ANTIMICROBIAL EFFECT OF ALBIZIA ZYGIA DC LEAF EXTRACTS ON SOME SELECTED MICROORGANISMS

BY

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IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M. Sc.) IN MICROBIOLOGY

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DEDICATION

This work is dedicated for the benefit of mankind.
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ABSTRACT

This study investigated the antimicrobial activities of methanolic, ethanolic and aqueous leaf extracts of *Albizia zygia* on some selected bacteria and fungi with a view to determining the possibility of harnessing the antimicrobial potential of the leaf as a cheaper substitute for conventional synthetic antibiotics.

The antimicrobial activities of *Albizia zygia* leaf extract on the test organisms was carried out using disc diffusion technique on Mueller Hinton agar and Potato Dextrose Agar for bacterial and fungal assay, respectively. The bored wells on agar plates previously seeded with standardized bacteria and fungal spores were filled with different concentrations (30 and 50mg/ml) of the extracts (Methanolic, ethanolic and aqueous) and incubated at 37 ºC for 24 h for bacteria and 28 ºC for 5 d for fungi. The diameters of zones of inhibition were measured. The minimum inhibitory and minimum bacteriocidal concentrations were equally determined using agar dilution method. The chemical group constituents present in the crude leaf extract were also monitored. The death rate of some selected bacteria by the methanolic leaf extract was determined as well as the protein and the potassium leakage from the bacterial cell. The cytotoxicity effects of the methanolic leaf extract on mice were monitored. The effect of the methanolic leaf extract on the metabolic activities (enzymes) Aspartate transaminase (AST) and Alanine transaminase (ALT) were also assessed using Randox enzyme kit.

The antibacterial sensitivity test showed that the extract was effective against both Gram-positive and Gram-negative bacteria, indicating the broad-spectrum antibiotic activity of the extract. The diameter of zones of inhibition ranged from 3 to 25 mm. The ethanolic and aqueous extracts had little or no effect on the bacterial isolates at the concentrations used. The antibacterial activity of the methanolic extract compared favorably with the reference antibiotic (streptomycin) used. All the
extracts showed no antimicrobial activity on fungal isolates. The chemical groups inherent in *Albizia zygia* were alkaloids, flavonoids, tannins, saponins and anthraquinone. The lowest Minimum Inhibitory Concentration (MIC) (3.75 µg/ml) of the plant extract was observed in *Clostridium sporogenes* while the highest MIC value (15.5 µg/ml) was observed in *Bacillus subtilis*. The Minimum Bactericidal Concentration (MBC) of the plant extract ranged from 7.5 µg/ml for *Clostridium sporogenes* to 30 µg/ml for *Bacillus subtilis*. The highest percentage of bacteria killed was 100% in *Bacillus cereus* treated with 60 µg/ml of methanolic extract at 120 min. The lowest percentage of bacteria killed was 28% and was observed in *K. pneumoniae* treated with 7.5 µg/ml of the extract at 120 min. The amount of protein and potassium ion detected in solution after treatment of the bacterial cells with the plant extract increased gradually with time for both *Bacillus subtilis* and *Klebsiella pneumoniae*. The highest amounts of protein and potassium ion leakages observed in *Bacillus cereus* were 40 µg/ml and 400 ppm respectively. The highest amounts of protein and potassium ion leakages observed in *Klebsiella pneumoniae* were 10.02 µg/ml and 370 ppm respectively. Enzymatic studies revealed that the leaf extract at high concentrations had negative effect on the enzymes aspartate transaminase (AST) and alanine transaminase (ALT) in the liver of the mice as the levels of these enzymes were found to increase significantly in serum (ALT 69.23 IU/ml and AST 168.45 IU/ml) but reduced drastically in the liver (ALT 30.61 IU/ml and AST 137.57 IU/ml). The levels of ALT and AST increased in the kidney with corresponding decrease in the serum confirming that the leaf extract does not have any lethal effect on the mice' kidney.

The study concluded that the methanolic extract of *A. zygia* showed better activities only on the bacterial isolates, hence could be formulated for empirical therapeutic measures against infections caused by the susceptible test bacteria.
CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Nature has always provided therapeutic means of treating microbial diseases. Nature has endowed several plants with metabolites which are of medicinal importance (Cowan, 1999). Modern scientific research claims that out of the millions of plant species, there are approximately 7000 species that are of medicinal value and the total sales of herbal medicine in China amounted to $2.5 billion. In Japan, there was a 15 fold increase in herbal medicine sales when compared to 2.5 fold increase of pharmaceutical products between 1974 and 1989 (WHO, 2006).

The countries of the Far East Asia have been able to meet 75% of their health care needs through the development and utilization of herbal medicine and traditional medicine practice. Meanwhile, only 40% of herbal medicines consumed in Nigeria are produced locally and the remaining 60% are imported from foreign countries. Again, the commercial interest to mass produce herbal medicine through research and development (R and D) and make them available for local and international use is lacking (Sofowora, 2008) and it would probably take a longer time for this to improve and grow significantly. In Nigeria, effort at local herbal medicine R and D is yet to yield the desired benefits due to low funding of R and D activities, lack of adequate infrastructural facilities, non-commercialization of most research results, low demand that constraints R and D investment and problems faced by herbal medicine researchers (Togola et al., 2008).

Due to incessant rise in the cost of over the counter drugs, the practice of ayurvedic medicine is gathering momentum. *Albizia zygia* is a cheap substitute to some of the known prophylactic drugs (Njakou et al., 2007). The application of *A. zygia* is of two categories; internal and external use.
1.2 Statement of Research Problem:

In contemporary times, incessant cases of microbial resistance to the available synthetic antibiotics have been recorded; there is an exigent need to promote the development of alternative antimicrobial substances of natural origin for the treatment of infections. Hence, this study intends to evaluate the antimicrobial potentials of *Albizia zygia* DC leaf.

1.3 Specific Objectives of Research:

The specific objectives of the research are to:

a) evaluate the antimicrobial potential of *Albizia zygia* DC leaf extracts on some selected microorganisms;

b) determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC);

c) monitor the death rate of all selected bacterial pathogens by the plant extracts;

d) monitor the protein and Potassium ion (K\(^+\)) leakage from the test organisms by the extracts; and

e) determine the acute toxicity of the plant extract in experimental animals

1.4 Expected Contribution to Knowledge: This study will provide useful information on the inhibitory potential, kill kinetics and acute toxicity of *Albizia zygia* leaf extracts.