

**STUDIES OF ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF
THE STEM BARK OF *Irvingia gabonensis* (Baill) ON SOME SELECTED BACTERIAL
AND FUNGAL ISOLATES**

ONUNDE Odunayo Abiodun

SCP11/12/H/0233

B.Sc.(Hons.) Microbiology, Kogi State University, Anyingba.

**A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY
OF SCIENCE, OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF
SCIENCE DEGREE IN MICROBIOLOGY**

2015

AUTHORISATION TO COPY**OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA,****HEZEKIAH OLUWASANMI LIBRARY,****POSTGRADUATE THESIS.****AUTHOR: ONUNDE Odunayo Abiodun****TITLE: STUDIES OF ANTIMICROBIAL ACTIVITIES OF METHANOLIC****EXTRACT OF THE STEM BARK OF *Irvingia gabonensis* (Baill)****ON SOME SELECTED BACTERIAL AND FUNGAL ISOLATES.****DEGREE: M.SC. (MICROBIOLOGY)****YEAR: 2015**

I, ONUNDE Odunayo Abiodun, hereby authorize the management of Hezekiah Oluwasanmi Library to copy my thesis in whole or parts, in response to the requests from individual researchers and organisations for the purpose of private study or research.

Signature: Date:

CERTIFICATION

This is to certify that this research work was carried out by **ONUNDE, ODUNAYO ABIODUN (SCP11/12/H/0233)**, in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

.....
Prof. Olu Odeyemi
(Supervisor)

.....
Date

.....
Dr. D.A Akinpelu
(Head of Department of Microbiology)

.....
Date

DEDICATION

This thesis is dedicated to God Almighty, the Alpha and Omega for seeing me through this programme.

ACKNOWLEDGEMENTS

Every praise and honor goes to God who made this programme a success and for his protection upon my life.

My sincere appreciation goes to my supervisor Prof. Olu Odeyemi who has given me all form of support throughout the programme and made me to understand the power of deductive reasoning in writing good thesis.

My special thanks go to the able Head of the Department of Microbiology the person of Dr. D.A. Akinpelu for his assistance. I also appreciate the efforts of the academic staff and non-academic staff of the Department of Microbiology Prof. A.K. Akonai, Dr. B.O. Omafuvbe, Dr. A.O. Shittu, Dr. M.K. Bakare, Dr. A.O. Oluduro, Dr. N. Torimiro, Dr. O.K. Awojobi, Dr. J.O. Omololu-Aso, Dr. S. Adeyemo, Mrs C.D. Fashina, Mr. O. Adedeji, Mrs M.O. Japhet, Mr. O.O. Olumide, Mr. T. Fadare, Mr. T. Abioye, Mr. O. Aregbesola and Mrs O. Bamigbade, Mr. P.O. Oni, Mrs O.T. Awotipe, Mrs A.A. Rafiu, Mrs E.O. Oyeyemi and Mr. Odejobi.

My appreciation also goes to Mr. Akinkunmi in Drug Research and Production Unit, Faculty of Pharmacy, O.A.U, for his assistance.

The success story of this thesis will not be completed without mentioning my guidance Mrs. Temitope Stella for all the assistance and selfless service rendered to me. I pray that Almighty God will reward you in a million fold.

I am very grateful to my parents, Mr. and Mrs. G.A. Onunde. You made it all count with your support and encouragements. My siblings (Aunty Yemi, Bro. Jide, Aunty Sade, Aunty Stella and Aunty Adesola). To my uncle Bro Kayode, I am most grateful.

I also want to appreciate Mrs. Feruke-Bello and Mrs. Fakorede. I also want to thank the numerous friends I made during the course of this programme, Adeolu, Tope, Yemikale,

Yetunde, Dayo, Sis. Adeola, Sis. Taiwo, Sis. Sade, Sis. Dayo, Bro. Bayo, Progress, Kenny,
Deji, Alice, Sis. Kenny, Titi and Mr. Paulfor being a wonderful set of people.

OBAFEMI AWOLOWO UNIVERSITY

TABLE OF CONTENTS

Title	Page
Title page	i
Authorization to copy	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Table of Contents	vii
List of Tables	xi
List of Figures	xiii
List of Plates	xiv
Abstract	xv
 CHAPTER ONE	
1.0 Introduction	1
1.1 Background of the Study	1
1.2 Statement of Research Problem	3
1.3 Specific Objectives of the Research	4
1.4 Expected Contribution to Knowledge	4
 CHAPTER TWO	
2.0 Literature Review	5
2.1 History of Medicinal Plants	5
2.2 Some Medicinal Plants and their Antimicrobial Activities	5
2.3 <i>Irvingia gabonensis</i>	10
2.4 Antimicrobial Agents	13
2.5 Mode of Actions of Atimicrobial Agents	14
2.5.1 Cell Wall Synthesis Inhibitors	14
2.5.2 Effect of Nucleic Acids	15
2.5.3 Competitive Inhibitors	16

2.5.4	Cell Membrane Inhibitors	17
2.6	Mechanisms of Antimicrobial Resistance to Antibiotics	18
2.6.1	Target Modification	19
2.6.2	Alteration in the Metabolic Pathway	19
2.6.3	Efflux Pumps and Outer Membrane Permeability	20
2.6.4	Antibiotic Inactivation by Hydrolysis	21
2.6.5	Antibiotic Inactivation by Redox Process	21
2.6.6	Antibiotic Inactivation by Group Transfer	22
2.7	Secondary Metabolites Produced by Plants	22
2.8	Classes of Secondary Metabolites	23
2.8.1	Tannins	23
2.8.2	Flavonoids	24
2.8.3	Alkaloids	24
2.8.4	Glucosinolates	25
2.8.5	Proanthocyanidins	26
2.8.6	Phenolic compounds	26
2.8.7	Terpenes or Terpenoids	27
2.8.8	Saponins	27
2.9	Microorganisms as a Causative Agent of Diseases	28
2.9.1	<i>Staphylococcus aureus</i>	29
2.9.2	<i>Escherichia coli</i>	30
2.9.3	<i>Bacillus cereus</i>	31
2.9.4	<i>Micrococcus luteus</i>	33
2.9.5	<i>Proteus</i> Species	33
2.9.6	<i>Salmonella</i> spp.	34
CHAPTER THREE		
3.0	Materials and Methods	35

3.1	Materials	35
3.1.1	Collection of the Plant Materials	35
3.1.2	Preparation of the Plant Extract	37
3.1.3	Test Microorganisms	37
3.2	Methods	37
3.2.1	Antibacterial Sensitivity Testing	37
3.2.2	Antifungal Sensitivity Testing	38
3.2.3	Determination of MICs of the Extract on the Test Isolates	38
3.2.4	Determination of MBC and MFC of the Extract	39
3.3	Fractionation of the Crude Extract	39
3.4	Determination of the Rate of Killing by the Extract	40
3.4.1	Determination of Protein Leakage by the Active Fraction	40
3.4.2	Determination of Potassium ion Leakage by the Active Fraction	41
3.5	Determination of the Antibigram of the Test Isolates	41
3.6	Phytochemical Screening of the Extract	42
CHAPTER FOUR		
4.0	Results	44
4.1	Yield of the plant used	44
4.2	Sensitivity patterns of the Methanolic and Aqueous Extract of <i>Irvingia gabonensis</i>	44
4.3	Yield of each fraction after fractionation	45
4.4	Sensitivity Patterns of Each Fraction after Fractionation	52
4.5	The Minimum Inhibitory Concentration of <i>Irvingia gabonensis</i>	52
4.6	The Minimum Bactericidal Concentration of <i>Irvingia gabonensis</i>	53
4.7	The Killing Rate of <i>E. faecalis</i> and <i>E. coli</i> by the Chloroform Fraction of <i>Irvingia gabonensis</i>	60
4.8	Protein Leakage from <i>E. faecalis</i> and <i>E. coli</i> by the Chloroform	

Fraction of <i>Irvingia gabonensis</i>	63
4.9 Potassium Ion Leakage from <i>E. faecalis</i> and <i>E. coli</i> by the Chloroform Fraction of <i>Irvingia gabonensis</i>	66
4.10 Antibiotic Susceptibility Patterns of the Isolates	69
4.11 Antibiotic Resistance Pattern of the Isolates	69
4.12 Phytochemical Screening Result	70

CHAPTER FIVE

5.0	Discussion and Conclusion	76
5.1	Discussion	76
5.2	Conclusion	83
5.3	Contribution to Knowledge	83
	References	84
	Appendices	100

LIST OF TABLES

Table Title	Page
Table 4.1 Yield of the Plant used	46
Table 4.2a Sensitivity Patterns of Bacterial Isolates to Methanolic and Aqueous Extract of <i>Irvingia gabonensis</i>	47
Table 4.2b Sensitivity Patterns of Fungal Isolates to Methanolic and Aqueous Extract of <i>Irvingia gabonensis</i>	49
Table 4.3 Yield of each Fraction after Fractionation	51
Table 4.4a Sensitivity Patterns of each Fraction Obtained on the Bacterial Isolates	54
Table 4.4b Sensitivity Patterns of each Fractions Obtained on the Fungal Isolates	55
Table 4.5a The MIC of the Methanolic Extract and Chloroform Fraction on the Bacterial Isolates	56
Table 4.5b The MIC of the Methanolic Extract and Chloroform Fraction on the Fungal Isolates	57
Table 4.6a The MBC of the Methanolic Extract and Chloroform Fraction on the Bacterial Isolates	58
Table 4.6b The MBC of the Methanolic Extract and Chloroform Fraction on the Fungal Isolates	59
Table 4.7a Sensitivity of Gram positive Bacterial Isolates to Five Classes of Antibiotics	71
Table 4.7b Sensitivity of Gram negative Bacterial Isolates to Five Classes of Antibiotics	72
Table 4.8a Antibiotic Resistance Pattern of the Gram positive Bacterial Isolates	73
Table 4.8b Antibiotic Resistance Pattern of the Gram negative Bacterial Isolates	74
Table 4.9 The phytochemical Screening Result	75

LIST OF FIGURES

Title	Page
Figure 4.1 The Percentage of <i>Escherichia coli</i> Killed by Chloroform Fraction of the Stem Extract of <i>Irvingia gabonensis</i>	61
Figure 4.2 The Percentage of <i>Enterococcus faecalis</i> Killed by Chloroform fraction of the Stem extract of <i>Irvingia gabonensis</i>	62
Figure 4.3 The Effect of Chloroform Fraction of the <i>Irvingia gabonensis</i> on Protein Leakage from <i>Enterococcus faecalis</i>	64

Figure 4.4	The Effect of Chloroform Fraction of the <i>Irvingia gabonensis</i> on Protein Leakage from <i>Escherichia coli</i>	65
Figure 4.5	The Effect of Chloroform Fraction of <i>Irvingia gabonensis</i> on Potassium ion Leakage from <i>Enterococcus faecalis</i>	67
Figure 4.6	The Effect of Chloroform Fraction of <i>Irvingia gabonensis</i> on Potassium ion leakage from <i>Escherichia coli</i>	68

LIST OF PLATES

Title	Page
Plate 2.1 The <i>Irvingia gabonensis</i> Nuts	12
Plate 3.1 The <i>Irvingia gabonensis</i> Tree	36
Plate 4.1 Sensitivity Pattern of <i>Bacillus cereus</i> to Methanolic Extract of Stem Bark Extract of <i>Irvingia gabonensis</i>	47
Plate 4.2 Sensitivity Pattern of <i>Rhizopus stolonifer</i> to Methanolic Extract of Stem Bark Extract of <i>Irvingia gabonensis</i>	49

ABSTRACT

This study investigated the antimicrobial activities of the methanolic extract of the stem bark of *Irvingia gabonensis* on some selected bacterial and fungal isolates. It also assessed the antimicrobial activities of the active fraction obtained from the methanolic extract of the stem bark of *Irvingia gabonensis* and determined the rate of kill, protein leakages as well as potassium ions leakages from the active fraction on the bacterial isolates. This was with a view to determining the antimicrobial properties of *Irvingia gabonensis* on some selected bacterial and fungal isolates.

The stem bark of *Irvingia gabonensis* was collected from Consecrated Farms Usi – Ekiti, Ekiti State, Nigeria. The stem bark of *Irvingia gabonensis* was air dried and grounded into powder. Exactly 1280 grams of the stem bark of *Irvingia gabonensis* were soaked in 10 litres of methanol and water respectively with regular shaking for four days. The filtrate obtained were consecrated and lyophilized and kept for further use. The antibacterial and antifungal activities of the plant were determined on fifteen bacterial and ten fungal isolates using agar well diffusion method at 35 mg/ml and 15 mg/ml respectively on Muller – Hinton agar and potato dextrose agar. The methanolic extract of *Irvingia gabonensis* was further fractionated using four organic solvent: n – Hexane, chloroform, ethylacetate and butanol according to their polarity. The rate of kill, the protein leakage as well as potassium ions leakage of the active fraction were determined on bacterial isolates. The phytochemical screenings of the extract was carried out and susceptibility patterns of the bacterial were also determined.

The methanolic stem bark extract of *Irvingia gabonensis* inhibited the growth of eleven bacterial isolates out of the fifteen bacterial isolates and one fungal isolate out of ten fungal isolates at 35

mg/ml. The zones of inhibition ranged from 14 ± 0.7 mm for *Salmonella typhi* to 22 ± 0.7 mm for *Bacillus cereus* (bacterial isolates) and 20 ± 0.0 mm for the *Rhizopus stolonifer* (fungal isolate). Out of the four organic solvent used for fractionation, the chloroform fraction of the methanolic stem bark extract of *Irvingia gabonensis* inhibited the growth of eleven bacterial isolates and one fungal isolate at 15 mg/ml. There was increase in the rate of kill of the isolates by the chloroform fraction of the methanolic stem bark of *Irvingia gabonensis* with increase in time and concentrations. The leakages of protein as well as potassium ions suggested that the cell membrane disruption was a mode of action for the chloroform fraction of the methanolic stem bark extract of *Irvingia gabonensis*. The phytochemical screening result revealed the presence of alkaloids, saponins, tannins, reducing sugar and triterpenes. The susceptibility patterns of the bacterial showed that the most of these bacterial isolates are multi resistance.

The study concluded that methanolic stem bark extract and chloroform fraction of the methanolic stem bark extract of *Irvingia gabonensis* showed a considerable antimicrobial activities against bacterial and fungal isolates.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Phytomedicine has been in existence for hundredsof years ever before the colonial administration and is still in use today with about 80% of the population depending on herbal medicine for its primary health care delivery (Ogbulie *etal.*,2007).However, the knowledge of medicinal plants has rapidly decreased due to influence of western lifestyles, reduction in number of traditional healers and lack of interest of the younger generations to carry on the tradition (Bussman*etal.*,2006).Recently, due to the resistance of the pathogens to the most of the available antibiotics this has led to the recognition of traditional medicine as an alternative form of health care and this has reopened the research domain for the biological activities of the medicinal plants (Arias *etal.*, 2004).

Herbal medicine is based on the use of plants, plant extracts and many of these plants are herbs and spices used to season foods and they yield useful medicinal compounds including those having antibacterial activities, antioxidant activities, antifungal properties and antimalarial properties (Lai and Roy, 2004). The use of herbs to treat disease is almost universal among developing societies (Edgar*etal.*,2002). Many of the drugs currentlyavailable to physicians have long history of theiruse as herbs.

According to World Health Organization, (2005) reported that, approximately 25% of the modern drugs used in the United States have been derived from plants.Treatments of infections prior to the beginning of the twentieth century were based on the medicinal folklore.An

estimated 25% of prescription drugs and 11% of drugs considered essential by the WHO are derived from plants and a large number of synthetic drugs are obtained from precursor compounds originating from plants (Rates, 2001). It has also been found that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs and 20% of the population of the US takes herbal products (Bent, 2008). In fast developing countries such as China and India, 80% of the populations depend on herbal treatment, thus the economic importance of medicinal plants is much more to countries such as India and China than to rest of the world (Joy *et al.*, 2001). Out of the 250,000 higher plant species on earth, more than 80,000 are medicinal. The drugs are derived either from the whole plant or from different organs like leaves, stem bark, root, flower and seeds. Some drugs are prepared from excretory plant product such as gum, resins and latex (Khan *et al.*, 2003).

Infectious diseases are the result of the invasion of the host system by pathogens which are not repelled or destroyed by the immune system. Though it was known in the nineteenth century that bacteria are the cause of many diseases, no effective antibacterial treatments were available then (Thurston, 2000). Infectious diseases account for approximately one-half of all deaths in tropical countries. Bacterial infections can be caused by a wide range of pathogens, resulting in mild to life-threatening illness. World Health Organization estimated that infections caused by microorganisms accounted for 45% of deaths in Africa and South-east Asia, these diseases were responsible for 48% of premature deaths worldwide (Okeke and Sosa, 2003). A study indicated that in Africa, 28% of children admitted to the hospital with bacteremia died and more importantly 26% of the hospital deaths were associated with bacteremia (Berkley *et al.*, 2005). This suggests that bacterial diseases may be responsible for more deaths in children than malaria in this area where malaria is endemic (Mulholland and Adegbola, 2005).

The prevalence of multiple antibiotics resistance developed by microorganisms against available synthetic antibiotics has increased parallelly in the last decade. The resistance problem demands that a renewed effort should be made to seek for antibacterial agents especially those of natural origin that will be effective against pathogenic bacteria that have developed resistance against the current synthetic antibiotics. Literature reports and ethno botanical records suggest that plants have tremendous potentials in the pharmaceutical industry as an important source of new compounds for antimicrobial drugs synthesis (Akinpelu *et al.*, 2008). Plants do synthesize many compounds which may be useful to them and sometimes not useful to them. Examples of these compounds are flavonoids, saponins, steroids, alkaloids, reducing sugars, tannins and terpenes. These compounds are referred to as secondary metabolites.

However, as stationary autotrophs, plants have to cope with a number of challenges, including engineering their own pollination and seed dispersal, local fluctuations in the supply of the simple nutrients that they require to synthesize their food, and the coexistence of herbivores and pathogens in the immediate environment (Kenndy and Wightman, 2001). Plants have therefore evolved in secondary biochemical pathways that allow them to synthesize a raft of chemicals, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks and nutrient deprivation (Reymond *et al.*, 2000). The secondary metabolite can be unique to specific genera or species. These secondary metabolites from medicinal plants serve as lead compounds in drug discovery and design (Ebi and Ofoefule, 2000).

1.2 Statement of Research Problem

The increase in antibiotic resistance of clinically important pathogens coupled with the emergence of new resistance strains with multidrug resistance have necessitated that alternative antimicrobial agents from natural origins be identified to combat these resistance

For more information, please contact ir-help@oauife.edu.ng

OBAFEMI AWOLOWO UNIVERSITY