ANTIMICROBIAL ASSESSMENT of *Icacina trichantha* Oliv Leaf Extract

BY

ALUKO, JOY BABASEWA

17010101016

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DECLARATION

I hereby declare that I wrote this project and that it is the result of my own research. It has never been submitted in any previous application for a higher degree at this or any other university. All citations and information sources in this project are clearly acknowledged with references.

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ALUKO, JOY BABASEWA   Date

CERTIFICATION

This is to certify that the content of this project entitled **ANTIMICROBIAL ASSESSMENT OF** *Icacina tuber extract,* was prepared and submitted by Aluko, Joy Babasewa with matriculation number 17010101016, in partial fulfilment of the requirements for the degree of Bachelor of Science in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria**.** The original research work was carried out by her under my supervision and is hereby accepted.

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(Signature and Date)

Dr. F.H. Ibadin

 Supervisor

_____

(Signature and Date)

Dr. O.E. Fayemi

Head of Department

DEDICATION

This project is dedicated to God, the giver of wisdom, knowledge and understanding. Also to my father, Late Pastor Josiah Aluko, who I am very sure is smiling on me now and my mother Mrs. Stella Aluko, whose undying love and strong support has brought me this far.

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ABSTRACT

Antimicrobial resistance is a major cause of the health and economic burden of inappropriate or excessive use of antimicrobials. Antimicrobials are antibiotics used to treat and prevent infections in humans, animals, and plants. The fight against drug resistance (MDR) has cost the world economy the most. Antibiotics, the mainstay and wonder drug of the 20th century, played a crucial role in the treatment of infectious diseases. Antimicrobial resistance is caused by improper, infrequent and irrational use of antibiotics. With 30-50 percent of existing drugs derived from plants, this has led to renewed interest in medicinal plants. The search for new and effective antibacterial compounds from natural sources, such as plants, has become an important part of overcoming this medical problem. *I. trichantha* has been shown to have analgesic, anti-inflammatory and anti-diabetic properties (Asuzu and Abubakar 1995; Asuzu 1999; Eshowbo 2010). However, no information was found about its antibacterial properties. Therefore, the aim of this study was to determine whether the methanolic extract of *Icacina trichantha Oliv.* The leaves have antibacterial properties. Fresh leaves of *I. trichantha* were collected from the University of Ibadan, Oyo State, Nigeria, washed, dried and extracted by maceration with distilled water and analytical methanol. Agar well diffusion was used to test the antimicrobial activity of the extracts. Test organisms *Salmonella* (SH1351) and *Escherichia coli* (SH70E1) were obtained from the Department of Biotechnology, Mountain Top University, Ibafo, Ogun State, Nigeria. However, the plant extract was ineffective against both test microbes.

Keywords: antibiotic resistance, antibiotics, *Icacina trichanta,* medicinal plant

CHAPTER 1

INTRODUCTION

1.1   Background to the study

Plants had been utilized in indigenous groups for over 5 millennia to deal with infections and illnesses. Nigeria has a wealthy medicinal plant heritage, and several research had been carried out to evaluate those plant life antimicrobial activities. Traditional medicinal techniques, including Chinese Traditional Medicine, Unani Medicine, Ayurvedic Medicine, and others, hire a extensive array of plant life which have been used for over 3000 years. There are as a minimum 121 regarded chemical substances which are nonetheless extracted from plant life and used everywhere in the world (Anon, 1982a). Medicinal plant life, mainly the ones used by conventional healers, produce pharmaceutical energetic chemical substances which have antibacterial, anti-helminthic, anti-fungal, antiviral, anti-inflammatory, anti-oxidant, and anti-diabetic properties (Rabah 2007; Ansari and Sitaram, 2011; Gupta et al.,2007; Karim et al.,2011). The look for new anti-contamination retailers has been studied within the subject of ethno-pharmacology for numerous decades (Reco et al. 1989a), and it compiled a listing of seventy five species for which the authors had set up the properties of the extract, in addition to the spectrum of and standards in the back of this properties, Rios et al. (1988) posted a evaluation of the experimental techniques used to observe the properties of each plant extracts and vital oils, recommending the diffusion approach for figuring out relative efficiency and setting up antimicrobial spectrum as it lets in exceptional lines for use in opposition to the extract at the identical plate. Antimicrobial resistance is certainly considered one among humanity's maximum urgent troubles today, and the fast unfold of drug-resistant microorganism is wreaking havoc everywhere in the world. Antimicrobials had been misused to the factor wherein they may be basically useless, with a number of those tablets associated with proof of risky facet outcomes in a few people, setting the fitness and monetary burden of antimicrobial resistance on a few people. Antibacterial hobby of medicinal plant life have stimulated a surge of interest (Ngwendson 2003). Antimicrobial resistance is split into categories: mobile stage resistance (CELR) and network stage resistance (CLR) (COLR). CELR happens whilst a set of organisms develops resistance to environmental strain thru endogenous gene mutation or horizontal gene switch of resistance determinants from different microorganisms, while COLR happens whilst a set of organisms develops resistance to environmental strain thru endogenous gene mutation or horizontal gene switch of resistance determinants from different microorganisms. (Cheng et al, 2016). In

healing plant life, antimicrobial resistance may be found. These studies may be prepared into a systematic paper to resource within the improvement of novel antimicrobial tablets. Many natural drugs also are used without enough protection precautions, and they may be ingested with the perception that they may be harmless, regardless of the reality that they may be incredibly toxic. *Icacina* tuber is a West African meals and medicinal plant endemic to Nigeria. The Igbos of Nigeria seek advice from it as "Urumbia" or "Eriagbo," while the Yoruba of western Nigeria seek advice from it as "Gbege" (to purify). In animal models, it shows an extensive spectrum of pharmacological actions. The antibacterial assessment of *Icacina* tuber is mentioned on this work.

1.2   Statement of the Problem

The emergence and spread of drug-resistant organisms that have created new resistance mechanisms known as antimicrobial resistance pose a threat to the availability of effective treatments for prevalent diseases. Although their antimicrobial resistance is not thoroughly or openly explored, traditional medicines are routinely employed instead of contemporary antibiotics since they are so effective.

1.3   Aim and Objectives of the Study

The aim of this research is to determine the antimicrobial activity of *Icacina trichantha*

The objective of the study is to;

- Ascertain the antibacterial capacity of *Icacina trichantha*
- Determine *Icacina trichantha's* root's antibacterial activity
- Use medicinal plants as antimicrobials by reducing antibacterial resistance to a minimum.

1.4   Significance of the Study

This project work was done to assess the antimicrobial activity present in root extracts of *Icacina trichantha*

CHAPTER 2
Literature Review

2.1   Medicinal Plants

Medicinal plants have medicinal properties or have favorable pharmacological effects on the human or animal body (Ram Singh, 2018). A medicinal plant is any plant that contains chemical substances in one or more of its parts that can be used for medicinal purposes or as precursors for the production of effective pharmaceutical drugs. In traditional African medicine and various forms of treatment around the world, medicinal plants have been used to treat a wide variety of ailments. Despite the availability of modern treatments in some places, herbal medicines continue to be popular for historical and cultural reasons, as well as their effectiveness and lower cost. When administered to humans, most potent herbal medicines have little or no toxic effects, while others are toxic to humans and animals and have the potential to harm specific organs. in the body. Medicinal plants can also be defined as (Sofowora 2008; Evans 2008):

a.   Plants or plants parts used medicinally in galenical preparations (e.g. decoctions, infusions, etc.) e.g. Cascara bark.

b. Foods, spices and perfumes used in medicine, e.g. Ginger.

c. Yarn mills, e.g. Cotton, jute, flax used to prepare wound dressings.

d. Microscopic plants, such as fungi and actinomycetes, are used to isolate drugs, especially antibiotics. *Streptomyces griseus* and ergot (*Claviceps purpurea* growing on rye) are two examples.

e. Plants are used to extract purified chemicals for medicinal use, directly or indirectly by the synthesis of hemi-healing compounds (e.g., synthesis of sex hormone hemi from diosgenin obtained from diosgenin) from sweet potato Dioscorea).

Ethnobotany is a branch of botany that studies, interprets, and documents the traditional uses of plants in different cultures. Herbs that can be used to heal or improve health include lily of the Valley, wild calla, passion flower, horseradish, and others. Medicinal plants are used for a variety of purposes, some of which have medicinal value (India's National Health Portal);

a) Plants such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, blackberry, and safflower are used to heal wounds, sores and boils.

b) To reduce fever and thermogenesis due to illness. (Black Pepper, Sandalwood, Safflower)

c) Turmeric to inhibit the growth of harmful germs, germs and bacteria.

d) Fingers and cloves are used in some cough syrups.

Medicinal plants have become an essential component of health systems around the world. This includes not only the use of medicinal plants for the treatment of diseases, but also as a potential source of raw materials for maintaining excellent health and living conditions. Herbal medicine is used for health care in many countries around the world, two thirds of the world's population depends on it. This is due to increased cultural tolerance, compatibility and adaptation to the human body, and fewer harmful effects. Some of the drugs believed to be plant-based include aspirin, atropine, artimesinine, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine (Sandberg and Corrigan). 2001).

Pathogenic bacteria and fungal infections pose a major risk to human health (Eswarappa 2009).

Infectious diseases continue to be a leading source of morbidity and mortality worldwide, despite widespread use of medicines and immunization programs (Bloom 2000). Antibiotic resistance has driven a search for new antimicrobial agents, particularly those derived from medicinal plants, as well as the appearance of unwanted side effects from certain drugs (Idu et al. 2007). Medicinal plants, according to the literature, are the cornerstone of traditional medicine. Indigenous plants serve as metabolite reservoirs, storing an infinite supply of vital chemicals with distinct biological properties (Tomoko et al. 2002). In affluent countries, more than a quarter of all recommended medicines are derived directly from plants (Newman et al. 2000). Antibacterial chemicals can be found in medicinal herbs in large quantities (Kubmarawa et al. 2007). Because of their perceived effectiveness, little adverse effects in clinical practice, and low cost, herbal drugs are routinely provided despite the fact that their physiologically active constituents are unknown (Eliza et al. 2009).

2.1.2 Phytochemical Constituents of Medicinal Plants

Herbs have medical potential because they contain chemical components that operate on the human body in a certain physiological way. Alkaloids, tannins, flavonoids, and phenolic

compounds are some of the most significant plant bio-active substances (Hill, 1952). These plants are sometimes added to food for pregnant women and nursing mothers for medicinal reasons as well as being used as spices and food plants (Okwu, 1999, 2001). Medicinal plants contain phytochemicals, which are bio-active organic chemical components present in cereals, vegetables, fruits, and other plant products that act as a defense against major chronic diseases in both the host metabolic or genetic malfunctioning disease and infectious disease (Esposito et al., 2016).

Phytochemical screening of medicinal plants is a typical method for detecting antimicrobial activity against a wide range of microbes, and it is based on the active elements of the plants, which are mostly secondary metabolites. Secondary metabolites are complex molecules with unknown roles that are widely used in human medicine, veterinary medicine, agriculture, scientific research, and a number of other sectors (Trease and Evans, 1978). Phytochemicals are classified into two categories: primary and secondary constituents, based on their activities in plant metabolism. Medicinal plants include a variety of bio-active substances such as saponins, tannins, and alkaloids, according to a chemical composition screening. (Ezike *et al*., 2016). Primary constituents include carbohydrates, amino acids, proteins, and chlorophyll, with secondary elements such as alkaloids, terpenoids, and phenolic compounds, as well as flavonoids, tannins, and other substances. Plant chemical constituents should be studied since they will be important in the production of complex chemical compounds (Mojab et al, 2003).

2.1.3   Characteristics of Medicinal Plants

In West Africa, particularly in Nigeria, medicinal plants have demonstrated distinct characteristics in the field of herbal therapy. In Nigeria, there are approximately 1000 medicinal plants, the majority of which have not yet been studied for their medicinal properties. Their medical activities could be critical in the treatment of current or future health issues. Some medicinal plants, known as synergic medicinal plants, can complement, harm, or neutralize their potential negative effects in the body; some are used in the treatment of complex cases, such as cancer diseases, and are referred to as official medicinal plants; others have the ability to prevent the emergence of certain diseases by reducing the side effects of synthetic treatment and are referred to as preventive herbal medicinal plants.

2.1.4 Mechanism of Action of Medicinal Plant

The plant kingdom is expected to include about 500,000 species, of which only a small percentage have been studied for their antibacterial activity (Mickymaray et al.). Because of their safety and usefulness, traditional herbal medicines can be cultivated by humans for generations without the need for today`s rigorous standards and testing. As a result, bio-active molecules derived from these medicinal plants appear to have a better chance of passing toxicity tests than molecules synthesized from scratch. Because traditional herbal medicines contain potential antimicrobial components beneficial to the formulation of medicinal substances for the treatment of diseases, an increased focus on traditional ethno-pharmaceuticals may lead to the discovery of new therapeutic agents. Today, research is increasingly focused on traditional medicine, with the aim of developing better pharmaceuticals for the treatment of diabetes, cancer and microbial infections (Subkalingam et al.) Medicinal plant extracts and their active components have been tested against bacteria, fungi, algae and viruses in various locations around the world (Casciaro et al.). Flavonoids, alkaloids, tannins and terpenoids are phytochemicals found in medicinal plants with antibacterial and antioxidant effects (Talib et al., 2010). Several species of plants have been extensively studied for their antibacterial properties. For instance, a range of Gram-positive and Gram-negative bacteria are resistant to the antibacterial effects of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs (Alzoreky et al., 2003; Castro. et al., 2008). Additionally, it has been demonstrated that Chinese and cassia extracts effectively stop the growth of Escherichia coli and other bacteria in preserved meat, juice, and milk (Mau et al., 2001). In a related study, Doddanna et al. (2013) studied the effects of different plant extracts on the growth of the fungus *Candida albicans*. The results showed that the alcoholic extract of curry leaves increased the growth of *C. albicans* with a 48-hour reduction of 24.05 ± 0.07. Furthermore, Nzeako et al. (2006) found that thyme oil extract can inhibit the growth of *Candida albicans* and *Pseudomonas aeruginosa* fungi.

The antibacterial activity of various traditional herbal medicines has been scientifically studied. Extracts from plant organs, such as from roots, stems, rhizomes, tubers, leaves, bark, flowers, fruits, and seeds, may contain antimicrobial compounds (Karalija et al.). It is well known that single plants are commonly used in traditional medicine to treat a wide variety of diseases and conditions (Dewapriya et al.). To discover possible innovative drugs, plant extracts with a long history of widespread use should be verified by modern techniques for activity against disease in humans. Many recent studies have examined the use of plant extracts

as natural preservatives (Fernández-López et al., 2005; Suppakul et al., 2016; Clarke et al., 2017).

Historically, crude extracts from various parts of medicinal plants, such as roots, stems, flowers, fruits, and branches, were widely used to cure various diseases in humans (Khan et al., 2013).

2.2   Antimicrobial Resistance

Antimicrobial resistance (AMR), according to the World Health Organization (WHO), happens when bacteria, fungi, and parasites adapt over time and stop responding to medications, making infections difficult or impossible to treat, raising the risk of disease transmission, serious illness, and death, and antimicrobial medications becoming ineffective due to drug resistance. AMR occurs naturally, most often as a result of genetic changes. People, animals, food, plants, and the environment all include antimicrobial resistant microbes.

Antimicrobial resistance (AMR) is the ability of bacteria and fungi to acquire resistance to antimicrobial (antibacterial or anti-fungal) drugs that are meant to stop them from multiplying. Antimicrobial resistance is exhibited by bacteria through innate (e.g., lack of drug target site) and acquired (e.g., enzymatic drug degradation) processes imparted by AMR genes (ARGs) acquired from other microorganisms via horizontal gene transfer (HGT) (transformation, transduction, and conjugation) (Schwarz et al., 2016). Poor hygiene, a lack of clean water, antibiotic abuse and overuse, insufficient surveillance and limited laboratory antimicrobial susceptibility testing, a lack of information or awareness, and other factors can all contribute to antimicrobial resistance (Ayukekbong et al, 2017).

According to the Centers for Disease Control and Prevention (CDC), antibiotic-resistant infections affect more than 2 million people in the United States each year, with at least 23,000 people dying as a result of the infection. A number of recent studies have summarized AMR data in Africa, the most recent of which was Leopold et al. (2014), which focused on Sub-Saharan Africa. Antibiotic resistance was common. Chloramphenicol resistance was found in Gram negative bacteria, for example. The widespread evolution of antimicrobial resistance since the widespread use of antibiotics for the treatment and prevention of infectious diseases has further been shown to be the result of an evolutionary response among microbes that has produced resistance to antibiotic administration for the treatment and prevention of infectious diseases through a visual representation.

It has been established over time that increased antimicrobial medication intake by humans and animals has resulted in resistance issues. Antibiotic overuse in humans can lead to the development of antibiotic-resistant microorganisms (Goossens, 2009). Antimicrobial use early in life increases the risk of infection with a drug-resistant organism, and people who have had greater antimicrobial exposure are more likely to contract resistant bacteria (Griffith et al., 2012). Antibiotics have long been used to treat or prevent disease in livestock. Antibiotics are routinely found in animal feed in quantities ranging from sub therapeutic to therapeutic, and the antibiotics used are from the majority of antimicrobial families used in humans. There is evidence that providing antibiotics to animals can result in the development of antimicrobial resistant microbes, which can subsequently be passed on to humans who eat those animals (Landers et al., 2012). Antimicrobial resistance patterns found in the animals are a reflection of the types and dosages of antibiotics given to the animals. Antimicrobial resistance can be passed from animals to humans via a variety of routes, the most prevalent of which is direct oral transmission (includes eating meat plus ingestion of faces in contaminated food or water). People coming into direct contact with animals is another common route (Wegener 2012).

2.2.1   Antimicrobial activity of medicinal plants in Nigeria

Novel antibacterial compounds could be produced by medicinal plants. Antimicrobial actions of phytochemicals have rekindled interest. Nigeria has a long history of medicinal herbs, and experts have been studying their antibacterial properties. These findings can be used to produce new antimicrobial medications, and the antibacterial characteristics of the plants can also be determined. In Nigeria, there is virtually little scientific information on indigenous medicinal herbs (Adebayo et al, 2011). There were few articles in the 1980s on the chemistry and antibacterial properties of Nigerian plants in the literature. However, there have been numerous studies and publications on this topic from 1999 to the present, which could be attributable to greater awareness, method developments, and a number of citations in local books indicating the need for such research (Van Vuuren et al, 2008). To analyze plants for antimicrobial activity, several stages have been taken, the first of which is the selection of plants for screening, which is crucial in order to prevent wasting time and resources. Plant selection can be done in one of four ways (Okigbo et al, 2009).

- Random selection followed by chemical screening
- Random selection followed by antimicrobial assays

- Follow-up of antimicrobial activity reports
- Follow-up of ethno-medical or traditional uses of plants against infectious diseases

Selected plants must be identified by plant experts or taxonomists and deposited in a reliable herbarium for future identification and research reproducibility. The type of solvent used and the degree of binding with other chemicals in the plant material determine the degree of extraction of plant bio-active components (Nasir et al 2015). Only a few antimicrobial screening studies on Nigerian plants looked at the root, stem, bark, fruit, and/or seed, with the majority focusing on the leaf. A good solvent for plant extraction in antimicrobial assays should have the following characteristics:

i. Low toxicity
ii. Low boiling point to facilitate removal from the chemical upon extraction
iii. Activity that serves as a preserver and the inability to dissociate or quench active principles

Table 1

Antimicrobial screening assays performed on extracts from Niger

Screening approach	Number of plants studied	Extract tested	Plant part analyzed	Assay
Antimicrobial activity	6	Ethanol	Leaf	AWD, MIC
Pharmacological study	5	Methanol	Leaf	AWD
Antimicrobial activity	3	Petroleum ether, methanol	Leaf	DD, MIC
Antibacterial activity	3	Methanol	Leaf	AWD, MIC
Pharmacological study	50	Ethanol	Various	MIC
Pharmacological	13	Methanol	Various	DD

Source: Journal of Intercultural Ethno pharmacology

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Nasir et al. proposed that in studies where the goal is to screen plants for antimicrobial activity, the process could begin with crude extract prepared from various organic and aqueous solvents, followed by fractionation using various organic solvents.

Agar Well Diffusion (AWD) and Minimum Inhibitory Concentration Assays are the two techniques used most frequently to examine the antibacterial activity of medicinal plants in Nigeria. AWD is similar to disk diffusion in that it indicates the concentration of plant extract that has the greatest microbiostatic effect on the test organism. This approach relies on exposing a test organism to an identical volume of various test drug concentrations injected into wells of equal depth and diffusing into cultivated agar (Balouiri et al, 2016). The lowest concentration at which a bacterium cannot grow visibly under predetermined conditions is known as the minimum inhibitory concentration (MIC) of an antibacterial agent. Assays based on MIC methodology are widely used and an accepted criterion for determining organism susceptibility to inhibitors (Lambert et al, 2000).

Secondary metabolites and antibacterial activity were tested in *Carpolobia lutea*, *Curculigo pilosa*, and *Strophanthus hispidus*, three Nigerian medicinal plants historically used in the treatment of infectious infections and other disorders. Saponins were identified in all three plants, as were cardenolides in *C.lutea* and *S.hispidus*, but only *C.lutea* had measurable levels of alkaloid.

The majority of the test organisms, including *Staphylococcus aureus* NCTC 6571, *Bacillus subtilis, Escherichia coli* NCTC 9001, P*seudomonas aeruginosa* NCTC 6570, *Aspergillus niger,* and *Candida albicans,* were inhibited by the crude extracts of the plants at concentrations ranging from 10 to 100 mg/ml, according to the agar well diffusion method. In this investigation, *S.hispidus* extracts were the most active (Journal of Pharmacy & Bioresources 116-119, 2005).

In a different study, coliforms isolated from surface waters in Akure, Nigeria, were used to investigate the antibacterial activity of *Jatropha curca*s (LINN) using growth inhibition indices based on the agar plate technique. The percentage recovery of the extract was 19.17 percent, 18.10 percent, and 18.10 percent for hot water, ethanol, and acetone, respectively. Except for *Klebsiella pneumonia* and *Escherichia coli*, aqueous extracts of *J.curcas* outperformed standard antibiotics (gentamycin) on coliform bacteria, leading to significantly stronger antibacterial activity compared to organic extracts. As a result, aqueous extract of *J.curcas* could be used as an antibacterial agent against coliform diseases (International Journal of Biomedical Science, 2014).

The methanol extracts of three Nigerian medicinal plants also demonstrated antibacterial efficacy against five clinical bacterial isolates, including two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and three Gram-negative bacteria (*Pseudomonas aeruginosa, E. coli, and Klebsiella pneumonia*) (Chikezie et al., 2015).

E. coli was sensitive to aqueous extracts, whereas *S. aureu*s and *Salmonella* species were sensitive to ethanol extracts. Alcoholic extracts from the leaves of two Diospyros species *(D. barteri* and *D. monbuttensis*) demonstrated antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, whereas two fungal species investigated in the study, *Aspergillus niger* and *Candida albicans*, were resistant to both *Diospyros spp* (Suleiman et al., 2008).

A study of the activity of aqueous and ethanolic extracts of *Zingiber officinale* and *Allium sativum* on selected foodborne pathogens (*Salmonella species, Bacillus cereus, E. coli*, and *Staphylococcus aureus*) revealed multidrug resistance (Ola-Fadunsin and Ademola, 2014).

Azadirachta indica (A. Juss.) bark extracts demonstrated significant antibacterial activity against all 14 multidrug-resistant *Salmonella typhi* strains with zone diameters of 18–31 mm at concentrations of 25–400 mg/ml in acetone and ethanol extracts (Okpe et al., 2016).

Calotropis procera, and Asclepiadaceous flowering plant, has been used in traditional medicine in a variety of ways. This medication is used to treat diseases such as eczema, skin infections, leprosy, and syphilis, as well as malaria.

Crude methanolic extracts of *Spondias mombin* (bark and leaves) with diameters of 11.00 0.47 mm and 15.00 0.47 mm, respectively, were found to have anticandidal effects. The

extracts' phytochemical content varies, but it includes terpenoids, alkaloids, glycosides, saponin, and flavonoids (Agbaje and Onabanjo 1994).

Three lignans were extracted from *Justicia flava* VAHL: (+) isolariciresinol, helioxanthin, and justicinol. *J. root flava* also yielded 8-demethylorosunol and orosunol, two new 1-aryl-2, 3-naphthalide lignans. As a result, further research and optimization of these novel compounds may aid in the development and manufacture of a new class of antimicrobial medicines that are both effective and safe (Bongomin et al., 2019)

2.2.2 Origin of Resistance

Susceptibility and resistance are frequently measured in terms of the minimal inhibitory concentration (MIC) (MIC). Susceptibility is defined as a range of average MICs across distinct bacterial species for a specific drug. A species is said to be innately resistant to a treatment if its average MIC falls within the resistant range. Bacteria can acquire resistance genes from related and unrelated organisms, and the level of resistance varies depending on the species and genes acquired (Coculescu 2009). There are two types of resistance: natural and acquired.

- **Natural Resistance:** This might be intrinsic (always present in the species) or induced (caused by the environment) (not always present in the species). Intrinsic resistance is a bacterial trait that can be found across species, is unrelated to horizontal gene transfer, and is independent of previous antibiotic exposure. The most common bacterial mechanisms implicated in intrinsic resistance are reduced outer membrane permeability (most notably, in gram negative bacteria, the lipopolysaccharide, LPS) and natural efflux pump activity. Multi-drug-efflux pumps are another source of induced resistance (Cox and Wright, 2013).

- **Acquired Resistance**: All three of the fundamental processes for gaining genetic material—transformation, transposition, and conjugation—can be used by bacteria to acquire the genetic material that causes resistance (all of which are referred to as horizontal gene transfer-HGT). Mutations in the bacteria's chromosomal DNA are possible. It's possible that the acquisition will be either temporary or permanent. Plasmid-mediated transmission of resistance genes is the most prevalent method of obtaining foreign genetic material; bacteriophage-mediated transfer is uncommon.

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Acinetobacter spp., for example, are intrinsically competent, which means they can obtain genetic material from the outside world. Internally, insertion sequences and integrins may transport genetic material around, and stresses (such as hunger, UV radiation, chemicals, and other environmental factors) are common causes of genetic changes in bacteria (substitutions, deletions etc.). Bacteria have a mutation rate of one per 106 to 109 cell divisions, with the majority of these mutations causing cell death (Davies and Davies, 2010). Mutations encoding drug targets, drug transporters, drug transporter regulators, and antibiotic-modifying enzymes are the most common types of antimicrobial resistance mutations (Martinez 2014). The fact that taking antibiotics causes resistance is one of the most confusing elements of antimicrobial resistance. Even low or very low antimicrobial concentrations (sub-inhibitory) can select for high-level resistance in succeeding bacterial generations, boost the ability to acquire resistance to other antimicrobial agents, and encourage the movement of mobile genetic elements (Blázquez et al., 2012).

2.2.3   Mechanisms of Antibacterial Activity and Resistance

- An agent's antibacterial activity is primarily attributed to two mechanisms: chemical interference with the synthesis or function of vital components of bacteria, and/or circumvention of conventional antibacterial resistance mechanisms. There are numerous targets for the antibacterial agents that are present;
- Bacterial protein biosynthesis
- Bacterial cell-wall biosynthesis
- Bacterial cell membrane destruction
- Bacterial DNA replication and repair
- Inhibition of a metabolic pathway

Bacteria can also develop resistance to antibacterial agents via a variety of mechanisms. Some bacterial species have an intrinsic resistance to one or more classes of antimicrobial drugs, which leads to resistance across the board in that bacterial species' strains. One major source of concern is that bacteria develop resistance, in which initially susceptible bacterial populations become resistant to the antibacterial agent. Antibacterial resistance mechanisms include (Khameneh et al, 2016);

- Activation of Efflux Pump: Efflux pumps act as an export or efflux mechanism, resulting in antimicrobial drug resistance across a broad spectrum. Antibacterial agents may be effective and accumulate at exact concentrations when they reach their target site of action. Because the antibacterial chemical is pushed out quicker than it can permeate into the bacterial cell, the intrabacterial concentration is substantially lower than the effective value. In bacteria, efflux pumps come in a wide range of sizes and forms. In bacteria, efflux pumps are classified into five families;

1. The ATP-binding cassette (ABC) family
2. The multidrug and toxic compound extrusion (MATE) family
3. The small multidrug resistance (SMR) family, the major facilitator superfamily (MFS)
4. The resistance-nodulation-cell division (RND) family

The majority of these efflux pump families are one-component pumps that move substrates through the cytoplasmic membrane. A periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) work together to efflux substrate over the entire cell envelope by the RND family of multi-component pumps, which are virtually exclusively found in gram negative bacteria (Poole 2007). In gram negative bacteria, various members of the efflux family can work as multicomponent pumps in conjunction with other cellular components. MacB, a member of the ABC family, functions as a tripartite pump to extrude macrolide medicines (MacAB-TolC). EmrB, an MFS member, functions as a tripartite pump (EmrAB-TolC) in E to extrude nalidixic acid (Jo *et al*., 2017).

Eps are capable of removing molecules from bacteria that are both lipophilic and amphipathic, as well as one kind of substrate and/or a variety of structurally different antibacterial compounds present in numerous drug-resistant bacteria. In bacteria, efflux pump genes are chromosomally encoded.

Some are expressed constitutively, while others are triggered or over-expressed in response to particular environmental stimuli or when a suitable substrate is present (high-level resistance is usually achieved through a mutation that alters the transport channel). The efflux pumps are responsible for removing harmful molecules from the bacterial cell, however many of them can transport a wide range of chemicals (multi-

drug [MDR] efflux pumps). Many of these pumps' resistance capabilities are influenced by the carbon source available (Blair *et al*., 2015).

- Destroying the Antibacterial agent: The chemical degradation of antibiotics or antibacterial drugs is the second process of bacterial resistance, with the goal of changing the chemical formula from the previous one. The degradation is mediated by targeting the beat lactam ring of penicillins, cephalosporins, and carbapenems with the hydrolytic enzyme beta lactamase (Blair et al). Because each enzyme molecule can hydrolyze 103 antibiotic molecules per second, it may be claimed that the production of 105 enzymes via resistant bacteria destroys 100 million molecules of the targeted antibiotic per second, rendering it completely worthless.

- Modification of antibiotics: Other antibiotic classes, such as aminoglycosides, represent a different mechanism of resistance than the previous ones. These agents are deactivated by modifying functional groups at three different sites using three different types of modifying enzymes. Because they are unable to bind to ribosomes, these modified products have a significantly lower affinity for RNA and have caused protein synthesis to be halted (Shaw et al).

- Altered Target: Drug-binding site alteration is a different type of resistance mechanism where the antibacterial agent's target site is built in a way that prevents the antibacterial activity of the agent from reacting with it, drastically reducing the antimicrobial activity of the agent. Reprogramming the target structure is a form of resistance that is seen in a number of resistant bacteria (Waish et al). Antimicrobial medications can target a variety of components in the bacterial cell, and the bacteria can modify those targets to enable resistance to those medications. Changes in the structure and/or number of PBPs are one mechanism of resistance to -lactam medicines, which are virtually exclusively employed by gram positive bacteria (penicillin-binding proteins). PBPs are transpeptidases that help cells build peptidoglycan in their walls. The amount of drug that can bind to that target is affected by changes in the quantity of PBPs (increase in PBPs with decreased drug binding ability or decrease in PBPs with normal drug binding capacity). A structural

alteration (e.g., the acquisition of the mecA gene in S. aureus) can reduce or completely block drug binding (Beceiro *et al*., 2013).

Glycopeptides (such as vancomycin) hinder cell wall construction, while lipopeptides (such as daptomycin) depolarize the cell membrane. These medications are intrinsically resistant to Gram negative bacteria (thick LPS coating) (Randall *et al*., 2013). Vancomycin resistance in enterococci (VRE—vancomycin-resistant enterococci) and *Staphylococcus aureus* has become a major problem (MRSA). Resistance is achieved by the acquisition of van genes, which causes changes in the structure of peptidoglycan precursors, resulting in a reduction in vancomycin binding ability (Cox and Wright., 2013). Calcium is required for the binding of daptomycin. Gene mutations (e.g., mprF) shift the charge of the cell membrane surface to positive, blocking calcium binding and thus daptomycin binding (Stefani *et al*., 2015).

Resistance to drugs that target ribosomal subunits can arise from ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal subunit methylation (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) most commonly involving erm genes, or ribosomal protection (aminoglycosides, macrolides (tetracyclines). The ability of the medication to bind to the ribosome is hampered by these mechanisms. Drug interference varies dramatically between these pathways (Kumar *et al*., 2013).

Changes in DNA gyrase (gram negative bacteria, such as gyrA) or topoisomerase IV are the main mediators of resistance to drugs that target nucleic acid production, such as fluoroquinolones (gram positive bacteria, e.g. grlA). These alterations alter the structure of gyrase and topoisomerase, reducing or eliminating the drug's capacity to bind to these proteins (Redgrave *et al*., 2014).

Mutations in enzymes involved in the folate biosynthesis route (DHPS—dihydropteroate synthase, DHFR—dihydrofolate reductase) and/or overproduction of resistant DHPS and DHFR enzymes (sulphonamides—DHPS, trimethoprim—DHFR) produce resistance to drugs that disrupt metabolic pathways. Vancomycin resistance has become a major issue in enterococci (VRE—vancomycin-resistant enterococci) and *Staphylococcus aureus* (MRSA). Sulphonamides and trimethoprim bind to their respective enzymes because they are structural analogs of natural substrates (sulphonamides—p-aminobenzoic acid, trimethoprim—dihydrofolate).

These drugs work by binding to the active site of the enzymes and inhibiting them competitively. The active site of these enzymes is frequently mutated, and the ensuing structural alterations in the enzyme interfere with drug binding while allowing the natural substrate to bind (Vedantam *et al.*, 1998).

2.3   *Icacina tricantha*
2.3.1   Nomenclature, Classification, and Taxonomy
Domain: Eukaryota

Kingdom: Plantae

Phylum: Angiosperms

Subphylum: Eudicots

Class: Asterids

Order: Icacinales

Family: Icacinaceae

Genus: Icacina

Species: *Icacina trichantha*

Icacina trichantha Oliv. belong to the family Icacinaceae. It is a perennial shrub, with broadly elliptic, simple and alternate leaves and large underground tuber. It is a common weed of field crops, forest regrowth and waste areas (Akobundu and Agyakwa 1998).Since it is recognized as a very useful household medicine for emergency treatment, the plant is widely utilized in rural regions. Therefore, almost all houses have the macerated tuber in ethanol, which is preserved in corked bottles (Asuzu and Abubakar 1995).

Icacinaceae are a family of flowering plants consisting of trees, shrubs, and lianas, primarily of the tropics with around 55 genera totaling over 400 species. They belong to the order Icacinales. The family was alter split into four other families in three different orders so that Icacinaceae is now left with 23 genera and 160 species.

1

1. Description

It is a shrub that can grow up to two meters high. It is characterized by the large, fleshy, yam-like underground tubers, as big as several kilograms in weight, which are often consumed by tribal people.

2. Range

Western tropical Africa- Nigeria

3. Habitat

Forest and jungle vegetation. The plant is also reported to have become a weed of rice-paddies

4. Edible Uses

The root is edible on its own and can be dried and ground into a white powder known as gbe-wutu that is used in soups or added to a dish called igbalo that is formed from roasted watermelon seeds (*Citrullus lanatus*). Some people also treat it as a famine-food eating the flour only after prolonged maceration and repeated washing. The tuber is rich in starch and can be eaten fresh or processed into flour to make soup, pastes or porridges. The traditional way of preparation involves cleaning, slicing, and soaking in water for several days to soften it and leach out the bitter components. The tuber is then pulverized after it has been dried under sunlight and it is sieved to produce a grayish-white or creamy-yellowish flour (Umoh et al). The fruit of *Icacina trichantha* is a drupe with a soft sweet outer pulp which is also edible.

5. Medicinal

The plant is considered to be aphrodisiac and is also used externally to heal soft tumors. Additionally, castor-oil seeds are reportedly wrapped in the leaf. The tuber is not flammable and when it burns, it produces intense heat. In Western Nigeria and the surrounding regions, *I. trichantha* is a typical home remedy for treating food poisoning in cases of emergency and first aid (Mbatchou et al). Additionally, the leaves and seeds can be utilized to treat asthma and hypertension by being crushed and macerated in local gin (Ajibesin et al). Traditional healers utilize tubers to cure a variety of illnesses, including as diarrhea, food poisoning, malaria, toothaches, rheumatism, as well as to cause nemesis and abortion (Ariwaodo et al). Additionally, the juice from the tuber can be utilized to cure mumps (Ubom et al).

2.3.2 Antimicrobial activity of *Icacina trichantha*

The antibacterial activity of *I. trichantha* leaf was initially discovered in *Pseudomonas aeruginosa* and *Escherichia coli* (Timothy et al.); both the water and ethanol and water extracts were found to be efficacious against *Staphylococcus aureus*, *Candida albicans*, and *Klebsiella pneumoniae* (Shagal et al). Other studies have reported that hexane and ethyl acetate extracts are effective against *E.coli, P.aeruginosa*, and *K.oxytocoa* (Otun et al).

In 1990, the first pharmacological report on *I. trichantha* was published. In mice, Asuzu and colleagues demonstrated that an aqueous extract of the tuber caused diarrhea, and that the extract also potentiated pentobarbital-induced loss of righting reflex (Asuzu et al). Pentobarbitone resting time in rats was extended, guinea pigs were given a local anesthetic effect, and rats and mice were protected from pentylenetetrazole poisoning. In mice pretreated with pentylenetetrazole, a 50 percent methanol extract of the leaf was found to have anti-convulsant properties, as well as increasing pentobarbitone-induced sleeping time. (Asuzu et al).

Increased number of retches in guinea pigs indicated that a methanol extract of the tuber had emetic effect, and the extract also prevented histological development of the liver and kidney intoxicated with carbon tetrachloride. Paracetamol-induced liver damage in rats was reversed in rats using an ethyl acetate extract of the leaf portion (Udeh et al).

In rats, a methanol extract of *I. trichantha* leaf showed hepatoprotective efficacy against arsenic intoxication, lowering serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and -glutamyltransferase enzyme activities (Samuel et al). The number of micronucleated polychromatophilic erythrocytes in the bone marrow smears of Icacina-treated rats was found to be lower than in non-treated arsenic-intoxicated controls in the same experiment (Samuel et al). The authors hypothesized that *I. trichantha* leaf extract had hepatoprotective and anti-genotoxic properties. In vitro cytotoxic and genotoxic actions of an aqueous leaf extract were described in a recent study, with chromosomal abnormalities in onion root tip cells (Timothy et al).

A chloroform extract of the tuber inhibited ear edema in croton oil-treated mice and paw edema in carrageenan-treated rats, indicating anti-inflammatory activity (Asuzu et al).

In vitro screening of *I. trichantha* leaf, wood, and root parts revealed moderate levels of antioxidant activity in the 2,2-diphenyl-picryl-hydrzyl radical assay (Oke et al). The leaf's

antioxidant activity was found to be related to its total phenol content (Sofidiya et al), and a hexane extract was also found to be active in three nearly identical reports (Otun et al).

In alloxan-induced diabetic mice, the anti-diabetic potential of *I. trichantha* leaf extract was observed (Ezeigbo et al). In rats treated with alloxan, the tuber extract was also found to be active. Thus, oral treatment for up to 21 days resulted in a reduction in fasting blood glucose levels, as well as improvements in serum biochemical parameters such as HDL-cholesterol, LDL-cholesterol, triglyceride, total proteins, ALT, AST, and alkaline phosphatase levels. In addition, the histopathological changes in pancreatic tissues were alleviated (Onakpa et al).

It was discovered that an aqueous extract of the tuber killed termites in a concentration- and time-dependent manner (Edori et al).

A phytochemical analysis of *I. trichantha* leaf extract revealed the presence of alkaloids, tannins, phenols, and saponins (Shagal et al). Stearolic acid, oleic acid, and erucic acids were discovered to be fatty acid components (Otun et al). Alkaloids, tannins, saponins, steroids, carbohydrates, and cardiac glycosides have been found in the tuber (Shagal et al).

In three species of *Icacina, I. claessensii, I. mannii*, and *I. guessfeldtii*, early phytochemical studies confirmed the presence of diterpenes and diterpene alkaloids. Icaceine and demethylicaceine, two novel diterpene alkaloids isolated from *I. guessfeldtii* leaf and root, have been found. Icacinol was extracted from the root of *I. claessensii*, and *icacenone* was recovered from the root of *I. mannii* (On'okoto et al). X-ray diffraction investigation validated the last two structures. These molecules have a skeleton of (9H)-pimarane or (9H)-17-nor-pimarane, which is unusual in the pimarane family of diterpenes.

2.4 Review of method
2.4.1 Minimum Inhibitory concentration
The minimum inhibitory concentration (MIC) of an antimicrobial (such as an antifungal, antibiotic, or bacteriostatic) drug that inhibits visible growth of a microorganism after overnight incubation is defined in microbiology. Following the isolation of a pure culture, MICs can be assessed using broth dilution techniques or plates of solid growth medium, also known as agar (as seen in the "Kirby-Bauer Disk Susceptibility Test" method) (in liquid growth media). For instance, similar doses of bacteria are cultivated in wells of liquid media with progressively lower quantities of the drug in order to estimate the MIC through broth dilution. The antibiotic's minimum inhibitory concentration lies between the concentrations

of the final well in which no bacteria grew and the following lower dose, which permitted bacterial growth. MIC values can also be measured experimentally using a variety of commercial methods. The lowest concentration of an antibacterial agent, measured in milligrams per liter (g/mL) that completely prevents detectable growth of a test strain of an organism under well controlled in vitro conditions.

2.4.2 MIC Methods of Determination
The following techniques are employed:

1. Methods of dilution
   • Agar

   • Liquid medium

2. Antibiotic gradient methods
   • Strips impregnated with an antibiotic concentration gradient.

To obtain MIC values, all quantitative procedures use Mueller–Hinton (MH) medium in the form of agar (MHA) or broth (MHB).

Antibiotics are put to the test to check if they have any inhibitory effects. The filter paper disc (Kirby-Bauer) method (Bauer et al., 1966), the agar and broth dilution method (Wiegand et al., 2008), and the dilution method are a few of the techniques used to test antibiotics (Brown and Young, 1947; Bradshaw, 1979; Owuama, 2015). The minimum inhibitory concentration (MIC), which is the lowest antimicrobial agent concentration required to prevent microbial growth, and the minimum bactericidal concentration (MBC), which is the lowest antimicrobial agent concentration required to kill microorganisms, are both determined using the dilution method (Andrews, 2001). Dilution can be divided into two types: micro dilution and macro dilution. To calculate the MIC and MBC, microdilution and macrodilution techniques were applied (Lambert and Pearson, 2000; Eucast, 2003). The Dilution Method (DM), which entails inoculating an indicator bacterium into different antibiotic dosages, incubation for 18 to 24 hours, and then evaluating bacterial viability by subculturing on antibiotic-free agar media, is frequently used to determine MIC and MBC (Andrews, 2001; CLSI, 1998). Most frequently, samples from tubes with no turbidity or growth are injected into antibiotic-free agar medium to determine the MIC and MBC.

A Petri dish and extra time are required to prepare agar media. The dilution tube, a more effective new technique, was created as a result. DTM is used in the use of tubes to compute MIC and MBC, which is easier and less expensive because it does not require agar media.

2.5   McFarland standard

McFarland Standards are used as the reference when changing the turbidity of the liquid or bacterial suspension in the vial or tube in the microbiology lab. It is beneficial to maintain and/or ensure that the concentration of bacteria will fall within a given range in order to standardize microbiological tests. The McFarland standard can be produced in concentrations ranging from 0.5 to 4, and its concentration affects the cell count density.

However, 0.5 McFarland standard is typically utilized as the concentration for antimicrobial susceptibility testing and culture media performance testing. Antibiotic Susceptibility Tests can standardize the approximate quantity of bacteria in a liquid suspension or broth culture of bacterial cells by comparing the turbidity of the cultured test suspension to that of the McFarland standard. It contains a proper ratio of 1% barium chloride ($BaCl_2$) and 1% sulfuric acid (H_2SO_4). When these two chemicals interact, a fine barium sulphate precipitate forms, resulting in a turbid solution ($BaSO_4$).

Chapter 3
METHODOLOGY

3.1   Collection of plants

The plants were obtained from Ibadan University. The samples were taken with a clean pocketknife. The sample was preserved in a sterile Ziploc bag and transported to the lab.

3.2   Materials

The following materials were used for the experiment: Petri dishes, conical flask, and Inoculating loop, aluminum foil, measuring cylinder, cotton wool, cork borer, test tubes, Test tube racks, sensitivity discs, sterile filter paper and beakers. The following equipment was also used for the experiment: Autoclave, incubator, water bath, Bunsen burner, weighing balance, vortex, hot air oven, weighing balance micro pipettes, Pipette tips.

3.3   Processing and Extraction of the plant

The plant's roots were air-dried at temperatures below 40 °C and pulverized into fine powder with a laboratory milling machine, after which 500 g of the ground powder was extracted in the following order: n-hexane, ethyl acetate, chloroform, and methanol. In the maceration, 5 L of each solvent (volume per volume [v/v]) were used. The extract was condensed using a rotary evaporator (Buchi model R210, Switzerland), and then dried in a vacuum desiccator. The dry extract was ground into powder using a laboratory mill and sieved with a 250-mesh sieve.

3.4   Test Organisms

The antimicrobial activity of the extracts was determined using agar well diffusion. The test organisms were gotten from the Department of Biotechnology, Mountain Top University, Ibafo, Ogun state, Nigeria. All the isolates were checked for purity and maintained in nutrient agar. The bacteria isolates used includes *Salmonella* (SH1351), *Escherichia coli* (SH70E1)

3.5   Preparation of Agar

A weighing balance was used to weigh 7g of nutrient agar, which was then poured into a conical flask. 250ml of distilled water was measured and poured into the conical flask containing the nutrient agar; the mixture was stirred with a magnetic stirrer and autoclaved for 15 minutes. The media was removed from the autoclave and allowed to cool before being aseptically poured into sterile petri dishes. The media was allowed to solidify before being inverted and stored.

3.6   Preparation of MacFarland Standard

1g of anhydrous barium chloride ($BaCl_2$) in 100ml distilled water was mixed to make a 1 percent barium chloride ($BaCl_2$) solution. 1ml of concentrated H_2SO_4 was mixed with 99ml of distilled water to make a 1 percent sulfuric acid (H_2SO_4) solution. A 0.5 percent McFarland standard was made in a sterile test tube by combining 0.05ml or 50 microlitres of 1% ($BaCl_2$) in 9.95ml or 9950 microlitres of 1% H_2SO_4. The solution was thoroughly mixed with a vortex to form a turbid suspension. To prevent evaporation, the test tube was sealed with an aluminum foil cap.

3.7   Antibacterial activity and Minimum Inhibitory Concentration

Agar Diffusion Test

100 microliters of the test organism were dispersed into test tubes containing BHI and incubated at 37 degrees Celsius for 24 hours. This was done to get the test organisms to work. 10mg of the plant extract was diluted four times with 1000 microliters of sterile distilled water and placed in four sterile sample bottles labeled 800, 600, 400, and 200 microliters. The set plate was properly labeled with the appropriate concentration for each strain of the test organisms.

The antibacterial activity of the extracts was tested against the selected strains using the agar well diffusion method. Each sterile Petri plate received 20 mL of sterile nutritional agar medium, which was allowed to harden. The test bacteria cultures were standardized to 0.5 percent McFarland standard (NCCLS 1993) and evenly disseminated across the suitable substrate using a swab stick. Then, using a sterile cork borer, a 2mm well was drilled into the

medium (Bhargav et al. 2016). Concentrations of sample solutions were created, and then dilutions to the desired concentration (20 mg/mL) were performed.

These concentrations (20 microliters) were transferred to separate wells and incubated at 35°C for 24 hours. After the incubation period, the zones of growth inhibition (ZI) were observed and measured with a transparent ruler (Mbata, Debiao, & Saikia, 2008). Each test was repeated three times to ensure reproducibility. The diameter of the zone of inhibition was measured as the standard error of the mean (SEM) of the triplicate tests.

A clean, sterile perforator was used to create a mini sensitivity disc from sterile filter paper. For 30 minutes, the perforated filter paper was placed in 1.5ml Eppendorf tubes containing different volumes of extract. With sterile forceps, the perforated filter paper was removed from the tubes and placed in a sterile petri dish, which was then placed in an incubator at 37°C for 24 hours. The agar was poured into a sterile petri dish, 50 microliters of the test organisms were decanted into the plate, and the test organisms were spread with a hockey stick. The perforated filter paper was placed on the agar and incubated at 37°C for 24 hours.

CHAPTER 4

RESULTS AND DISCUSSION

These are the results obtained by using the concentrations listed below. 800 microlitres containing 10mg of extract and 800 microlitres of sterile distilled water, 600 microlitres containing 10mg of extract and 600 microlitres of sterile distilled water, 400 microlitres containing 10mg of extract and 400 microlitres of sterile distilled water, and 200 microlitres containing 10mg of extract and 200 microlitres of sterile distilled water. *E. coli* was resistant to the extract after 24 hours of incubation at 37°C because no zone of inhibition was observed. Salmonella, on the other hand, was sensitive to the extract. A noticeable zone of inhibition existed, which was both weak and strong. The plate containing 800 microliters of extract had a weak zone of inhibition with a diameter of 14cm, while the plate containing 600 microliters of extract had a weak zone of inhibition with a diameter of 13cm. The plate containing 400 microlitres of extract had a 12 cm diameter zone of inhibition. The plate containing 200 microliters of extract had a 12cm diameter zone of inhibition. The extract had a stronger effect at 200 microliters and a decreased effect at 800 microliters.

Each test was performed three times to guarantee repeatability. The second time the test was run, however, there was no zone of inhibition on any of the plates. *Salmonella* and *E. col*i were both resistant to the extract. The second time the test was performed, the plate containing the extract at the concentration with the two test species, *Salmonella* and *E. coli,* showed no zone of inhibition. There was no zone of inhibition on any of the plates. Both test organisms developed resistance to the plant extract.

Comparing an invitro antimicrobial test done on methanol extracts of *Icacina trichantha* Oliv. Leaf in the Department of Plant Biology and Biotechnology, University of Benin. The table below shows the MIC and MBC of the methanol extract of *Icacina* leaf.

Table 1: Minimum Inhibitory Concentration based on the experiment carried out

Test organism	Dilute concentration of extract(mg/ml)	MIC 1st Zone of inhibition	MIC 2nd Zone of inhibition	MIC 3rd Zone of inhibition
Escherichia coli	800	Nil	Nil	Nil
	600	Nil	Nil	Nil
	400	Nil	Nil	Nil
	200	Nil	Nil	Nil
Salmonella spp.	800	Nil	Nil	Nil
	600	Nil	Nil	Nil
	400	Nil	Nil	Nil
	200	Nil	Nil	Nil

Comparing an invitro antimicrobial test done on methanol extracts of *Icacina trichantha* Oliv. Leaf in the Department of Plant Biology and Biotechnology, University of Benin. The table below shows the MIC and MBC of the methanol extract of *Icacina* leaf.

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentrations of extracts of *Icacina trichantha* Leaf

Test Isolates	Concentration of Extract (mg/ml) Methanol Extracts MIC	Concentration of Extract (mg/ml) Methanol Extracts MBC
Staphylococcus aureus	6.25	6.25
Bacillus subtilis	12.5	25.0
Pseudomonas aeruginosa	3.125	6.25
Escherichia coli	3.125	6.25
Aspergillus niger	50	100
Candida albicans	25	50

This study found that *P. aeruginosa* and *E. coli* were more sensitive to the methanol extract than *S. aureus*. This is similar to the findings of Ramya and Devi (2011), although it contradicts previous findings that most antibacterial medicinal herbs attack Gram-positive strains more than Gram-negative pathogens due to permeability differences (Priscilla et al. 2007; Pavithra et al. 2010). Their ability to compound with cell walls could explain their broad spectrum activity against both gram positive and gram negative bacteria (Cowan 1999).

Possible factors that may affect zone of inhibition.

• **Pathogen susceptibility:** The antibiotic used is determined by the organism being tested. If the bacterium is antibiotic-resistant, it will not grow near the disk. They will, however, grow straight up to the disk if they are resistant.

• **Antibiotic diffusion effects:** An antibiotic's rate of diffusion through agar is not always the same. The concentration of antibiotic, Molecular weight of antibiotic, solubility qualities of antibiotic, pH and ionization, and binding to agar all influence the rate of antimicrobial diffusion through the agar. Molecules with a higher molecular weight diffuse at a slower rate than those with a lower molecular weight. Because of these considerations, each antibiotic has a distinct breakpoint zone size that indicates susceptibility to that antimicrobial chemical.

• **Agar depth:** If using dehydrated medium, the nutrient agar plates must be poured to a depth of four millimeters. Plates that are too shallow will yield false susceptible results because the antimicrobial ingredient will diffuse deeper than it should, resulting in bigger

inhibition zones. Plates poured to a depth of less than four millimeters, on the other hand, will yield false resistance findings.

• **pH:** During the test, the nutrient agar medium should be examined. Because most bacteria grow best in a pH range of 6.5-7.5, the agar medium should have a pH between 7.2 and 7.4 at room temperature. Exception The only human pathogen that thrives well beyond pH 8 is Vibrio cholerae. If the pH is too acidic, certain medications (such as amino glycosides, quinolones, and macrolides) will lose efficacy, while others (such as tetracyclines, novobiocin, and methioillin) will have excessive action, resulting in a smaller or wider zone of inhibition. The opposite consequences can be expected if the pH is too high.

• **Size of the inoculated organism:** The injected organism's size must also be standardized. The reasons for this are that if the inoculum is too tiny, the zone of inhibition will be bigger than expected, and if the inoculum is too large, the zone of inhibition will be smaller.

• **Other metals present:** Too much thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim, resulting in smaller and fewer identifiable zones of inhibition, or none at all. The findings of aminoglycoside and tetracycline tests against Pseudomonas aeruginosa will be influenced by an improper concentration of divalent cations (calcium and magnesium). Zone sizes will be reduced if the cation concentration is too high, while zone sizes will be increased if the cation concentration is too low. Excess calcium increases the growth of *P. aeruginosa's* daptomycin-resistant zone. Excess zinc ions may diminish carbapenem zone size against P. aeruginosa. The concentration of bacteria dispersed on the agar plate, drug antagonists, incubation temperature, incubation period, plate size, appropriate spacing of the disks, zone reading, and other factors all affect the zone of inhibition

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, the leaf of *I. trichantha* has been demonstrated to have a broad spectrum antibacterial function that is highly dependent on the solvent used for extraction. Despite its importance as a source of sustenance and medicine to West African tribal people, *I. trichantha* is an underutilized plant species. The scientific community has paid little attention to the genus *Icacina* as a whole. While there have been a few reports on pharmacological screening and chemical analyses of this plant, further in-depth research is needed to adequately assess its economic or medicinal potential.

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