

**COMPARATIVE ANALYSIS OF ACTIVITIES OF *CARICA PAPAYA* LINN. LEAF
EXTRACT AND SOME CONVENTIONAL ANTIBIOTICS AGAINST
SALMONELLA AND *SHIGELLA* SPP. ISOLATED IN FISH PONDS IN**

ILE-IFE.

BY

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**A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,
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DEPARTMENT OF MICROBIOLOGY

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OBAFEMI AWOLOWO UNIVERSITY

ILE-IFE, NIGERIA

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TITLE: Comparative Analysis of Activities of *Carica papaya* Linn. Leaf Extract and Some Conventional Antibiotics against *Salmonella* and *Shigella* spp. Isolated in Fish Ponds in Ile-Ife.

DEGREE: MASTER OF SCIENCE (M.Sc.) IN MICROBIOLOGY**YEAR: 2015**

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DEDICATION

I dedicate this work to Almighty God and to my parents Mr. and Mrs.Emoitologa, my aunty Mrs. C. Emoitologa andmy siblings: Anthony, Cyril and Israel.

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Abbreviations and Acronyms

CSIR	Council for Scientific and Industrial Research
NSSP	National Shellfish Sanitation Program
NARMS	National Antimicrobial Resistance Monitoring System
NDM	New Delhi metallo-beta-lactamase
STD	Sexually Transmitted Diseases
DSMZ	Deutsche Sammlung von Mikroorganismen und Zeukulturen (German Collection of Microorganism and Cell Cultures)
ATCC	American Type Culture Collection
NAERLS	National Agricultural Extension and Research Liaison Sources
WHO	World Health Organization
NAFDAC	National Agency for Food and Drug Administration and Control

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ABSTRACT

This study investigated the presence of *Salmonella* and *Shigella* spp. in fish ponds in Ile-Ife, Osun State, Nigeria and assessed the potential antibacterial activities of *Caricapapaya* Linn. leaf extract and some conventional antibiotics against these isolates. This was with a view to providing information on the comparative analysis of antibacterial activities of *Caricapapaya* Linn. leaf extract and some conventional antibiotics on the isolates.

Water samples were collected from the surface level of twelve fish ponds at a depth of 10 to 15 cm. The total bacteria counts, isolation and characterization of *Salmonella* and *Shigella* spp. were determined by culturing with tryptic soy agar and Salmonella-Shigella agar respectively following standard procedures. Physical parameters of water quality such as temperature, pH and total dissolved solids were determined *in situ* using mercury bulb thermometer and digitalized portable meters. The antibiotic resistance profiles of the bacteria isolated were determined by agar disc-diffusion technique. *Caricapapaya* leaves were collected, air dried and ground into fine powder. Aqueous methanol was used for extraction in a ratio of 3:2 (v/v). The potential antibacterial activity of the crude extract, Minimum Inhibitory Concentration MIC and Minimum Bactericidal Concentration MBC on the *Salmonella* and *Shigella* spp. isolated were determined. Cefotaxime (CFX) and amoxicillin-clavulanic acid (AMX) were the standard antibiotics used. The data collected in this study were subjected to Analysis of Variance (ANOVA) and the means were separated using Duncan's New Multiple Range Test (DNMRT) at 95% level of probability using SAS 9.0 software package.

The total bacterial counts of the studied fish ponds were in the range of 6.5×10^3 to 6.2×10^5 cfu/mL. Thirteen (13) *Salmonella* spp. and five (5) *Shigella* spp. were isolated from the

studied fish ponds. The antibiotic sensitivity of the bacteria isolated from the fish ponds showed that 94.4% of the isolates were multiple antibiotics resistant. Resistance was generally high to cefuroxime CPX (94.4%), cefixime CXM (83.3%), augmentin AUG (89.9%) and ceftizidime CAZ (77.8%). The crude extract of *Caricapapaya* showed activity against *Salmonella* and *Shigella* with the highest mean zone of inhibition 17.3 ± 0.30 mm and 16.25 ± 0.25 mm observed at a concentration of 120 mg/mL respectively. *Salmonella* and *Shigella* spp. appeared to be equally sensitive to the extract as shown by their MICs (≥ 30 mg/ml) and MBCs values (≥ 60 mg/ml). An Activity Index (AI) of ≤ 0.64 and ≤ 0.43 for cefotaxime CFX and amoxicillin-clavulanic acid AMX observed respectively also indicated that the cefotaxime CFX showed the highest antibacterial activity compared to amoxicillin-clavulanic acid AMX and *Caricapapaya* L. leaf extract against the *Salmonella* and *Shigella* spp. isolates.

This study concluded that *Salmonella* spp. were the more frequently isolated bacteria in the water samples compared to *Shigella* spp. The studied conventional antibiotics had more antibacterial potency compared to the *Caricapapaya* L. leaf extract against the *Salmonella* and *Shigella* spp. isolates.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Fish farming is fast becoming a means of livelihood in developing countries across Africa. Fish is a rich source of protein known for its high nutritional quality, relatively low fat content, saturated fat, cholesterol, high polyunsaturated fatty acids, protein and minerals such as calcium(Ca), phosphorus(P), sodium(Na), potassium(K) and magnesium(Mg) (HanyEl-Said, 2004).The natural habitat of fish is extremely prone to pollution that may be from domestic, industrial and agricultural discharges. Fishes take a large number of bacteria into their guts from water, sediment and food, thus, determining the bacterial contents of the water in ponds. The health of fish is dependent on the quality of the water in their aquatic environment (Pyakin and Krivoshein, 1986; Boon and Huisman, 1996;Noga, 2000). The microbial load of fish pond is not a problem but the presence of pathogens especially the faecal coliform is a major concern.

Most fish farmers engage in a lot of activities in an attempt to reduce marginal cost of production and increase their profits. Such activities include enrichment of water by the application of fertilizers and animal manure (Joseph and George, 2010). Some of these activities often lead to contamination of the ponds. It is well established that freshwater fish and their aquatic environment harbour human pathogenic bacteria, particularly members of the coliform group (Al-Harbi and Uddin, 2005). The use of antimicrobials/antibiotics in fish feeds by fish farmers as a way of immunizing and treating fishes of diseases are not without consequences (Pandey *et al.*, 2012; Madhuri *et al.*, 2012); the emergence of antibiotic resistance in bacteria can

be possibly tied to the fish food chain which can pose a major threat to public health. This should be a concern because antibiotic resistance determinants can be transferred to bacteria of human clinical significance (Blake *et al.*, 2003; Ibezim, 2005).

Salmonella spp. are rod-shaped, Gram-negative pathogenic bacteria found in water bodies in warm climatic zones which pose a great risk to human health (Heinitz *et al.*, 2000). It is a source of food and water-borne infection of most vertebrates. They cause a wide range of human diseases such as enteric fever, bacteremia and gastroenteritis. Gastroenteritis has the greatest adverse effect on children's growth and development (Black *et al.*, 1984). The majority of 1.3 billion annual cases of *salmonella* cause human gastroenteritis resulting from ingestion of contaminated food products such as undercooked beef, pork, eggs, shell fish and fish (Esaki *et al.*, 2004). *Shigella* spp. are facultative anaerobic Gram -ve rod-shaped bacteria belonging to the class *Enterobacteriaceae*. They cause diarrhoea which is sometimes bloody, fever, abdominal pains and sometimes cause serious complications such as reactive arthritis.

Plants have played major roles in the development of drugs. Nigeria has a vast plant resources base that has served both pharmaceutical and therapeutic purposes and has been proposed to have contributed to the health and wellbeing of the people. However, information on these plants is not well-documented (Gbile and Adesina, 1986). Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoctions from the plants. These medicinal plants are used in the treatments of diseases such as malaria, dysentery, diarrhoea, pyogenic infections, dental caries, and many other diseases (Gill, 1992). World Health Organization (WHO) defines medicinal plants as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration, or

other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Carica papaya belongs to the family of Caricaceae. Several species of Caricaceae have been used as remedy against a variety of diseases (Gill, 1992). Papaya plant (*Carica papaya* L.) is widely cultivated in Indonesia and Nigeria. The plant serves dual purposes of edibility and medicinal functions. Its fruits, leaves and flowers are edible. Its roots can be used as medicine for renal and urinary bladder problems, and its seeds have anthelmintic activity (Kermanshai *et al.*, 2001). Papaya leaf extracts have phenolic compounds such as protocatechuic acid, p-coumaric acid and caffeic acid. It produces natural compounds in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties (Walter, 2008; Ayoola and Adeyeye, 2010). A major bioactive compound known as papain is present in the milky juice of the papaya fruit, as well as all other parts of the plant. The juice has been used to make meat tender because of the papain it contains (Foster and Tyler, 1999). The seed when chewed has anthelmintic activities against intestinal worms (Kermanshai *et al.*, 2001). The root is chewed and the juices swallowed for cough, bronchitis and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotency. The development of antibiotics resistant bacteria has led to the use of alternative means (Andersson, 2003) and the extracts of *C. papaya* have been proven to have antimicrobial activity against a wide range of pathogenic bacteria and fungi (Anibijuwon and Udeze, 2009; Efunwole *etal.*, 2014). Hence, this study focuses on the comparative analysis of activities of *Carica papaya* Linn. leaf extract and some conventional antibiotics against *Salmonella* and *Shigella* spp. isolated in fish ponds in Ile-Ife, Osun state, Nigeria.

The objectives of this study were to

- (i) determine the total bacterial count of water samples from different fish ponds in Ile-Ife;
- (ii) isolate and identify *Salmonella* spp. and *Shigella* spp from the water samples;

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