Studies on Bioactive Potential of Ethanolic Leaf Extract of *Spondias mombin* on some Bacterial Isolates Obtained from Stool Samples of Diarrhetic Patients.

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FASOGBON, ALBERT OLUWASOGA

B. Sc. (Microbiology), University of Benin, Benin.

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CERTIFICATION

I certify that this work was carried out by FASOGBON ALBERT OLUWASOGA under my supervision and approved in partial fulfillment for the award of Master of Science Degree in Microbiology.

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Dr. (Mrs.) A. O. Oluduro Date
(Supervisor)

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Dr. (Mrs.) A. O. Oluduro Date
(Head, Department of Microbiology)

DEDICATION

This work is dedicated to God Almighty for his mercy over my life.

It is also dedicated to my blood sisters and brother; Mrs. A. A. Adewoyin, Mrs. G. O. Ajayi, Mrs. B. F. Adedire and Engr. S. K. Fasogbon.

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ABSTRACT

The antibacterial activity of ethanolic extract of *Spondias mombin* leaf on some bacteria that were associated with diarrhoea was investigated. This was with a view to determining the phytochemicals, the extent and the rate of killing of the test isolates by the crude and various fractions of the extract.

The leaves of *Spondias mombin* were harvested, dried in hot air oven at 45^{o}C and ground into a fine powder. The powered leaf was cold extracted using ethanol and distilled water in ratio 3:2 (v/v). The crude extract obtained was concentrated in *vacuo* and lyophilized. The extract was screened for phytochemicals and antibacterial activity against selected bacterial isolates. The extract was partitioned into n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions in order of their polarity. The antibacterial potentials, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the crude extract and the fractions were determined using agar diffusion and agar dilution techniques, respectively. The antibacterial activities of the crude and active fractions (n-hexane, ethyl acetate and butanol fractions) were compared with standard antibiotics- ampicillin and streptomycin (1mg/mL). The time-kill assay of the various active fractions on the representative Gram negative (*Escherichia coli*) and Gram positive (*Staphylococcus aureus*) bacterial isolates was determined using standard method.

The crude extract of the plant and various fractions obtained exhibited varying degree of antibacterial activities. The MIC of the crude extract ranged from 6.25 to 25.00 mg/mL while the MBC ranged from 12.50 to 25.00 mg/mL. The MIC and MBC of the active fractions against the isolates was 15.00 mg/mL. Phytochemical screening of the extract showed the presence of tannins, saponins, steroids, terpenes, alkaloids, flavonoids, and cardiac glycosides. The time-kill

assay reveals a minimum of 4.3% death rate at 1 x MIC after 15 min contact time with the active fractions and minimum of 64.0% death rate after 120 min at the same concentration (1 x MIC). The rate of kill increased with increased concentration of the extract (active fractions) and time interval.

The study concluded that the leaf of *Spondias mombin* investigated in the study area possessed antibacterial area possessed antibacterial property with broad spectrum of activity against both Gram negative and Gram positive test bacteria.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

There is an increase in the prevalence of multidrug-resistant (MDR) pathogens in clinical practice generated from misuse of antibiotics in clinical and veterinary practices. This has therefore raised a case of concern in health care delivery (Lewis and Stanley, 2012). As resistance to antibiotics becomes more common, a vicious circle develops wherein increasingly broad-spectrum agents must be prescribed empirically to ensure that initial antibiotic therapy is adequate to the task (Gary, 2003). Thus new, ever more powerful agents are needed for the treatment of MDR bacterial infection. This has now led to an intensive search for newer and more effective antimicrobial agents to deal with these problems. Such agents are now been sourced for from bioactive components of the medicinal plants (Gary, 2003). Infection caused by multidrug-resistant bacteria constitutes a serious problem for intensive care patients throughout the world (George, 1993). The mortality rate associated with multidrug-resistant Gram-negative enteric bacteria in these patients is high in some intensive care units (ICUs). It is likely that patterns of microbial infection and antibiotic resistance in ICU patients differ widely from one country to another and are often facilitated by the increasing use of inappropriate antibiotic therapy and immunosuppressive drugs (Asem *et al*., 1996). Occurrence of epidemics due to multi-drug resistant microorganisms and emergence of unknown disease causing microbes even at the moment, pose enormous public health concern (Iwu *et al*., 1999).

In addition, certain types of pathogens are becoming common in each local community and represent an important risk factor for the morbidity and mortality of ICU patients (Emori

and Gaynes, 1993). According to an estimate by the Center for Disease Control and Prevention (USA), 13,300 patients died of antibiotic-resistant bacterial infections in the US during 1992. An incredible 150% increase in the occurrence of drug-resistant pneumococci was noted between 1987 and 1994, while a twenty-fold increase in the frequency of hospital-aequired enterococci and resistant to vancomycin was seen between 1989 and 1993 (Saswati and Madhab, 2012). The frequency of methicillin-resistant *Staphylococcus aureus* rose from 2% in 1975 to 32% in 1992. By this time, resistance to virtually all the therapeutically useful antibiotics had been evidenced (Saswati and Madhab, 2012).

Reports from European countries and the United States of America indicate that extensive use of the new lactam and fluoroquinolone drugs has contributed to the rapid emergence of multidrug r esistant Gram-negative bacteria, particularly *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Acinetobacter* spp. (New, 1993). Numerous studies have shown that multidrug-resistant bacteria, in particular aerobic Gram-negative bacteria, easily colonize the gastrointestinal tract and respiratory tract of hospitalized patients (Verhoef *etal*., 1993). In addition, it is well known that multidrug-resistant bacteria are becoming increasingly prevalent in the hospital environment as a result of the extensive use of antibiotics (Verbist, 1993). Two decades back in early 90s, multidrug-resistant Gram negative bacteria such as *Acinetobacter baumanii, Psuedomonas aeruginosa* and *Klebsiella pneumoniae* were the major cause of infection in ICU patients in Jordan (Asem *et al*., 1996).

Medicinal plants are of great importance to the health of individuals and communities and thus showing them to contain certain chemical substances that produce a definite physiological action on the human body. Plants can synthesis many different types of secondary metabolites, which have been subsequently exploited humans for their beneficial role in a

diverse array of applications (Balandrin *et al*., 1985). The most important of the bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Acamovic and Brooker, 2005). Screening techniques of biologically active medicinal compounds have been conducted on well known species of plants used in traditional medicine and most plants have shown antimicrobial activity (Arunkumar and Muthuselvam, 2009). Many of the indigenous medicinal plants are used as spices and food plants and they are also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 2001; Okwu, 1999). One of the great advantages of medicinal plants is that they are readily available and have very low side effects (Wadkar *et al*., 2008). New drugs of herbal origin discovered through ethno pharmacological studies have shown interesting results (Ricardo *et al*., 2004). For example, plant oils containing terpenes have shown increasing promise invivo, against multiple drug resistant species of bacteria. Cinnamon oil has shown broad-spectrum activity against *Pseudomonas aeruginosa* (Prabuseenivasan *et al*., 2006). There is global resurgence in the use of herbal preparations in some developing countries like Nigeria and now it is being gradually integrated into the primary and secondary health care systems (El-Mahmood and Ameh, 2007). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002).

Aloe vera has been used to treat various skin conditions such as cut, burns and eczema. It is also confirmed that sap from *Aloe vera* eases pain and reduces inflammation (Arunkumar and Muthuselvam, 2009).

According to the World Health Organisation (WHO), up to 80 percent of the people still rely mainly on traditional remedies for their ailment (Arunkumar and Muthuselvam, 2009). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing (Igbinosa *et al*.,

2009; Meyer, 1992). Traditional medicine is a reliable alternative approach to healthcare delivery