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

ONUNDEOdunayo 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

OBAFEMI AWOLOWO UNIVERSITY

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 Date

(Supervisor)

…… ……

Dr. D.A Akinpelu Date

(Head of Department of Microbiology)

DEDICATION

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

ACKNOWLEDGEMENTS

Every praise and honor goes to God who made this programme a successand 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 andProduction Unit, Faculty of Pharmacy, O.A.U, for his assistance.

The successstory 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-Belloand 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.

TABLE OF CONTENTS

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

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 filterate 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 isolatesat 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, thechloroform 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 *etal*.,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 *etal.,*2003).

Infectious diseases are the result of the invasion of the host system by pathogens which are not repelled or destroyed by theimmune 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 infectionscan be caused by a wide range of pathogens,resulting in mild to life-threatening illness.World Health Organizationestimated 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).Thissuggests 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 microorganismsagainst available synthetic antibiotics has increased parallely in the last ecade. The resistance problem demands that a renewed effortshould be made to seek for antibacterial agents especially those of natural origin that will be effective against pathogenic bacteria that have developed resistant 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 synthesize (Akinpelu *etal.,* 2008).Plant 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 tripetens. These compounds are referred to as secondary metabolites.

However, as stationary autotrophs, plant have to cope with 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 Wigthman, 2001).Plants have therefore evolved in secondary biochemical pathways that allow them to synthesis a raft of chemicals, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks and nutrient depravation(Reymond*etal.,*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).

1.2Statement of Research Problem

The increase in antibiotic resistance of clinically importance pathogens coupled with the emergence of new resistance strains with multidrug resistance have necessitated that alternative antimicrobialagents from natural origins be identified tocombat these resistance

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