DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL EVALUATION OF LEAF EXTRACTS FROM *Carica papaya*

By

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CERTIFICATION

This is to certify that this project work presented by ADELODUN WASILAT OMOTAYO wit h matriculation number ND/23/SLT/PT/0649 has been read, approved and submitted to the Department of Science Laboratory Technology (Chemistry Unit), Institute of Applied Sciences, Kwara State Polytechnics, Ilorin.

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DEDICATION

This project work is dedicated with thanks to Almighty God for His unending blessings u

pon me.

ACKNOWLEDGEMENT

I sincerely thank Almighty God for granting me the strength, good health, and wisdom required to complete this project work successfully.

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May the Almighty continue to bless and guide you in all your endeavours.

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ABSTRACT

This study investigated the phytochemical composition and antimicrobial activity of methanol leaf extracts of *Carica papaya*. The leaves were collected, air-dried, and pulverized before methanol extraction, yielding 1.102 g from 1325 g of plant material. Phytochemical screening revealed the presence of proteins, fats and fixed oils, carbohydrates, amino acids, glycosides, phenolic compounds, tannins, and alkaloids, while anthraquinones were absent. The antimicrobial efficacy of the extract was tested against clinical strains of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* using the agar well diffusion method. The extract demonstrated inhibitory activity against all tested organisms, with *Staphylococcus aureus* showing the highest susceptibility (1.2 cm zone of inhibition). However, the extract's effectiveness was lower compared to standard antibiotics (ofloxacin and ampicillin). These findings suggest that *Carica papaya* leaf extract possesses significant phytochemical constituents with potential antimicrobial properties.

CHAPTER ONE

INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) has emerged as a significant global health concer n, threatening the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. According to the World Health Organization (WH O), AMR is responsible for an estimated 700,000 deaths annually, and this number could rise to 10 million deaths per year by 2050 if no action is taken (WHO, 2019). In Nigeria, th e situation is particularly alarming. The overuse and misuse of antibiotics, coupled with i nadequate healthcare infrastructure, have accelerated the emergence and spread of resis tant pathogens. A study by Olayemi *et al.* (2019) highlighted that over 60% of bacterial i nfections in Nigerian hospitals are resistant to commonly used antibiotics, posing a sign ificant challenge to public health.

Given the escalating threat of AMR, there is an urgent need to explore alternative a ntimicrobial agents. Medicinal plants have historically been a valuable source of therape utic compounds. *Carica papaya*, commonly known as pawpaw, is one such plant that ha s been traditionally used for its medicinal properties. Various parts of the plant, including the leaves, seeds, and roots, have been utilized in folk medicine to treat a range of ailmen ts (Akinmoladun *et al.,* 2020).

Recent scientific studies have corroborated the antimicrobial potential of Carica p apaya. For instance, Aina *et al.* (2017) conducted a comparative phytochemical screenin g and antimicrobial activity assessment of aqueous and methanol leaf and bark extracts

of Carica papaya. The study revealed that methanol extracts exhibited significant antimicrobial activity against pathogens such as Staphylococcus aureus and Bacillus subtilis, with zones of inhibition ranging from 31.00±1.106 mm to 45.50±1.125 mm.

Furthermore, Jaji *et al.* (2021) performed a phytochemical analysis of Carica papaya leaf extracts and identified the presence of bioactive compounds such as flavonoids, alkaloids, tannins, steroids, saponins, and terpenoids. These compounds are known for their antimicrobial properties, suggesting that *Carica papaya* could be a viable source of alternative antimicrobial agents.

1.2 Problem Statement

The increasing prevalence of antimicrobial-resistant pathogens poses a significant threat to public health, particularly in developing countries like Nigeria. The limited availability of effective antibiotics and the high cost of new drug development necessitate the exploration of alternative sources of antimicrobial agents. Despite the traditional use of *Carica papaya* in treating various infections, there is a lack of comprehensive scientific studies validating its antimicrobial efficacy, particularly concerning methanolic extracts. This gap in knowledge hinders the potential integration of *Carica papaya*-derived compounds into mainstream antimicrobial therapies.

1.3 Justification

The urgent need to identify and develop novel antimicrobial agents is underscored by the alarming statistics associated with AMR. Nigeria's high mortality rates linked to resistant infections highlight the inadequacy of current treatment options (Olayemi et al., 2019). Exploring the antimicrobial properties of Carica papaya aligns with global efforts t

o harness natural products in combating resistant pathogens.

Previous studies have demonstrated the presence of bioactive compounds in Carica papaya with antimicrobial potential (Aina et al., 2017; Jaji et al., 2021). However, comprehensive analyses focusing on methanolic extracts and their efficacy against a broad spectrum of microorganisms remain limited. This study aims to fill this gap, providing scientific validation for the traditional use of Carica papaya and contributing to the global search for effective, plant-based antimicrobial agents.

1.4 Aim and Objectives of the Study

1.4.1 Aim

To evaluate the phytochemical constituents and antimicrobial activities of methanolic extracts from Carica papaya leaves.

1.4.2 Objectives

i. To perform qualitative and quantitative phytochemical analyses of methanolic extracts from Carica papaya leaves.
ii. To assess the antimicrobial efficacy of these extracts against selected bacterial strains.
iii. To compare the antimicrobial activities of the extracts with standard antibiotics to determine their relative effectiveness.

1.5 Significance of the Study

This study holds significant promise in addressing the pressing issue of AMR by exploring alternative, plant-based antimicrobial agents. By scientifically validating the antimicrobial properties of Carica papaya, the research could pave the way for the develop

ment of novel therapeutics derived from natural sources. Additionally, the findings may c ontribute to the conservation and sustainable utilization of medicinal plants, promoting biodiversity and traditional knowledge systems.

1.6 Scope of the Study

The study focuses on the extraction of phytochemicals from *Carica papaya* leave s using methanol as a solvent. It encompasses the qualitative and quantitative analysis of these phytochemicals and evaluates the antimicrobial activities of the extracts agains t selected bacterial and fungal strains. The research is confined to laboratory-based anal yses and does not extend to clinical trials or in vivo studies.

1.7 Definition of Key Terms

i. **Antimicrobial Resistance (AMR):** The ability of microorganisms to withstand t he effects of medications that once could successfully treat them.
ii. **Phytochemicals:** Bioactive compounds produced by plants, often contributing to their color, flavor, and resistance to diseases.
iii. **Methanolic Extract:** A solution obtained by using methanol to extract soluble c ompounds from plant materials.
iv. ***Carica papaya*:** A tropical plant species known for its edible fruit and medicina l properties.
v. **Antimicrobial Activity:** The ability of a substance to kill or inhibit the growth of microorganisms.

CHAPTER TWO

LITERATURE REVIEW

2.1 MEDICINAL CHEMISTRY

Medicinal chemistry, also called pharmaceutical chemistry, is the field of pharma ceutical sciences which applies the principle of chemistry and biology to the creation of knowledge leading to the introduction of new therapeutic agents. It is a discipline at the i ntersection of chemistry, especially synthetic organic chemistry and pharmacology and various other biological specialties, where they are involved with design, chemical synthe sis and development for market of pharmaceutical agents or bio-active molecules (drug s) (Andrew & Simon, 2015). It also involves synthesis of new compounds, investigations of their relationships between the structure of natural or synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, incl uding enzymes and DNA, the determination of their absorption, transport and distributio n properties and studies of the metabolic transformations of these chemicals into other chemicals (Edward, 2022). This branch of chemistry plays a major role in drug research and development, taking advantage of newer techniques and increased knowledge of dif ferent branches of related sciences.

Medicinal plant is an integral part of medicinal chemistry. Medicinal plant is any plant whose one or more of their organs contains substances that can be used for thera peutic purposes or which are precursors for the synthesis of useful drugs. A term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to des cribe whole plant or parts of plant which have medicinal properties (Sofowora *et al.*, 201

3). Medicinal plants have been used since prehistoric times as food, dietary supplements or medicines. Drug research sometimes makes use of ethnobotany to search for pharmacologically active substances, and this approach has yielded hundreds of useful compounds (Ahn, 2017). These drugs include aspirin, digoxin, quinine and opium, which are used, respectively, for the treatment of fever, arrhythmias, malaria and suppression of pain. Other products of natural product have also proved their sole role in coping with a number of deadly diseases including cancer and the diseases associated with viral onslaught viz hepatitis, AIDs etc.

Medicinal chemist, therefore, attempts to design and synthesize a pharmaceutical agent that has a desired biological effect on the human body or some other living system, such compound is called drug. Therefore, the primary objective of medicinal chemistry is the design and discovery of new compounds that are suitable for use as drug.

2.2 MEDICINAL PLANTS AS SOURCES OF ANTIMICROBIAL AGENTS

Medicinal plants have long been recognized as valuable sources of therapeutic agents, particularly in the realm of antimicrobial compounds. In recent years, the exploration of plant-derived antimicrobials has gained momentum, driven by the escalating challenge of antimicrobial resistance (AMR) and the need for novel, effective treatments.

Plants produce a diverse array of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which exhibit antimicrobial properties. These bioactive constituents can inhibit the growth of or kill pathogenic microorganisms through various mechanisms, such as disrupting microbial cell walls, interfering with protein synthesis, and inhibiting nucleic acid replication (Cowan, 1999). The complexity

and diversity of these compounds make plants a rich reservoir for potential antimicrobial agents

Recent studies have highlighted the efficacy of various medicinal plants against a broad spectrum of pathogens. For instance, a comprehensive review by Silva and Fern andes (2021) discusses the antimicrobial activity of plant-derived components and their possible mechanisms of action, emphasizing their chemical potential in combating micr obial infections. Similarly, research by Rahman and Akhter (2018) demonstrated the anti bacterial properties of *Carica papaya* seed extracts, affirming their potential as antimicro bial agents.

The antimicrobial properties of medicinal plants are not only of academic interest but also hold practical significance, especially in regions with limited access to conventi onal antibiotics. In many developing countries, traditional medicine remains a primary so urce of healthcare, and the use of medicinal plants is deeply ingrained in cultural practic es. The World Health Organization (WHO) estimates that approximately 80% of th e world's population relies on traditional medicine for their primary healthcare needs (WH O, 2013).

In the context of Nigeria, the rich biodiversity and ethnobotanical knowledge prese nt opportunities for the discovery and development of plant-based antimicrobials. Studie s have documented the use of various indigenous plants for treating infections, and scie ntific investigations have begun to validate these traditional practices. For example, rese arch by Oloyede et al. (2018) on *Carica papaya* leaf extracts revealed significant antimicr obial activity, supporting its traditional use in managing infectious diseases.

The exploration of medicinal plants as sources of antimicrobial agents aligns wit h global efforts to address the AMR crisis. By investigating the phytochemical constituen ts and antimicrobial efficacy of plants like *Carica papaya*, researchers can contribute to t he development of alternative treatments that are both effective and accessible. Such en deavors are particularly crucial in regions where the burden of infectious diseases is hig h, and the availability of conventional antibiotics is limited.

2.3 PHYTOCHEMISTRY

Phytochemistry is the study of chemical compounds produced by plants, particul arly, secondary metabolites, synthesized as a measure for self-defence against insects, p ests, pathogens, herbivores, ultraviolet exposure and environmental hazards (Chukwuebu ka *et al.*, 2018). It takes into account the structural compositions of metabolites, the bios ynthetic pathways, functions, mechanisms of action in the living systems as well as its medicinal, industrial and commercial applications (Chukwuebuka *et al.*, 2018). It is simpl y referred to as the study of phytochemicals.

Phytochemicals, from the Greek word 'phyto' meaning plants, are biologically acti ve naturally occurring chemical compounds found in plants, which provides health benef its for humans, further than those attributed to macronutrients and micronutrients (Hasle r, 1999). They are natural bioactive compounds in plants that work with nutrients to prot ect them against pathogenic attack. They are produced by plants through primary or sec ondary metabolism. They have biological activity in the plant host and play a role in plan

t growth and defence against competitors, pathogen or predators (Molyneux *et al.*, 200 7). These chemical constituents are usually concentrated in different parts of plant such as the roots, stems, leaves, flowers, fruits of seeds (Moranchian, 2000).

2.3.1 PRIMARY METABOLITES

Primary metabolites are compounds that are directly involved in the normal growt h, development, and reproduction of an organism. They are essential for cellular function s and are typically produced during the active growth phase of cells. These metabolites i nclude carbohydrates, proteins, lipids, and nucleic acids, which form the fundamental bu ilding blocks of life. In the context of medicinal chemistry, primary metabolites play an in dispensable role in cellular metabolism and provide the biochemical precursors for seco ndary metabolite biosynthesis, many of which are pharmacologically active (Nelson et a l., 2017).

Carbohydrates are among the most common primary metabolites and serve as a primary energy source through glycolysis and the citric acid cycle. Glucose, for instance, is central to cellular respiration and provides the energy currency ATP that drives various biochemical processes (Berg et al., 2015). In addition, polysaccharides such as starch an d glycogen act as energy storage molecules, while structural carbohydrates like cellulose are essential for maintaining cell wall integrity in plants.

Proteins, another crucial category of primary metabolites, are composed of amino acids linked by peptide bonds. These biomolecules serve structural, enzymatic, and regul atory functions within the cell. Enzymes, which are specialized proteins, catalyze bioche mical reactions, including those involved in the biosynthesis of both primary and second

ary metabolites. Moreover, some amino acids such as tryptophan and phenylalanine are not only essential for protein synthesis but also act as precursors to a variety of seconda ry metabolites, including alkaloids and neurotransmitters (Croteau et al., 2000).

Lipids, which include fats, oils, phospholipids, and steroids, function as structural components of cell membranes and energy reserves. Phospholipids are particularly vital as they form the lipid bilayer of cell membranes, ensuring compartmentalization and fun ctionality of cells. Additionally, sterols such as cholesterol are precursors to biologically i mportant molecules like steroid hormones and vitamin D, which have extensive medicina l relevance (Voet et al., 2016).

Nucleic acids, primarily DNA and RNA, carry genetic information and are involved in protein synthesis and cell regulation. Nucleotide derivatives such as ATP, NAD^+, and FA D also function as coenzymes and energy carriers, playing critical roles in metabolic reac tions. Without these molecules, cellular processes such as replication, transcription, and translation would be impossible (Nelson et al., 2017).

In industrial and pharmaceutical biotechnology, the production and manipulation of pri mary metabolites have significant implications. For example, ethanol and lactic acid, bot h primary metabolites, are produced by microbial fermentation and have wide applicatio ns ranging from biofuels to food preservation. Furthermore, advancements in metabolic engineering have enabled the modification of metabolic pathways to overproduce specif ic primary metabolites or channel them toward the biosynthesis of valuable secondary metabolites, such as antibiotics and anticancer agents (Nielsen & Keasling, 2016).

2.3.2 SECONDARY METABOLITES

Secondary metabolites are natural products synthesized mainly by bacteria, fungi and plants. They are molecules of low molecular weight with diverse chemical structures and biological activities (Olga, 2021). The medicinal effects of herbals is due to seconda ry metabolites. They provided lead compounds for the production of medications for tre ating various diseases from migraine up to cancer (Rehab & Amira, 2017). The secondar y metabolites are known to possess antioxidant (Wong *et al.*, 2009), antibacterial and an tifungal (Hamid *et al.*, 2011), anti-inflammatory (Kumar *et al.*, 2008), radio-protective acti vity (Kurkina, 2018). The research of Bello *et al.* (2020) showed that the nanoparticles of plant extracts also possess biological activities at an improved rate. The major class of t hese chemicals are terpenes, alkaloid and phenolic compounds.

The mechanism by which an organism biosynthesises these compounds is uniqu e to an organism or is an expression of the individuality of a species and it is termed 'sec ondary metabolism'. The most common pathways taken for this energy consuming react ion are performed through the pentose for glycosides, polysaccahrides; shikimic acid for phenols, tannins, aromatic alkaloids; acetate-malonate for phenols, alkaloids; and meval onic acid for terpenes, steroids and alkaloids (Wallingford, 1999). The most important bu ilding blocks employed in the biosynthesis of secondary metabolites are derived from ac etyl-CoA, shikimic acid, mevalonic acid and 1-deoxylulose-5-phosphate (Ginsburg, 2004). The biosynthetic scheme of plants secondary metabolites is showed below.

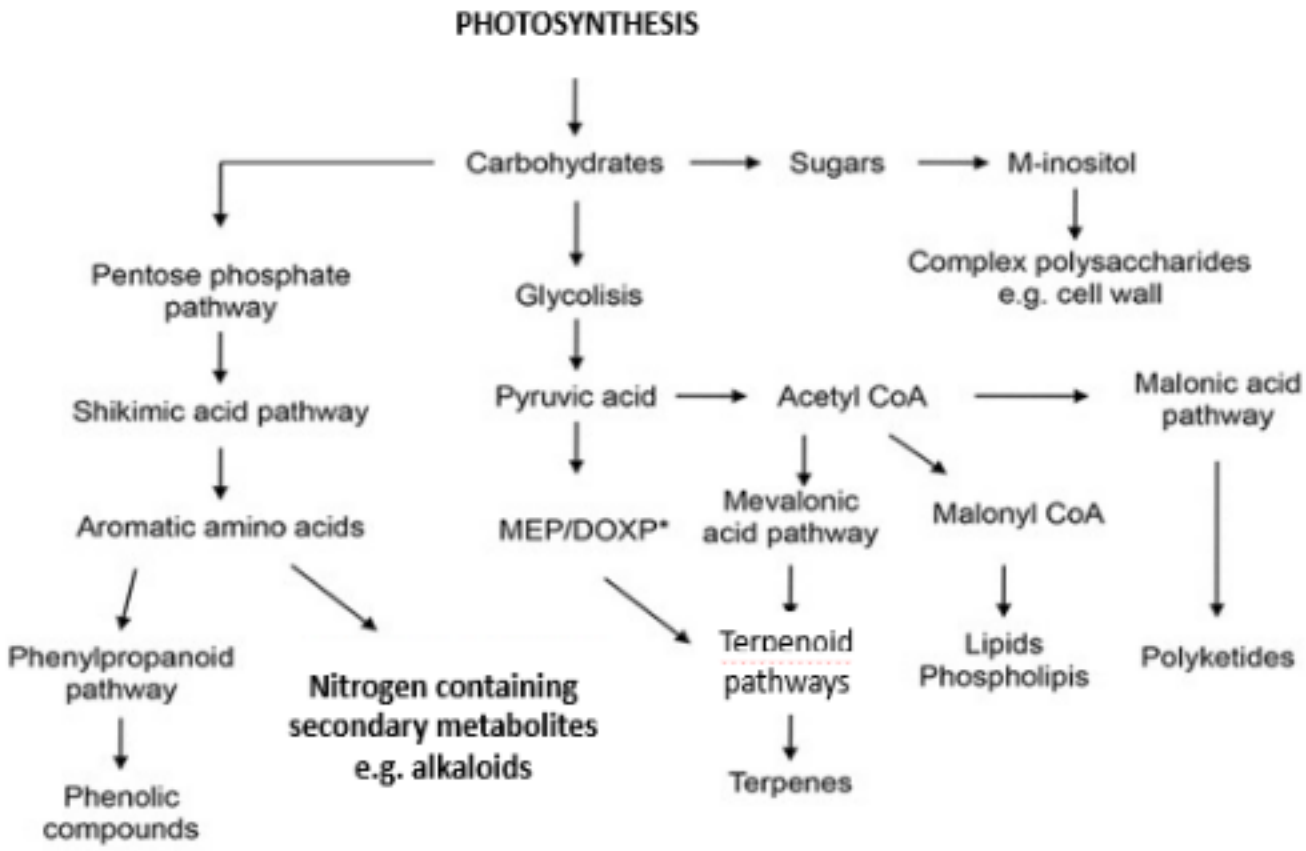

Fig. 2.1: The Biosynthetic Scheme of Plants Secondary Metabolites (Ginsburg, 2004)

2.4 EXTRACTION PROCESS

Extraction is the process that allows separating secondary metabolites from the p lant by using solvents of different polarity. It is usually carried out with different solvents of increasing polarity in order to extract as much as possible the most active component s with highest biological activity since plant matrices are naturally complex and contains a wide range of compounds that have various physical and chemical properties. Broadly, extraction involves the separation of medicinally active portions of plant or animal tissu es from the inactive or inert components by using selective solvents in standard extracti on procedures. The products so obtained from plants are relatively impure liquids, semis olids or powders intended only for oral or external use (Sukhdev *et al.*, 2008).

The initial step during extraction is the preparation of plant tissues. The extraction