OBAFEMI AWOLOWO UNIVERSITY

STUDIES ON BIOACTIVE POTENTIALS OF STEM BARK EXTRACT OF*Dracaenacinnabari*Balf. ON SOME BACTERIAL AND FUNGAL ISOLATES.

BY

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CERTIFICATION

I certify that this work was carried out by RAJI Hubaydah Bolatito under my supervision and approved in partial fulfilment for the award of Master of Science in Microbiology.

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DEDICATIONsss

This research work **is dedicated to Almighty God, the Beneficient and the Merciful**. It is also dedicated to my little boy **MUHAMMED AWWAL IDUNNUMI OLADAPO and my late** father Mr. Ahmodu Raji.

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ABSTRACT

This study assessed the bioactive potentials of the stem bark extract of *Dracaena cinnabari* on some bacterial and fungal isolates that were associated with human diseases. This was with the view to determining the mechanisms of action of potent fractions on the test isolates and the phytochemical compounds that were responsible for the bioactive action of the extract.

Fresh stem bark of *D. cinnabari* was collected from Ile-Ife, Nigeria. The sample was dried in hot air oven at 40^{o}C and then ground into fine powder. The powdered stem bark was cold extracted using methanol and distilled water in the ratio 3 to 2. The crude extract obtained was concentrated *in vacuo* and lyophilized. The methanolic crude extract was screened for phytochemicals and tested for antimicrobial activity against all the bacterial isolates. The extract was partitioned into n-hexane, chloroform, ethyl acetate and butanol. The antimicrobial potentials, minimum inhibitory concentrations (MIC) and the minimum bacteriocidal concentration (MBC) of the extract and those of fractions were determined. Finally the rate of killing, potassium ions and nucleotides leakages from the test cells were determined using different fractions.

The crude extract of the plant and the two active fractions (n-hexane and chloroform) at a concentration of 30 mg/mL and 10 mg/mL respectively, exhibited antibacterial activities against the test isolates. The MIC of the crude extract ranged between 0.94 and 15 mg/mL while that of n-hexane and chloroform fractions ranged between 0.16 and 5 mg/mL. All the fungal isolates tested against the crude extract were resistant to it. The MBC ranged between 1.88 to 15 mg/mL and 0.31 to 5 mg/mL for the extract and the fractions respectively. The results of the antibacterial activities of the stem bark extracts compared favourably with the activity of standard antibiotics, streptomycin and ampicillin. The phytochemical screening of the extract showed the presence of tannins, flavonoids, saponins,

steroids, reducing sugars, cardiac glycosides and terpenoids. The time kill assay reveals that the percentage of the cells killed increases with increase in the concentrations of the fractions as well as contact time intervals. The time-kill assay reveals a minimum of 8.4% killed at 1 x MIC after 15 min contact with the fractions and minimum of 61.1% killed after 120 mins. Varying amount of potassium ions as well as nucleotides were leaked from the test cells by n-hexane and chloroform fractions.

In conclusion, this study established the possibility of developing antimicrobial agents of natural origin to combat the effect of probable multiple resistance to antimicrobial compounds by some pathogens currently being experienced in health care industries.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The emergence and spread of antibiotic resistant bacteria pose increasingly difficult therapeutic problems, particularly in hospitals. Intensive research efforts have been devoted to the development of alternative antimicrobial agents to combat this problem (Darwish *et al.*, 2002). Also, the problem of bacterial resistance to commonly used antibiotics has necessitated the search for newer and alternative compounds for the treatment of drug resistant infectious. High cost of conventional drugs, particularly in resource poor communities of the African continent has led to the increased use of plants as an alternative for the treatment of infectious (Sibanda and Okoh, 2008a). Infectious diseases are currently the world's leading causes of premature deaths, killing almost 50,000 people every day (WHO, 2002). The control of these diseases has pose new challenges because of the emergence of multiple drug resistance among several pathogens to some of the antimicrobial drugs commonly used in the treatment of infectious diseases. The problem is further compounded by the indiscriminate use of antibiotics (WHO, 2002). The problem is further compounded by the indiscriminate use of antibiotics (Davis, 1994). Moreover, the clinically useful antibiotics in use have major setbacks. Apart from the narrow spectrum of antimicrobial activity many of them have been found to be neurotoxic,

nephrotoxic, ototoxic or hypertensive and few others cause severe damage to the liver and cause bone marrow depression (Chong and Pagano, 1997).

The plant kingdom has served as an inexhaustible source of useful of drugs, foods, additives, flavouring agents, lubricants, colouring agents and gums from time immemorial (Parikh *et al*., 2005). The therapeutic power of herbs has been recognized since creation of the universe and botanic medicine is one of the oldest practiced professions by mankind (Kambizi and Afolayan, 2001). Medicinal plants have been found useful as antimalarial, antisickling, anti-helminthic, anti-microbial, anti-convultant, anti-hypertensive and anti-schistosomal (molluscicidal) agents (Prescott *et al*., 2002). The medicinal actions of plants are unique to particular plant species or groups, consistent with the concept that the combination of secondary products in a particular plant is taxonomically discrete (Parekh *et al*., 2005). Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nascimento *et al*., 2000). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment (Joy *et al*., 2001). The characteristic of the plants that inhibit microorganisms are important for human health which have been researched in laboratories since 1926 (Ates and Erdogrul, 2003). Medicinal plants have been used since time immemorial in virtually all societies as source of medicine to combat various ailments including infectious diseases (Alli *et al*., 2011). The World Health Organization reported that over 85% of the population in Sub-Sahara Africa, including Nigeria still depend on herbal traditional medicine for their healthcare (WHO, 2002). The organization advocates the exploitation of those aspects of it that provides safe and effective remedies for use in primary health care. The organization emphasized, in particular, the importance of scientific investigations into herbal medicine (Alli *et al*., 2011).

Hoareau and Da Saliva (1999) noted that medicinal plants and herbal remedies are reemerging medicinal aids whose contribution and significance in the maintenance of good health and well-being is widely accepted. Alves and Rosa (2007) also attested to the important role of the folk or traditional medicinal use of plants in modern drug discovery. They noted that there are about 121 pure chemical substances extracted from about 130 species of higher plants in the modern pharmacopeias throughout the world. Out of these, 89 plant-derived drugs currently used in modern medicine were originally discovered through the study of traditional cures and folk knowledge of indigenous people.

Medicinal plants contain large varieties of chemical substances with important therapeutic properties that can be utilised in the treatment of human diseaess (Akinpelu *et al*., 2011). They have an almost infinite ability to produce aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Kambizi and Afolayan, 2011).

Most are secondary metabolites, of which at least 12,000 have been isolated, a number projected to be less than 10% of the total (Van Wyk *et al*., 1997). In many cases, these substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores. Some of the compounds like, trepenoids- give plants their odours and, quinones and tannins are responsible for its pigmentations. Many compounds are responsible for plant flavour (e.g., the trepenoids capsaicin from chili peppers) and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Pecere *et al*., 2000). The isoquinoline alkaloid emetine from the underground part of *Cephaelis ipecacuanha*- has been used for many years as an amoebicidal drug as well as for the treatment of abscesses due to the spread of *Entamoeba histolytical* infections (Iwu ***et al***., 1999).

Another important compound of plant origin with a long history of use is quinine- an alkaloid which occurs naturally in the bark of Cinchona trees. Apart from its continued usefulness in treatment of malaria, it can also be used to relieve nocturnal leg cramps (Iwu *et al*., 1999). Similarly, plants have made important contributions in the areas beyond anti-infective, such as