nigeria), and Goron or biri (in Hausa, Northern Nigeria) (Mahunu et al., 2019). *Irvingia gabonensis* is widely cultivated in West African countries including southwest and southeast Nigeria, southern Cameroon, Côte d'Ivoire, Ghana, Togo, and Benin, to produce its edible fruit whose seed is used in the preparation of local delicious viscous soup for swallowing yam and cassava puddings. *Irvingia gabonensis* seeds are also a good source of nutrients including a variety of vitamins and minerals such as sodium, calcium, magnesium, phosphorus, and iron. It is also a rich source of flavonoids (quercetin & kaempferol), ellagic acid, mono-, di-, and tri-O-methyl-ellagic acids, and their glycosides which are potent antioxidants (Sun & Chen, 2012).

Irvingia gabonensis is an ethno-medicinal plant that has been used for traditional therapeutic purposes (Ainge & Brown, 2016). Various parts of *I. gabonensis* have found use in the treatment of a variety of ailments, for example, in the treatment of diarrhoea, gastrointestinal, liver conditions, yellow fever, relieve body pains, sterility, hernia, urethral

discharge, as an antidote for poisoning and for reduction of breastfeeding period. It has also been found to fight obesity, reduce body fat, lower body cholesterol and control appetite. The seed has been known to reduce blood glucose levels in subjects with obesity while the bark has been reported to have analgesic effects. The leaf and root were documented to have inhibitory properties against microorganisms (Nworie et al., 2016).

2.2 Microorganisms

2.2.1 *Escherichia coli*

Escherichia coli are bacterium that is commonly found in the gut of humans and other warm-blooded animals. National Center for Emerging and Zoonotic Infectious Diseases reported that most strains of E. coli are harmless. However, few are known to contaminate food. Symptoms of disease include abdominal cramps, pains, bloody diarrhea, and nausea. Fever and vomiting may also occur. Most individuals recover within two weeks, even though in a few cases the disease may become extremely dangerous (Mustapha, 2013).

The development of resistance by *Escherichia coli* due to increasing in the use of antimicrobial agents has led to the use of medicinal plants extracts against it. Medicinal plant extracts have shown to have antimicrobial activity against enteropathogenic Escherichia coli found in food material (Fullerton et al., 2011). Traditional products used in food preserving (spices) have antimicrobial activity against multiple antibiotic resistant Escherichia coli isolated from water (Rahman et al., 2007). Other studies carried out on

plants with a medicinal value such as Allium sativum has shown antimicrobial activity against Escherichia coli (Ziarlarimi et al., 2011).

2.2.2 *Staphylococcus aureus*

Staphylococcus aureus is Gram-positive bacteria causes a variety of pyogenic (pus-forming) infections and toxinoses (microbial toxins) in humans. *Staphylococcus aureus* causes superficial skin lesions such as pimples or boils and more serious infections such as osteomyelitis and endocarditis (Mustapha, 2013). It is an important community-acquired infections, nosocomial infections of surgical wounds and also, the most common cause of hospital acquired infection such as surgical wounds and *S. aureus* in hospitals are becoming increasingly resistant to antibiotics. Medicinal plant extracts have shown a wide range of antimicrobial activity against both bacterial and fungal pathogens (Manvi et al., 2010).

Studies carried out had shown that some edible plants extracts have antimicrobial and great synergistic activities when used against pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and other bacteria organisms (Das et al., 2012).

2.2.3 *Salmonella typhi*

Salmonella typhi is a Gram-negative bacterial pathogen that causes gastroenteritis in humans. In developing countries, it is mainly associated with causing typhoid fever (Watson & Holden, 2010). Typhoid fever is a major cause of death around the world in a limited setting and globally remains as one of the most infectious diseases (Buckle et al., 2012). The disease is estimated to be responsible for about 26.9 million infections and 269,000 deaths in 2010 (Buckle et al., 2012). Studies carried out have shown that herbal extracts and dietary spices from medicinal plants have antimicrobial activity against *Salmonella typhi* (Shan et al., 2017). Other studies have shown that herbal extracts from medicinal plants not only have antimicrobial activity on *Salmonella typhi* found in vegetables but also against other disease-causing bacteria pathogens such as enteropathogenic Escherichia coli and Listeria monocytogenes (Cutter, 2010).

2.3 Phytochemicals in medicinal plants

Phytochemicals can have complementary and/or overlapping mechanisms of action in the body, including antioxidant effects, modulation of enzyme actions, stimulation of the immune system, modulation of hormone metabolism, anti-bacterial and antiviral effect, interference with DNA replication and physical action whereby some may bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls (Ngoci et al., 2011).

2.3.1 Alkaloids

Alkaloid is a plant-derived compound that is toxic or physiologically active. Some alkaloids such as *isopteropodine, pteropopine* have anti-microbial activity whereby they act by promoting white blood cells to dispose harmful microorganisms and cell debris. Highly aromatic planar quaternary alkaloids like *berberine, piperine* and *harmane* work by intercalating the DNA and cell wall. Others, by simulating neurotransmitters such as acetylcholine, dopamine and serotonin, they affect central nervous system (CNS) at the synapses. They also act as narcotics, as antimalarial, as topical anesthetic for ophthalmology; in treating hypertension, neuralgia, rheumatism, motion sickness, and also in extending the life of hormones (Ngoci, et al., 2011). They have analgesic activity and hence used to alleviate pain incases of boils, septic wounds, and complains such as headaches, abdominal pains and eye conditions. They also have antineoplatic activity, for example, indole alkaloids are used in leukemia and Hodgkin's disease chemotherapy. They act by terminating and depolymerization of protein microtubules that form the mitotic spindle in cell division. This process helps in terminating the tumor cells from separating or dividing and henceforth resulting to reduction of cancer. Nevertheless, some types of alkaloids are hallucinative, addictive, and toxic and hence used as arrow poison for hunting wild game (Ngoci et al., 2011).

Alkaloids are a group of naturally occurring compounds that contain nitrogen and can be neutral or have weakly acidic properties. They may also sometimes contain oxygen, Sulphur, more rarely other elements such as chlorine, bromine, and phosphorus. They are

mainly secondary metabolites of plants but can also be produced by a variety of organisms including bacteria, fungi, and animals (Kittakoop et al., 2014). They dissolve in water poorly but readily dissolve in organic solvents (Shi et al., 2014). They are divided into five major groups namely: true alkaloids (contain nitrogen in heterocyclic and originate from amino acids), proto alkaloids, polyamine alkaloids, peptide and cyclopeptides alkaloids and pseudoalkaloids. They have a wide range of pharmacological activities such as antiasthma, antimalarial, anticancer, cholinomimetic, vasodilatory, antiamyhyrithic, analgesic, antibacterial and antihyperglycemic activities (Cushnie & Lamb, 2014). Some alkaloids have been known to possess psychotropic and stimulant activities and have been used as recreational drugs and entheogenic rituals.

Alkaloids have great antimicrobial activity against bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aureginosa* (Maatalah et al., 2012). Some of the bioactive components of alkaloids such as morphine and cordine have been found to be active not only against bacterial and fungal pathogens but also trypanosomes and plasmodia. Some of the Alkaloids found in dietary food materials have also been found to contain microbiocidal and antidiarrheal effect in the small intestines where they show the ability to intercalate with the microbial genetic material. Other studies carried out on alkaloids extracted from a variety of medicinal plants in Nigeria showed a great antimicrobial activity against both Gram-negative and Gram-negative bacteria and also showed great antifungal activity (Garba & Okeniyi, 2012).

2.3.2 Saponins

These are service active agents with soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells. They have a host of biological roles including boosting respiratory system as expectorant, and hence activity against cough. They also have anti-protozoa activity whereby they act by reacting with cholesterol in the protozoal cell membranes causing cell lysis, e.g. Yucca saponins are effective against protozoan Giardia lamblia. They serve as vaccine boosters by acting as adjuvant. They have anti-inflammatory, emetics, antiviral, antifungal, insecticidal, molluscicidal, piscidal and anti-bacterial activity (Ngoci et al., 2011).

Studies carried out have shown medicinal plant extracts fractions rich in saponins are effective against microorganisms such as *Escherichia coli, Salmonella typhi, Aeromonas hydrophilia* and other fungal pathogens such as Candida albicans (Deshpande et al., 2013). Saponins antimicrobial activity is attributed mainly to its capability of lysing microorganism's membranes rather than the surface tension of the extracellular medium (Asl, 2008). Apart from antimicrobial activity, saponins have shown other biological properties with its cytotoxic activity on cancer or tumor cells being considered the most important one (Yokosuka & Mimaki, 2009). Other plants are known to produce steroidal saponins for example cholestane glycosides which are known to have a broad spectrum of biological activity such as cytotoxic activity, antifungal, antibacterial and in vivo antitumor activities (Li et al., 2012).

2.3.3 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. They have physiological role by acting as antioxidants through free radical scavenging activity, chelation of transition metals, inhibition of prooxidative enzymes and lipid peroxidation (Ngoci et al., 2011), hence modulating oxidative stress and preventing degenerative diseases. They also inhibit tumor growth by inducing apoptosis and inhibiting mutagenecity of carcinogens (Ngoci et al., 2011).

They exhibit anti-microbial activity by complexing nucleophilic proteins by hydrogen bonding, covalent bonding, and nonspecific interactions. The main targets for complexing are cell wall and cell membrane adhesion proteins, hence inactivating microbial adhesion which is the first step in establishment of infections. They also cause cell wall/membrane disruption (Ngoci et al., 2011). This also inactivates microbial enzymes and cell envelope transport proteins by processes that may involve reaction with sulfhydryl groups of proteins. They also accumulate /complexes metal ions (e.g. cobalt, manganese, iron, copper, etc.) necessary for microbial growth as co-factors and activators of enzymes. They also inhibit viral reverse transcriptase (Ngoci et al., 2011).

Toxicity to microorganisms in phenolic compounds depends on the site and the number of hydroxyl groups, with evidence that increased hydroxylation results to increased toxicity (Ngoci et al., 2011). They have endocrine role by interacting with estrogen receptors. They are also anti-inflammatory, molluscicidal and hence important in the control of schistosomiasis. They also have antidiarrhoeal, anti-septic anti-fungal properties,

anti-parasitic, anti-irritant properties and also used in curbing hemorrhage, in wound healing, and improving vascular health by suppressing peptides that harden arteries (Ngoci et al., 2011). Also, they have economic role of tanning leathers in leather industry. Nevertheless they affect intake and digestibility of feeds among livestock, and excess can be carcinogenic on normal tissues (Ngoci et al., 2011).

Antimicrobial activity of tannins has been tested in various fields of medicine providing positive results such as antioxidant activities, anticarcinogenic activities and antimutagenic properties. Tannins have been used in inhibiting the growth of many fungi, yeasts, bacteria and viruses. Studies carried out have shown that tannins have antibacterial activity (Akiyama et al., 2011). Some of the bioactive compounds of tannins such as catechin and pyrogallol found in vegetable tannins have been found to be toxic to microorganisms. Tannins have been found not only effective against pathogenic microbes but also have a significant value as a cytotoxic and an antitumor agent (Joshi et al., 2013).

2.3.4 Flavonoids

Flavonoids or bioflavonoids are secondary metabolites of plants that chemically have a general structure of 15 carbon skeleton consisting of two phenyl rings and a heterocyclic ring. There are over 500 groups of flavonoids that have been characterized from various plants according to their chemical structure (Ververidis et al., 2017). They are usually subdivided into anthoxanthins, flavanones, flavanols, flavans, and anthocyanidin (Zhao et al., 2012).

In plants they are responsible for floral pigmentation, ultraviolet ray's filtration in higher plants and symbiotic nitrogen fixation. They are also known to have inhibitory activities against organisms that cause plant diseases for example Fusarium oxysporum. Flavonoids have been known to possess antimicrobial activity against bacterial, fungal and viral microorganisms. They are usually known for their antimicrobial activity of inhibiting the synthesis of the nucleic acids, tampering with the integrity of the cytoplasmic membrane function and the energy metabolism process (Cushnie & Lamb, 2005).

Flavonoids from some medicinal plants have been found to inhibit the synthesis of the nucleic acids, cause permeability of the inner bacterial membrane and a dissipation of the membrane potential of Gram negative and Gram positive bacteria (Cushnie & Lamb, 2005). Some of the bioactive components that have been isolated from flavonoids have been found to contain antifungal, antibacterial and insecticidal activities (Abdel et al., 2013). Previous studies carried out have shown that when mixed with antibiotics they have synergistic activity and suppress many pathogenic microorganisms in numerous in vitro and in vivo studies (Cushnie & Lamb, 2011; Manner et al., 2013). Additional in vivo studies have shown that flavonoids can be used as pharmaceutical drugs for bacterial infections or through the dietary intake to offer protection against infection (Zamora et al., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION AND LOCATION

Irvingia gabonensis (Africa bush mango) leaves, bark and stem was collected from Ago-Iwoye in Ijebu-north local government of Ogun State. Isolate used (*Staphylococus aureus, Escherichia coli and Samollena typhi*) were obtained from Hegada scientific service limted, Ibadan Oyo state.

3.2 Preparation of Aqueous and Ethanolic Extract

The samples were soaked in each solvents (Aqueous and Ethanol) for 72 hours and it was filtered with whatman filter paper and the filtrates were collected and kept inside refrigerator for further analysis.

3.3 Phytochemical Screening

3.3.1 Qualitative Analysis

The ethanolic and aqueous extracts were subjected to phytochemical screening namely, alkaloids, tanins, phenols, flavonoids, saponins, terpenoids, triterpenes, phytosterols, glycosides, steroids, glycosides, Phlobatannin and carbohydrates according to the method of Evans WC & Trease, 1994

3.3.2 Test for alkaloids: 300 ml extract was digested with 2 molar HCl. The acidic filtrate was mixed-with amyl alcohol at room temperature and the alcoholic layer was examined. Pink color indicated the presence of alkaloids.

3.3.3 Test for tannins: 5ml of extract was added to few drops of 1 % lead acetate. A yellow precipitate indicate the presence of tannins.

3.3.4 Ferric chloride test for phenolic compounds and tannins: About 2.0 ml of each extract was measured in a test tube and 0.01 moldm-3 Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated presence phenolic compounds and tannins.

3.3.5 Lead acetate test for flavonoids: To 1ml each of the extracts were dissolved in ethanol and few drops of 10% lead acetate solution were added. Appearance of yellow precipitate indicates the presence of flavonoids.

3.3.6 Foam test for saponins: Small amount (1ml) of the extract were taken in test tubes with little quantity (1.0ml) of water and shaken vigorously. Appearance of foam persisting for 10 mins indicated presence of saponin.

3.3.7 Test for terpenoids (Salkowski test): 5 ml of each extract was mixed in 2 ml. of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a monolayer of reddish brown coloration of the interface that show positive results for the presence of terpenoids.

3.3.8 Test for triterpenes: 300ml extract mixed with 5ml chloroform and warmed for 30 minutes. To chloroform solution small volume of concentrated sulfuric acid was added and mixed properly. The appearance of red color indicated the presence of triterpenes.

3.3.9 Salkowski reaction test for phytosterols: To 0.55ml each of the extracts in a testube was was added 1.0ml of cocentrsted H_2SO_4 (conc.) from the sides of the test tube and then 10ml chloroform. Appearance of reddish brown colour in chloroform layer incates the presence of phytosterols.

3.3.10 Test for cardiac glycosides: 5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1m' of conc. H_2SO_4. A brown ring of the interface indicated a deoxy sugar, characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

3.3.11 Test for steroids: 0.2ml of acetic anhydride was added to 5ml of ethanolic extract of each sample with 2ml of H_2SO4. The color change from violet to blue or green indicated the presence of steroids.

3.3.12 Keller-Killiani test for glycosides: About 1 ml of glacial acetic acid, few drops of 0.01 moldm-3 Ferric chloride solution and H_2SO4 (conc.) slowly through the sides of the test tubes were added to the extracts. Appearance of reddish brown ring at the junction the liquids indicated the presence of de-oxysugars.

3.3.14 Test for Phlobatannin: Sample was boiled with 1% aqueous HCl to produce red precipitate indicating the presence of phlobatannin (Harbone, 1998).

3.3.15 Molisch's test for carbohydrates: About 5ml each of the extracts was mixed with Molisch reagent and then added H_2SO4 conc. along the sides of the test tubes to form layers. Appearance of reddish violet ring on the interference indicated the presence of carbohydrates.

3.4 Quantitative Analysis

The phytochemical analysis **(Quantitative)** of ethanolic and aqueous extract was determined in accordance with the method described by (Singleton and Rossi, 1965)

3.4.1 Determination of Oxalate

1ml of each extract was measured. 75ml of 1.5N H_2SO_4 was added to the sample inside the 100ml conical flask, it was stirred for 1 hour using a magnetic stirrer, it was filtered with Whatman filter paper and 25ml of the extract was pipetted into another conical flask, 25ml of the extract when hot was titered against 0.1N $KMnO_4$ solution to a faint pink coloured point. Titre value was recorded Oxalate = (Titre value x 0.9004) mg/g according to the method of Harbone, (1973).

3.4.2 Determination of Phytate

1ml of each extract was measured into a conical flask. 50ml of 2% HCl was added and soak for 3hrs and it was filtered through a Whatman filter paper. 25ml of the extract was pipetted and was filtred into a conical flask. 5ml of 0.3% Ammonium Thiocyanate solution were added and 53.5ml of distilled H_20. 0.005M standard ferric chloride ($Fecl_3$) solution was titrated until a reddish brown persists for 5 minutes. The titre value was recorded.

3.4.3 Determination of Alkaloid

1ml of each extract was measured into a flask. 40ml of 10% acetic acid in ethanol (10% acetic acid in ethanol is 10ml of acetic acid into 90ml of ethanol) was added and was allowed to let it stand for 4hrs at 28°c. It was filtered and the filtrate was heated in a hot plate to one-quarter of its original volume by evaporation. The remaining concentrate was

treated with drop wise addition of 5ml Conc. Ammonium hydroxide (until all the alkaloid was precipitated).A dried filter paper was weighed and was used the filter the precipitate through the pre-weighed filter paper. The filter paper while still inside the funnel was washed with 2% ammonia solution which was dried in the oven at 55°c and allowed to cool and the dried filter paper was weighed.

3.4.4 Determination of Flavonoid

1ml of each extract was measured into a conical flask. 50ml of 2M HCl was added and was allowed to boil for 30 minutes. It was cooled and filtered. 5ml of the extract or filtrate was pipetted into another flask and 5ml of ethyl acetate was added starting with a drop to obtain a precipitate. A dried filter paper was weighed and was used to filter the precipitate through the pre-weighed paper which was dried in the oven at 55oc and was allowed to cool. The dried filter paper was weighed. Harborne, (1973)

3.4.5 Determination of Saponins

1ml of the extract was measured into a flask. Addition of 30ml of n-hexane was De-fatted in which 30ml of methanol was added to the residue filter. 30ml of methanol was added to the residue filter another addiction of 30ml of methanol to the residue filter for the third time and the concentrate was heated or filtrate to one-quarter (1/3). 100ml of cold acetone was added and put in the fridge for 50mins while a dried filter paper was weighed

and dried in the oven at 55^0C was allowed to cool and the filter paper was re-weighed after drying.

3.4.6 Determination of Tannin

3.4.6.1 Tannin standard stock solution (100ppm)

0.1g of tannic acid salt was dissolved in 1000ml vol flask and made up to mark with distilled H_20 0.1g of tannic acid = 100ml (1 Litre) = 100ppm.

3.4.6.2 Tannin working standard (10ppm)

10ml from the stock (100ppm) was pipetted into 100ml vol flask and made up to mark. From this 10ppm, prepared 0ppm, 0.5ppm, 1.0ppm, 1.5ppm, 2.0ppm & 2.5ppm into 50ml volumetric flask pipetting 0ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml & 2.5ml respectively into 50ml vol flask. 0.2ml of extract was measured and 10ml of 70% acetone was added. It was place in an ice bath for 10mins and was shaked with oscillator for 15mins which was allowed to cool for 30mins and was filtered into a conical flask. 0.5ml of the filtrate was pipetted into 50ml volumetric flask with 0.5ml of distilled H_20 to sample and 1ml distilled water into another flask as blank. 0.5ml folin–Dennis reagent was added to sample and standard. 2.5ml of 20% sodium carbonate solution were added to sample standard and mixed properly and made up to mark with distilled H_2O. It was allowed to stand for 40minutes for a bluish–green colour to develop. The absorbance at 725nm (Singleton & possi, 1965).

3.4.7 Determination of Total Phenolics

0.2ml of extract was measured into a conical flask. Extracted samples (10ml 50%methanol was added) and heated in water bath at 80°C for 30 mins and was allowed to cool. Contents were transferred to centrifuge tubes and whiz for 5 mins at top speed. 0.2ml of sample + 2.8 ml distilled water + 0.25 ml Folin-Ciocalteau reagent + 1 ml Sodium Carbonate was pipetted and was allowed to sit for 15 mins and read in a spectrophotometer at 760nm. (Singleton & Rossi, 1965).

3.4.8 Cyanogenic Glycosides (Cyanide Determination Method) Alkaline Picrate Method (Wang and Filled) Extraction of Sample

This extraction was according to Wang and filled method. About 5ml of extract was dissolved in 50ml distilled water in a conical flask and the cyanide extraction was allowed to stay overnight. The extraction was filtered and the filtrate was used for the analysis.

3.4.9 Preparation of Alkaline Pictrate Solution

1g of alkaline pictrate was weighed and 5g of sodium carbonate with little quantity of warm distilled water. The mixture was transferred into 200ml volumetric flask and made up to mark with distilled water.

3.4.9.1 Standard Preparation

0.25g KCN salt was weighed and dissolved in 100ml volumetric flask as stock cyanide standard solution (1000ppm)

3.4.9.2 Working Standard

10ml was pipetted into another 100ml flask and made up to mark (100ppm). 100ppm was pipetted from 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml into a 50ml flask and a blank of on standard solution was prepared. The above gave 1ppm, 2ppm, 3ppm, 4ppm, 5ppm and 0ppm (blank).

4ml alkaline pictrate solution was added, prepared above and made up to mark with distilled water. A reddish brown colour was developed; it was incubated in a water bath at 60°C for 5mins and was read spectrophotometrically at 490nm.

3.4.10 CYANIDE DETERMINATION

1ml of the extracted sample was prepared in a corked 50ml flask and 4ml alkaline picrate solution was added to made up to mark if needed (i.e. if the reddish brown colour was obtained) and incubated in a water bath at 60°C for 5mins. The absorbance was read spectrophotometrically at 490nm. The blank containing 1ml distilled water and 4ml alkaline picrate solution was read and was made up to 50ml if sample was treated the same way.

3.5 Anti-microbial Sensitivity Testing

3.5.1 Agar Well Diffusion Method

The anti-microbial sensitivity screening of ethanolic and aqueous extract was determined in accordance with agar-well diffusion method described by (Irobi et al., 1996; Russell and Fur, 1997) with little modification. The bacterial and fungi isolates were subculture into nutrient broth and potatoes dextrose broth for 18-24hrs. The 18hour-old culture organisms were standardized using McFarland standard (106 cfu/mL of 0.5 McFarland standard). One hundred microliters of each of the standardized organisms suspension was evenly spread on Mueller-Hinton agar medium using a sterile glass spreader. Sterile cork borer was used to bore holes into the agar medium allowing about 5 mm distance to the edge of the plate. The cultured plates were treated with the aqueous and ethanolic extract used. The plates were allowed to stand on the laboratory bench for one hour to allow for proper diffusion of the extracts into the medium. The plates were then incubated at 37°C for 24 hours after the plates were observed for zones of inhibition.

CHAPTER FOUR

RESULTS AND DICUSSIONS

4.1 RESULTS

Table 1 showed the phytochemicals results of *Irvingia gabonensis* (Africa bush mango) leaves, *Irvingia gabonensis* (African bush mango) bark and *Irvingia gabonensis* (Africa bush mango) stem extracts reveals. The presence of phlobatannin, glycoside, titerpenoids, steroids, phytosteroids, triterpenes, terpenoids, saponins, flavonoid, phenol, tannins, alkaloids, phytate and CHO, with the absence of oxalate in both aqueous extract and ethanolic extract. Phlobatannin, Glycoside, titerpenoids, steroids, phytosteroids, triterpenes, terpenoids, saponins, flavonoid, phenols, tannin, Alkaloids, phytate and CHO are the active compounds presence in Aqueous and ethanolic extracts with absence of triterpenes and oxalate in both Aqueous Extracts and ethanol extracts of *Irvingia gabonensis* (African bush mango) bark extracts. The aqueous extracts confirm the presence titerpenoids, steroids, phytosteroids, terpenoids, saponins, flavonoid, phenol, tannins, phytate, CHO while ethanolic extracts confirm the presence of titerpenoids steroids, phytosteroids, triterpenes, saponins, tannins and phytate and the absence of phlobatannin, glycoside, triterpenes, terpenoids in ethanolic extracts for *Irvingia gabonensis* (Africa bush mango) stem extract.

The phytochemical screening of chemical constituent result of *Irvingia gabonensis* (Africa bush mango) leaves, *Irvingia gabonensis* (African bush mango) bark and *Irvingia gabonensis* (Africa bush mango) stem extract in Table 2. Phytate is confirmed the highest

active compound in aqueous extract and flavonoids is confirmed the highest active compound in ethanolic extract of *Irvingia gabonensis* (Africa bush mango) leaves, in the Aqueous extract of *Irvingia gabonensis* (Africa bush mango bark) extract confirm the presence of phytate as the highest active compound while in the ethanolic extract of *Irvingia gabonensis* (Africa bush mango) bark extract confirm the presence of flavonoids as the highest active compound, Flavonoids is the highest active compound in Aqueous extract but phytate is the highest active compound in ethanolic extract.

The order of zone of inhibition for aqueous extract against *S. aureus* of ABM.B and ABM.S were found to be 6.00, 6.00. ABM.B has the maximum zone of inhibition while ABM.S has no zone of inhibition. ABM.B has the highest zone of inhibition which is 6.00.The extract of ABM.B and ABM.S has no zone of inhibition,

There is no zone of inhibition for ethanolic extract of ABM.S against *S. aureus*. ABM.B and ABM.S has no zone of inhibition, ABM.S has the lowest zone of inhibition. ABM.S has no zone of inhibition.

Table 1: Phytochemical constituents of *Irvingia gabonensis* (Africa bush mango) leaves, bark and stem

Parameters	Leaves (Aqueous Extract)	Bark (Aqueous Extract)	Stem (Aqueous Extract)	Leaves (Ethanolic Extract)	Bark (Ethanolic Extract)	Stem (Ethanolic Extract)
Phlobatannin	+	+	-	+	+	-
Glycosides	++	+	-	++	+	-
Titerpenoids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Phytosteroids	++	+	+	++	+	+
Triterpenes	++	+	-	++	-	+
Terpenoids	++	+	+	++	+	-
Saponin	+	++	+	+	+	+
Flavonoid	++	+	+	++	+	-
Phenol	++	++	+	++	++	-
Tannin	++	+	+	++	+	+
Alkaloids	+	+	-	+	+	-
Oxalate	-	-	-	-	-	-
Phytate	++	+	+	+	+	+
Carbohydrate	++	++	+	+	+	-

Key: (+++) strongly positive, (++)positive, (+)trace, (-)not detected

Table 2: Phytochemical constituents of *Irvingia gabonensis* (Africa bush mango) leaves, bark and stem

Chemical compound	Leaves (Aqueous Extract)%	Bark (Aqueous Extract)%	Stem (Aqueous Extract)%	Leaves (Ethanolic Extract)%	Bark (Ethanolic Extract)%	Stem (Ethanolic Extract)%
Saponin	0.890	1.420	0.685	0.730	1.000	0.850
Flavonoid	2.945	2.615	6.620	7.430	4.535	-
Alkaloid	4.005	1.620	-	2.475	2.245	-
Phenol	0.377	0.158	0.037	0.424	0.053	-
Tannin	0.082	0.027	0.009	0.058	0.023	0.004
Glycosides	1.389	0.242	-	0.693	0.242	-
Oxalate	0.045	0.045	0.090	0.090	0.045	0.045
Phytate	4.944	3.298	3.502	4.738	3.420	3.583

Table 3: Zone of inhibition of Aqueous plant extract

Extracts / Isolate	ABM.L	ABM.B	ABM.S
S. aureus	5.00	6.00	NZ
E. coli	2.00	6.00	NZ
S. typhi	2.00	NZ	NZ

KEY: ABM.L= African Bush Mango Leaf, ABM.B=African Bush Mango Bark, ABM.S=African Bush Mango Stem, NZ= No Zones of Inhibition.

Table 8: Zone of inhibition of Ethanolic plant extract

Extracts / Isolate	ABM.L	ABM.B	ABM.S
S. aureus	9.00	11.00	NZ
E. coli	6.00	NZ	NZ
S. typhi	7.00	NZ	1.00

KEY: ABM.L= African Bush Mango Leaf, ABM.B=African Bush Mango Bark, ABM.S=African Bush Mango Stem, NZ= No Zones of Inhibition.

4.2 DISCUSSION

The results of the preliminary phytochemical screening of the leaves, bark and stem of *Irvingia gabonensis* (African bush mango) is positive in aqueous and ethanolic extract .In the Bark of *Irvingia gabonensis* (Africa bush mango), It has more traces in aqueous extract, while in stem, It has more traces in aqueous extract, Than ethanolic extract comparable to result obtained by Abulude, F.O. et al., (2009), Whose results revealed that ethanol has been shown to be a stronger extract than water. Phytate increase the bioavailability of flavones and it is able to inhibit the replication of HIV cell lines and Flavonoids are important for human health because of their antioxidant, antibacterial, antiviral, and anti-inflammatory activities, Flavonoids as the highest active compound function as signal molecules, and antimicrobial defensive compounds. (Panche, 2016).

It has been revealed that all the tested plant extracts possesses antimicrobial properties. *S. aureus* and *E. coli* (gram positive) was more susceptible to various extracts; aqueous and ethanolic extracts and thus higher size of diameter of inhibition zones. It was previously reported that plant extracts displayed stronger antimicrobial effect on gram positive strains than on their gram negative counterparts (Okigbo & Omodamiro, 2008; Chekole et al., 2016). *S. typhi* was not inhibited by aqueous as shown in Table 7, but was inhibited by ethanolic extracts as shown in Table 8. This is supported by previous studies reported by Adetunji et al. (2013); Adetutu et al, (2011), and Moreno et al. (2006) which showed that the ethanolic and acetone extracts possesses more antimicrobial activity than aqueous extract. The extracts obtained from aqueous in this study displayed weak or no

antimicrobial activity in ABM. B against S. typhi and ABM.S against *S. aureus, E. coli* and *S. typhi* and displayed stronger antimicrobial activity in ABM.L against the three isolates (*S. aureus, E. coli, S. typhi*). The extracts obtained from ethanol in this study displayed weak antimicrobial activity in ABM.B against *E. coli* and *S. typhi* and ABM.S against *S. aureus* and *E. coli*. Water is used as a solvent in traditional medicinal probable because it is more convenient to prepare in household environment using decoction. Eloff (1998) reported that due to the ability of water to extract nonpolar compounds, the water preparation is usually not suitable for antimicrobial discovery. Another reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents (Boer et al., 2005; Doughari et al., 2007). The presence of alkaloids, steroids, glycosides, flavonoids, tannins, saponins, phlobatannins, triterpenoids, phytosteroids, triterpenes, terpenoids, oxalate, phytate, CHO and phenolic in the extracts of the plant may explain the reason for its antimicrobial actions since the antimicrobial properties of most of the phytochemical have previously been documented.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Irvingia gabonensis (leaves, bark and stem) extract exhibited the highest antimicrobial activity at a minimum concentration against *S. aureus*. The results provide justification for the use of this plant in folk medicine to treat various infectious diseases.

5.2 RECOMMENDATION

Since these plants sample possessed bioactive compound that provide justification for its use in folk medicine, Further work should be carried out in order to isolate the active compounds for further antimicrobial, pharmacological and clinical testing.

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