PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF CURRY LEAVES

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CERTIFICATION

This is to certify that this research work was carried out *by* **ETCHIE EVAN EBUKA** the matriculation number **LSC1806700** from the department of Microbiology, Faculty OF Life Sciences**,** University Of Benin.

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APPROVAL

This project work was carried out by ETCHIE EVAN EBUKA in partial fulfilment of the award of Science, B.sc (Hons.) degree in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This research work is dedicated to Almighty God for his mercies, love and grace he has shown to me. I also dedicate this research work to my mother, father and friends for their untiring support seeing me through my university stay, amidst all stress and expenses.

TABLE OF CONTENT

TITLE **PAGE**

CHAPTER 3

CHAPTER 4

CHAPTER 5

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Curry-leaf tree is scientifically known as *Murraya koenigii*. It used as spice in Nigerian local cuisine and use as a medicinal plant in India. In recent years, greater attention has been paid to the use of plants and their extracts in traditional medicines and home remedies in the treatment of diseases and infections. This research project aim at investigating the phytochemical and antimicrobial activity on some clinical isolates which are of gastrointestinal origin.

Curry leaves were obtained from local market at New Benin, in Edo state. Pure clinical isolates of *Escherichia coli, Staphylococcus aureus* and *klebsiella* were obtained from the Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH). Identification of test organisms was based on their cultural, morphological and biochemical properties. Methanol, water and ethanol were used to extract the phytochemicals of the curry leaves. The leaf powder of the study plant was dissolved in various solvents and phytochemical analysis was carried out to test for the presence of various phytochemical constituents of the curry leaves.

The antimicrobial activity of the curry leaves extracts on the test isolates were examined using disc diffusion method. Zone of inhibition such as the minimum and maximum antimicrobial concentration of the extracts were determined. Antibiotic sensitivity test was carried out on standard antibiotics sensitivity disk. The antimicrobial effects of the curry leaves extracts was compared to standard antibiotics such as Tarivid, Augmentin, Amoxillicin, Pefloxacin, ampiclox, Streptomycin, Septrin, Erythromycin, Sparifloxacin, Ciprofloxacin, and Chloramphenicol etc.

Phytochemical test revealed alkaloid, carbonhydrate, Tannins and terpenoid in all three solvents for extract.

Cardiac glycosides was present only in methanol and ethanol extract of the sample while saponins was absent from all sample extract of the curry leaves. With all three (3) solvent extracts of the *M. koenigii* (curry leaves), minimum antimicrobial activity and zone of inhibtion at 25% aqueous concentration of the curry leaves extract against *E. coli*, while the overall maximum antimicrobial activity was recorded at a 100% aqueous concentration *against Staphylococcus aureus*. In the case of *Klebsiella pseudomonas*, all extracts failed to show antimicrobial activity.

Keywords: Antimicrobial leaves extract, *M. koenigii*, Phytochemicals, Susceptibility.

CHAPTER ONE

1.0 INTRODUCTION

The curry plant is scientifically known as *Murraya koenigii* or *Bergera koenigii*. It is a tropical and sub-tropical tree in the family *Rutaceae* (the rue family, which includes rue, citrus fruit, and satinwood), native to Asia (Plummer *et al*., 2021). This plant is also sometimes called sweet neem, although *M. koenigii* belongs to another neem family, *Azadirachta indica,* which share some similarities with the *Meliaceae* family.

Its leaves which is known as curry leaves, is used in varieties of dishes in many continents of the world, but predominantly utilized in the Indian subcontinent. Fresh curry leaves are an essential part of Indian cuisine and traditional Indian medicine. On the south and west coasts of India, they are often used in cooking, usually fried with vegetable oil, mustard seeds and chopped onions in many recipes. In Ayurvedic and Siddha medicine curry leaves is also used as herb in which they are believed to possess anti-disease properties, but there is no high-quality clinical assertion for its effectiveness and recommended use in modern medical practice. (Norman *et al*., 2012)

Classification

Kingdom:	Plantae
Sub kingdom:	Tracheobionta
Division:	Magnoliophyta
Sub-division:	Angiosperms
Class:	Eudicots
Sub-class:	Rosidae
Order:	Sapindales
Family:	Rutaceae
Genus:	*Murraya*
Species:	*Murraya koenigii*

In Nigeria, Curry leaves is added as spice and condiment to local food such as stew and soup. Fresh curry leaves are valued as seasoning in many part of the world. Though available dried, the aroma and flavor is of great culinary value. The oil can also be extracted and used in the production of scented soap.

Plants are the source of potentially useful contraptions for the development of new chemotherapeutic agents. The presence of important phytochemicals provides the plant with incredible benefits such that they help resist and fight against different diseases. In the same vein, these phytochemical properties are utilized in production of certain antimicrobial drugs and other therapy which are of medical importance to human. Phytochemicals are regarded as secondary metabolites produced at very minimal quantity, since the plant may only have little need for them. They can be produced naturally in whole parts of the plant body such as the leaves, stem, root, bark or flower, etc. (Tiwari *et al*., 2011). The curry plant is a rich source of carbazole alkaloids. Phytochemical screening over the years has revealed the presence of carbohydrates, alkaloids, steroids and flavonoids in the root extracts of the plant.

The quantity and quality of phytochemicals present in plant parts may differ from one part of the plant to another (Lahlou *et al*., 2004). The type of phytochemical present in the plant will determine its effectiveness in treatment of certain microbes. The first step towards this end is a phytochemical investigation of the plant material, followed by the *in vitro* antibacterial activity assay (Tona *et al*., 1998).

By keeping this in mind, this present investigation was conducted to study the phytochemical constituents, followed by the antibacterial activity in different solvent extracts of *M. koenigii* (curry leaves) against three clinical isolate, namely; *Eschericia coli* of gastrointestinal origin, *Staphylococcus aureus* and *klebsiella spp.*

The results obtained from this study will help determine the potency of the phytochemicals of curry leaves on pathogenic agents of gastrointestinal origin such as *E.coli*, *Staphylococcus aureus* and also *klebsiella spp.* which are of economic importance to human health. Based on the antimicrobial resistance and susceptibility testing, we would be able to determine the effective use of curry leaves on the treatment of certain infections and diseases relating to *E.coli*, *Staphylococcus aureus* and *klebsiella.*

1.1 AIMS AND OBJECTIVES OF THE RESEARCH

1. To determine the phytochemical composition of *Murraya koenigii* (curry leaves).
2. To determine the phytochemical and antimicrobial activities of the extracts of the curry leaves against clinical isolate of gastrointestinal origin e.g *Escherichia coli* and *Klebsiella.*
3. To determine the minimum and maximum antimicrobial activity of *Murraya koenigii* (curry leaves) against clinical isolate; *Escherichia coli* of gastrointestinal origin, *Staphylococcus aureus* and *Klebsiella.*

CHAPTER TWO

2.0 LITERATURE REVIEW

Curry leaves, scientifically known as *Murraya koenigii*, are a prevalent fragrant herb found abundantly not only in Sri Lanka but also in various other Asian nations. The curry trees typically attain heights of 4-6 meters and have trunks reaching a diameter of 40 centimeters. Although the tree bears small, glossy black berries that are edible, it's important to note that their seeds possess toxic properties. In the realm of Indian cuisine, curry leaves hold a pivotal role, often used as a seasoning to infuse a distinct aroma into each dish. Beyond their aromatic properties, these leaves also offer probiotic advantages for human well-being.

Among curry leaves enthusiasts, particularly those from Gujarat and South India, the appreciation for these leaves is pronounced, as they are extensively incorporated into everyday culinary preparations. Their usage prominently enhances the flavor profiles of dishes such as poha, sambhar, dokhla, dal, upma, nariyal chutney, and kadi. Enriched with notable nutrients such as calcium, phosphorous, iron, and vitamins C, A, B, and E, curry leaves assume significant medicinal value. Their attributes encompass combating infections, enhancing hair and skin quality, and regulating blood sugar levels, all the while promoting optimal digestion. The leaves exhibit a mildly robust and subtly acidic taste, and these qualities, encompassing flavor, taste, and medicinal properties, persist even upon drying.

2.1 Morphological Characteristic of *Murraya koenigii* (curry leaves)

The standard variety of *Murraya koenigii* is recognized for its rapid growth rate, boasting attractive leaves that are a rich shade of deep green. On the other hand, the compact variant takes on a shrub-like form, characterized by sprawling branches that create a bushy appearance. Its leaves are a pale shade of green, somewhat taller than those of the regular type, and emit their own distinct aroma. The brown variety stands out as the most aromatic, showcasing a dense leaf structure that is notably small and colored in dark brown.

Murraya koenigii typically manifests as a modestly spreading shrub, reaching a height of approximately 2.5 meters. Its stem displays hues ranging from dark green to brownish tones. When the bark is carefully peeled away lengthwise, the underlying white wood becomes visible. The central stem boasts a diameter of around 16cm. Each leaf, measuring approximately 30 cm in length, carries 24 leaflets and exhibits a reticulate venation pattern. The flower, with its white funnel-like shape, exudes a pleasantly aromatic quality. When fully opened, the flower averages a diameter of 1.12cm and possesses bisexual characteristics. The fruits, which vary in shape from round to oblong, measure between 1.4 and 1.6cm in length and 1 to 1.2cm in diameter. Upon complete ripening, the fruit adopts a black hue and acquires a lustrous surface, while the inner pulp takes on a shade reminiscent of wisteria blue. The seeds, tinted a vibrant spinach green, measure 11mm in length and weigh around 445mg (Singh *et al*., 2014).

2.2 Distribution

Murraya koenigii is distributed and cultivated across the expanse of India. Its presence spans regions from Sikkim to Garhwal, encompassing Bengal, Assam, the Western Ghats, and Travancore-Cochin. The seeds possess a natural propensity to germinate under the shelter of shade or partial shade. This variety of curry leaves can be encountered in the damp woodlands situated at elevations ranging from 500 to 1600 meters, particularly prominent in areas such as Guangdong, southern Hainan, and southern Yunnan. Beyond India, it extends its habitat to Bhutan, Laos, Sri Lanka, Thailand, Nepal, and Vietnam. As a result of migration from South India, curry leaves have also made their way to places like Malaysia, South Africa, and Reunion Island (Singh *et al*., 2014).

2.3 Phytochemistry

Mature curry leaves exhibit a moisture content of 63.2%. The protein content constitutes approximately 1.15% of nitrogen, while the carbohydrate content accounts for 14.6% of total sugars. Furthermore, the leaves contain a total ash content of 13.06%. Among the bioactive constituents present in curry leaves are oxalic acid, resin, and carbazole alkaloids. Notably, significant bioactive compounds include koenigin, bicyclomahanimbicine, cyclomahanimbine, murrayastine, coumarin, koenidine, and pypayafolinecarbazole. These compounds showcase noteworthy pharmacological activities. In the volatile oil fraction, the predominant components are bicyclomahanimbicine and amahanimbicine, constituting a major portion of the volatile oil content (Ganesan *et al*., 2013).

2.4 Culinary and Nutritional Value of Curry Leaves

Because of its aromatic, flavor-enhancing, and nutritional attributes, curry leaves are incorporated into a diverse array of dishes worldwide, spanning continents from Africa and Asia to South America. Curry leaves are particularly abundant in essential nutrients such as copper, minerals, calcium, phosphorus, dietary fiber, carbohydrates, magnesium, and iron. Additionally, these leaves frequently contain a variety of vitamins and amino acids. Depending on the intended culinary application, the leaves can be subjected to processes like drying or frying, although the fresh form remains widely popular (Mamta and Kanika, 2019).

The nutritional composition is outlined as follows: Carbohydrates contribute 60%, while moisture content stands at 5.86%. Protein content accounts for 3.81%, with an ample magnesium content of 568mg. A mere 0.1mg of calories is present, alongside 1.5mg of potassium. The leaves offer 0.50% Vitamin A, an impressive 2218mg of calcium, 0.50mg/100gm of Vitamin C, and 0.5mg/100gm of Vitamin B1 (Mamta and Kanika, 2019).

2.5 Traditional Uses of Curry Leaves

Recently, there has been increased attention towards incorporating Curry leaves into traditional remedies and natural treatments. However, limited research has been undertaken to comprehensively assess the medicinal and pharmacological potential of curry leaves in terms of promoting well-being and addressing ailments (Balakrishnan *et al*., 2018). Both essential oils and fresh leaf powder derived from curry leaves are valuable in seasoning culinary preparations and crafting ready-to-eat foods. The essential oil extracted from leaf sources, due to its heightened antimicrobial properties (Erkan *et al*., 2012), can also serve as a fragrance and flavor enhancer in traditional practices. An intriguing technique involves boiling fresh curry leaves with a mixture of coconut oil until they transform into a dark residue, yielding an effective hair tonic for maintaining natural hair color and promoting hair growth. Across traditional practices, curry leaves have been embraced for their roles as antidiarrheal, antifungal, blood-purifying, anti-inflammatory, and antidepressant agents (Joshi *et al*., 2018).

Historical applications of the curry leaf plant are extensive in South Asian cuisine due to its distinctive taste and aroma, with its use as a household remedy dating back generations (Chauhan *et al*., 2017). These fragrant leaves are widely employed to enhance the flavor of curries, thereby stimulating appetite and aiding digestion (Kataria *et al*., 2013). Locally, the leaves find usage in the treatment of external wounds, burns, and even to counteract the effects of venomous animal bites. They are also employed for addressing rheumatism (Tan *et al*., 2017). Roasted leaves are a remedy for preventing vomiting, while finely ground leaves mixed with buttermilk offer relief from stomach discomfort and can function as a gentle laxative when consumed on an empty stomach (Patel *et al*., 2019). A mixture of fresh leaf juice, lime, and sugar is used to alleviate morning sickness, while root juice consumption provides relief from kidney-related pain (Nishan and Subramanian, 2015). The stem is utilized for dental hygiene, contributing to gum strengthening (Yankuzo *et al*., 2011). The fruit's properties counteract astringency, while root juice aids in alleviating kidney pain. Moreover, the plant's antitumor, hypoglycemic, and anti-hypercholesterolemic effects have been identified (Kumari and Papiya, 2014). Curry leaves are employed to address issues like piles, body heat, inflammation, and itching (Bhandari, 2012). In traditional Ayurveda, various parts of the curry leaf are used to combat coughs, hypertension, hepatitis, rheumatism, and hysteria (Ghasemzadeh, 2014). Additionally, the traditional Ayurvedic practice involves boiling curry leaves with coconut oil until reduced to a concentrated state, which is then used as a hair tonic to maintain hair color and stimulate hair growth (Gunjan *et al*., 2015).

2.6 Industrial Uses

The essential oils derived from the plant possess versatile applications, finding utility in sun protection creams and erythema formulations. Furthermore, they have potential for employment in aromatherapy within the soap and cosmetic sector (Mittal *et al*., 2017). The curry leaf plant contributes to a few industrial products, including volatile oil, crystalline glycoside, and murragin extracted from its flowers. Key components like beta-carotene, folic acid, riboflavin, calcium, and zinc contained within curry leaves hold relevance for oral health and could be incorporated into the production of mouthwashes (Math and Balasubramaniam, 2014). India significantly exports curry leaf oil, characterized by its yellow, clear, and transparent appearance, extracted from the plant's seeds (Joseph and Peter, 1985). Extracts from the plant's stem are well-suited for formulating creams aimed at skin lightening and enhancing rough skin (Dhongade *et al*., 2013). Extracts obtained through petroleum ether and acetone from the leaves offer potential as larvicides against Aedes aegypti mosquitoes (Nishan and Subramanian, 2015).

2.7 Modern Medical Significance of Curry Leaves

Over the course of time, a series of research endeavors and experimental studies have been undertaken to highlight the medical potency and consequential value in treating specific ailments, thus contributing to human health improvement. Medicinally, these leaves have been employed to manage diarrhea, dysentery, and to avert instances of vomiting. Additionally, both the leaves and fruits yield essential oils that serve as fixatives for perfumed soaps. The tonic, stomachic, and carminative properties of the leaves, root, and bark have been acknowledged. The root juice has been recognized for its efficacy in relieving renal pain (Kirtikar *et al*., 2013).

Curry leaves are notably rich in nutrition and encompass several medicinal attributes, including antidiabetic, antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic properties, attributed to a range of essential phytochemicals, minerals, and trace minerals. *M. koenigii* has shown promise in reducing the reliance on medications for various metabolic and non-communicable diseases like diabetes, cancer, cardiovascular conditions, and obesity (Samanta *et al*., 2018). This is particularly crucial since a majority of these diseases are linked to elevated mortality and morbidity rates (Hu *et al*., 2020). The current thrust of research has shifted toward exploring the bioactivities of natural products from herbal origins.

In the global food industry, greater emphasis is now placed on natural preservatives due to the growing occurrence of resistance among pathogenic strains to chemical food preservatives. The investigation of plant extracts and products for their antimicrobial potential has demonstrated that higher plants hold the potential to serve as sources of novel antibiotic prototypes.

Many of the presently used antimicrobial agents come with adverse effects, including risks like blood cancer, gastrointestinal complications, organ damage, toxicity, hypersensitivity, immunosuppression, and the presence of residual compounds in tissues, posing significant public health concerns. Moreover, synthetic broad-spectrum antibiotics tend to be expensive and inaccessible to the economically disadvantaged. Additionally, pathogens are increasingly developing resistance against a significant portion of these synthetic drugs.

Numerous plant constituents have exhibited efficacy as remedies for various diseases, contributing to the identification of around seven thousand pharmaceutically valuable compounds in Western pharmacopeia, several of which are crucial drugs. The utilization of plant extracts and phytochemicals with recognized antimicrobial attributes holds significant promise in therapeutic applications.

According to a WHO survey, around 80% of populations in developing countries primarily rely on traditional medicine, often involving the use of plant extracts. Exploration of plants primarily focuses on discovering novel secondary metabolites or phytochemicals that offer protective functions for human consumers. In recent years, substantial efforts have been devoted to investigating the pharmacological benefits of curry leaves through scientific methods.

The curry leaf tree is a versatile species, with different parts utilized in folk medicine to address various ailments. It has been proven to possess noteworthy wound-healing capabilities and exhibits antioxidant activity, boasting a high degree of radical-scavenging properties. Curry leaves hold significant disease-remedial potential, with various plant parts – leaves, roots, and bark – being employed as tonics to promote digestion and alleviate flatulence, as well as antiemetics. The leaves and roots possess a bitter, cooling nature and serve to reduce fever. Root juice is applied for managing renal pain. Curry leaves, when boiled in milk and transformed into a paste, hold promise for treating venomous bites and skin eruptions (Sim and Teh, 2021).

The aromatic bioactive components present in curry leaves retain their attributes, including flavor, even after the drying process (Amna *et al.,* 2019). These slightly bitter leaves are characterized by a pungent aroma and weak acidity. They are embraced as antihelminthics, analgesics, digestives, and appetizers in Indian cuisine (Bhandari *et al*., 2022). In traditional usage, green curry leaves are applied to address a range of issues, such as piles, inflammation, itching, fresh cuts, dysentery, bruises, and edema. The roots are found to have mild purgative qualities, serving as stimulating agents for general body aches. The bark finds application in the treatment of snakebites (Goutam *et al*., 2018). The essential oil extracted from curry leaves has been reported to possess antioxidative, hepatoprotective, antimicrobial, antifungal, anti-inflammatory, and nephroprotective activities in animal models (Ma *et al*., 2016). The medicinal attributes of curry leaves can be attributed to various chemical constituents, including diverse carbazole alkaloids and other key metabolites like terpenoids, flavonoids, phenolics, carbohydrates, carotenoids, vitamins, and nicotinic acid present across different parts of the curry leaf plant.

With an escalating demand for plant-based medicines, health products, pharmaceuticals, food supplements, and cosmetics, the common curry leaf tree, *Murraya koenigii*, emerges as a multi-faceted source of medicinal products. Utilized across folk medicine, distinct parts of the plant offer treatment for an array of maladies. Notably, the plant has demonstrated significant wound healing capabilities and impressive antioxidant activity marked by radical-scavenging properties. The chemical constituents found within crude leaf extracts of *M. koenigii* hold considerable pharmacological value for human health (Ahmed *et al*., 2010).

Phytochemical screening serves as a method to reveal components or properties in plants that are readily available for bioactivity or ethnomedical applications. Plant-derived antimicrobials hold substantial therapeutic potential due to their capacity to address concerns with fewer side effects than synthetic antimicrobials (Iwu *et al*., 2019). Thus, it is foreseeable that phytochemicals displaying effective antibacterial properties could be harnessed for bacterial infection treatments (Balandrin *et al*., 2018). As antioxidants and antimicrobial agents, various extracts from numerous plants have garnered interest both in research and the food industry, as they could serve as natural alternatives to synthetic additives (Deba *et al*., 2018). The significance of medicinal plants lies in their effectiveness, minimal side effects, and relatively lower costs when compared to synthetic drugs (Raj *et al*., 2011).

Medicinal plants are abundant in essential phytochemicals and secondary metabolites that contribute to their antimicrobial, anti-inflammatory effects, and other known biological activities. The observed phytochemical compounds in these extracts have been reported to possess psychological activities and medicinal importance. The substantial biological activities of each phytochemical may enhance the identification of novel antibiotic and antifungal agents against pathogens associated with the *M. koenigii* species.

Alkaloids play a vital role in analgesic, neuroprotective, antimicrobial, and antimalarial actions (Hassan *et al*., 2020). Plant-derived flavonoids exhibit antidiarrheal, antimicrobial, antioxidant, and anti-inflammatory properties. Polyphenolic flavonoids create complexes with bacterial cell walls, thereby exerting biological effects (Hassan *et al*., 2020). Other compounds such as steroids and tannins demonstrate potent antimicrobial properties (Hetty et al., 2019). Terpenes function as antimicrobial agents by weakening microorganism tissues and cell walls. Additionally, they possess anticancer and antidiabetic properties (Cox-georgian *et al*., 2019).

Therefore, individual phytochemicals contribute various biological activities, including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, and anticancer effects (Abulude *et al*., 2007). Some of the applications of curry leaves, also known as *Murraya koenigii*, are explored in detail below;

2.7.1 Anti-Inflammatory Properties

Alcohol-based extracts obtained from the stem bark and the crude root of *Murraya koenigii* exhibit anti-inflammatory properties, as indicated by (Darvekar *et al*. 2011). Inflammatory responses, which encompass tissue damage, cell injury, pathogen-induced infections, and biochemical alterations, are a biological reaction. Within neurological disorders, inflammation involves crucial elements such as mast cells, microglia, ependymal cells, astrocytes, and macrophages. Notably, microglia, a type of neuronal support cell functioning as resident macrophages distributed throughout the brain, respond actively to inflammation by altering their morphology. They participate in eliminating damaged neurons and pathogens. Ethanol-based extracts derived from curry leaves have demonstrated substantial analgesic and anti-inflammatory activities, validated through the examination of carrageenan-induced hind paw edema in albino rats (Gupta *et al*., 2020). Another investigation also substantiated the anti-inflammatory potential of curry leaf extracts in the context of carrageenan-induced paw edema (Darvekar *et al*., 2017). Moreover, this study highlighted the analgesic attributes of curry leaves, drawing on various experimental models.

Curry leaf extracts effectively alleviate pain, evident in scenarios such as intraperitoneal acetic acid injection and subplantar formalin injection-induced discomfort in mice. The analgesic effect was corroborated through observations of writhing responses and late-phase pain responses. Additionally, the study noted that higher concentrations (20 and 40 mg/kg, administered orally) managed to mitigate early-phase inflammatory responses prompted by formalin (Mani *et al*., 2019).

2.7.2 Antidiabetic Properties

Especially prevalent in developing nations, medicinal plants play a pivotal role in addressing diabetes mellitus due to their economic viability. Diabetes mellitus, a metabolic disorder, is emerging as a significant menace to human well-being. In recent years, numerous phytochemicals with anti-diabetic attributes have been isolated from various plants. The presence of alkaloids within curry leaves' foliage has been investigated, revealing inhibitory effects on the aldose reductase enzyme, glucose utilization, and other enzyme systems that contribute to extended anti-diabetic effects (Patel et al., 2012).

Furthermore, curry leaves were subjected to assessment for their α-glucosidase inhibitory property, and findings indicated successful inhibition of α glycosidase. Alpha-glucosidase inhibitors find widespread use in treating type 2 diabetes patients. A study reported a significant reduction in blood glucose levels resulting from an ethanolic extract of curry leaves. This reduction in blood glucose is attributed to the antioxidant characteristics and insulin mimetic effects of curry leaves. Moreover, curry leaves demonstrated robust antioxidant effects by diminishing malondialdehyde (MDA) levels, elevating glutathione (GSH) levels, and markedly reducing the homeostatic model assessment (HOMA)-insulin resistance index. Collectively, the evidence underscores the antidiabetic potency of curry leaves and their capacity for antioxidant effects in rat models (Husna et al., 2018).

2.7.3 Antioxidants Properties

The most potent antioxidant and free radical scavenging activity has been identified in the alcohol-water (1:1) extract of *Murraya koenigii* (Ningappa *et al*., 2018). Extracts containing natural antioxidants from plant origins have garnered attention as promising therapeutic options for mitigating and treating diseases, particularly neurodegenerative disorders, cardiovascular ailments, cancer, and other related conditions. Within curry leaves, various natural bioactive compounds, such as mahanine, mahanimbine, isolongifolene, koenimbine, girinimbine, isomahanine, koenoline, and O-methylmurrayamine, manifest exceptional antioxidant attributes (Rehana *et al*., 2017). Reactive oxygen species (ROS), including singlet oxygen (O_2), hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and the hydroxyl radical (OH), often arise as byproducts of cellular metabolic reactions and exogenous influences. These ROS disturb homeostasis, leading to oxidative stress formation, which in turn triggers cell death and tissue damage (Brand *et al*., 2014). Elevated ROS levels can inflict harm on biomolecules such as nucleic acids, proteins, and lipids. Despite the presence of antioxidant defense systems like enzymatic and non-enzymatic antioxidants, unchecked ROS accumulation during the lifespan fosters the development of age-associated ailments like cancer, atherosclerosis, and arthritis.

The extracts derived from curry leaves showcase robust antioxidant potential. Rao assessed the antioxidant activities of water and ethanol extracts of curry leaves through the α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging assay, employing quercetin as a positive control. Yogesh et al. gauged the antioxidant prowess of curry leaves berry extracts using DPPH free radical scavenging and reducing power assays. Outcomes revealed that curry leaves berry extract outperformed recognized antioxidants like butylated hydroxytoluene, ascorbic acid, and tannic acid in free radical scavenging (Yogesh *et al*., 2018). Tomar et al. evaluated total antioxidant activity in acetone and petroleum ether extracts from young and mature curry leaves, utilizing H2O2 scavenging activity as a measure. The maximum activity of 151.58% was recorded in the acetone extract of mature leaves, and for young leaves in petroleum ether, the value was 72.23% (Rajesh *et al*., 2017). Additionally, the fruit extract of curry leaves exhibited antioxidant properties, with EC50 values of 2.6 mg/mL for the DPPH assay, 2.9 mg/mL for NO radical, 3.1 mg/mL for TBARS, 2.7 mg/mL for the reducing power assay, and 3.3 mg/mL for H_2O_2, comparable to the EC50 value of 5 mg/mL shown by vitamin C as the positive control. The potent antioxidant activities demonstrated by curry leaf extracts likely stem from the presence of flavonoids and phenolic derivatives. The aforementioned studies collectively underscore the high antioxidant potential exhibited by various curry leaf extracts and highlight the plant's capability to serve as a natural source of potent antioxidant compounds with therapeutic applications against ROS-induced human diseases.

2.7.4 Anticancer Activity (*In Vivo* and *In Vitro*)

Curry leaves demonstrate the presence of secondary metabolites with promising potential for being developed into anticancer agents. In a particular investigation, the cytotoxic efficacy of three distinct extracts - hexane, ethyl acetate, and methanol - sourced from curry leaves was assessed against the HeLa cell line. The findings revealed that these extracts exhibited potent cytotoxic properties against HeLa cancer cells. These outcomes underscore the in vitro anticancer potential of curry leaves as corroborated by (Amna *et al*., 2019). Further substantiation of curry leaves' anticancer attributes has emerged from studies involving rodent cancer cell lines and diverse in vivo cancer models (Pei *et al*., 2018).

2.7.5 Mitochondrial Dysfunction

Mitochondria serve as the primary hub for cellular high-energy metabolism, earning them the moniker of the cell's powerhouse. In addition to this crucial function, mitochondria assume responsibility for calcium homeostasis regulation and are key players in counteracting free radicals and governing programmed cell death via the apoptosis-signaling pathway. The impairment of mitochondria brings about a cascade of repercussions including diminished adenosine triphosphate (ATP) production, escalated generation of reactive oxygen species (ROS), compromised calcium buffering, mitochondrial DNA (mtDNA) damage, morphological changes in mitochondria, and disruptions in mitochondrial fission and fusion processes. These collective events ultimately culminate in cellular demise (Beal et al., 2019).

It is presently understood that a significant portion of ROS production is attributed to mitochondrial complexes I and III, which likely results from the liberation of electrons by NADH and Dihydroflavine adenine dinucleotide ($FADH_2$) incorporated into the electron transport chain (ETC). Mitochondrial dysfunction stands as a hallmark of various chronic diseases and the aging process, characterized by ETC efficiency decline and a decrease in high-energy molecule synthesis. These encompass neurodegenerative conditions like Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease, cardiovascular diseases, autoimmune disorders, diabetes, and others (Ischiropoulos and Beckman, 2013).

For neuronal cell viability, mitochondria assume a pivotal role as indispensable organelles. Perturbations in the mitochondrial membrane potential (MMP) are triggered by complex I inhibition, resulting in excessive ROS formation. Pro-apoptotic elements are presumed to instigate the permeabilization of the outer mitochondrial membrane through the creation of oligomeric pores, facilitating the release of apoptogenic molecules from the intermembrane space. Recent investigations have scrutinized the neuroprotective capabilities of compounds such as isolongifolene and structurally akin derivatives including girinimbine, murrayazoline, and O-methylmurrayamine A, derived from *M. koenigii*. Through diverse in vitro assays, it was revealed that these bioactive agents possess the capacity to reinstate MMP levels and rectify mitochondrial impairments (Balakrishnan *et al*., 2018).

2.8 Phytochemical Properties

Numerous studies have been conducted over time to explore the phytochemical properties of curry leaves, showcasing their advantageous composition and effectiveness in microbial inhibition within food contexts.

In a recent investigation involving freshly prepared ethanolic extracts of *Murraya koenigii*, a comprehensive assessment of phytochemical constituents was conducted to ascertain their antibacterial attributes. The phytochemical analysis revealed a significant presence of various primary and secondary metabolites responsible for the antibacterial properties. Notably, the studied curry species demonstrated a phytochemical profile encompassing alkaloids, flavonoids, triterpenes, tannins, and unsaturated steroids as the most prominent types of phytochemicals. However, a lack of saponins was found in both extracts, which is consistent with previous findings that suggested the absence of saponins in the tested curry leaf species (Abeysinghe *et al*., 2021; Vats *et a*l., 2011).

Raaman performed a phytochemical analysis on curry leaf extracts, revealing the presence of diverse compounds including alkaloids, glycosides, saponins, proteins, amino acids, fixed oils, phenolic compounds, tannins, flavonoids, gum, and mucilage (Rashmi *et al*., 2018). Raaman collected mature leaves of *M. koenigii* from the Kattakada area in Thiruvananthapuram district, Kerala. The leaves underwent drying and crushing to produce powdered form, which was then subjected to extraction with three different solvents: water, acetone, and chloroform. The powdered leaves (10 grams) were combined with 100 ml of distilled water, boiled for approximately two hours, and subsequently filtered. The outcome of the phytochemical assessments of the three extracts indicated that the acetone and water extracts exhibited more positive outcomes for glycosides, oils, saponins, and flavonoids. The phytochemical analysis unveiled the presence of six compounds: flavonoids, glycosides, oils, saponins, phenolics, and gum/mucilage. The acetone and chloroform extracts showed the presence of flavonoids, glycosides, oils, and saponins (Raaman, 2018).

2.9 Antibacterial Properties and activity

The unregulated utilization of antibiotics is fostering the emergence of drug-resistant bacterial strains, posing severe threats to public health due to the scarcity of effective treatment options. Consequently, the search for novel antimicrobials remains imperative. In tandem with conventional antibiotics and synthetically derived drugs, there is a growing exploration of potent compounds sourced from natural herbal medicines as an alternative. This is driven by the potential for these natural remedies to yield fewer side effects and exhibit lower toxicity owing to their inherent origins (Deb *et al*., 2015).

Efforts to combat microbial infections while minimizing side effects are complex endeavors. As part of this pursuit, the ongoing exploration of impactful molecules from natural herbal sources, alongside traditional antibiotics and synthetic drugs, persists. Curry leaf extracts have showcased notable antibacterial effects against a diverse array of microorganisms. Specifically, methanol and ethanol extracts of curry leaves have demonstrated efficacy against bacterial strains such as *Escherichia coli (E. coli), Staphylococcus, Streptococcus*, and *Proteus*. This positions curry leaves as a potentially effective natural remedy for averting various bacterial infections when integrated into everyday meals (Qais *et al*., 2019). Additionally, pyranocarbazoles isolated from curry leaves have exhibited antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* (Josh *et al*., 2018). Furthermore, silver nanoparticles synthesized from curry leaves have shown therapeutic efficacy against multidrug-resistant bacteria (Qais *et al*., 2019).

Curry leaves' essential oil has demonstrated antibiofilm properties against *Pseudomonas aeruginosa*, with a significant 80% reduction in biofilm formation observed upon treatment. Gas chromatography-mass spectrometry (GCMS) analysis identified substances like spathulenol, cinnamaldehyde, and linalool as potential contributors to these antibiofilm effects (Sankar and Rai, 2016). Moreover, studies have revealed the antibacterial impact of curry leaves on uropathogenic bacteria isolated from clinical samples (Rath and Padhy, 2014). An evaluation of curry leaves' antibiotic action against Mycobacterium species exhibited appreciable effectiveness akin to first-line anti-tuberculosis drugs. Notably, curry leaves' ethanol extract demonstrated significant synergistic antibacterial activity when combined with the anti-tuberculosis drug rifampicin (Naik *et al*., 2014).

The antimicrobial attributes of flavonoids isolated from aqueous extracts of *Murraya koenigii* have been documented. In vitro assays involving the ethanol extract of curry leaves demonstrated antibacterial activity against clinical isolates of *E. coli* and *Pseudomonas aeruginosa*. The antibacterial activity displayed variation based on extract concentration, with higher concentrations showing heightened effectiveness. This aligns with earlier findings that extracts from various parts of *M. koenigii*, containing secondary metabolites like phenols, essential oils, terpenoids, alkaloids, and flavonoids, possess strong antibacterial properties (Rashmi, Jyoti Bisht, and Naveen G, 2018).

Another study by Abeysinghe *et al*. (2021) assessed the antibacterial potential of *M. koenigii* against three bacterial strains. The study identified notable inhibitory activity against *Staphylococcus aureus* and *Escherichia coli* at a 1.0 mg/mL concentration. This study highlighted the positive correlation between total phenolic content (TPC) and total flavonoid content (TFC) with higher antibacterial and antioxidant activity. The methanolic leaf extracts exhibited varying degrees of antibacterial activity against selected bacterial species, with ciprofloxacin serving as the reference antibiotic (Abeysinghe *et al*., 2021).

Neethu (2006) conducted a qualitative phytochemical screening of crude leaf extracts using water, alcohol, and chloroform solvents. The analysis revealed the presence of glycosides, alkaloids, oils, saponins, and flavonoids. In an antimicrobial assessment against bacterial and fungal strains, the curry leaf extract exhibited higher antibacterial than antifungal activity. These findings underscored the rich repository of primary and secondary metabolites in curry leaves that contribute to their antimicrobial attributes.

2.10 Antifungal Properties and activity

Diverse research endeavors have underscored the antifungal attributes of curry leaves. Notably, the essential oil extracted from the leaves has exhibited antifungal properties. The potency of the antifungal activity emanates from the intricate molecular compositions and varied action mechanisms of curry leaf phytochemical constituents, encompassing alkaloids, terpenoids, flavonoids, phenolics, tannins, and saponins, which are renowned for their antimicrobial efficacy. Multiple investigations corroborate the traditional utilization of this plant as an antifungal agent. The observed in vitro antifungal activity aligns with the historical application of curry leaves in folk medicine for the management of conditions like diarrhea, dysentery, and skin eruptions. The bioactive compounds within curry leaves notably possess the capacity to impede mycelial growth, thereby fostering antifungal effects. The spectrum of antifungal action of curry leaves encompasses a broad range of pathogenic fungi, including *Penicillium notatum, Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Mucor mucedo,* and *Penicillium funiculosum*, discerned through alterations in growth characteristics, mycelial morphology, and spore traits. Within this context, the ethanolic extract of curry leaves has demonstrated distinct effects on hyphal morphology, characterized by augmented branching and the development of truncated, elongated hyphal branches with swollen tips, attributes common to antifungal agents. Prominent bioactive compounds such as girinimbine, murrayanine, and marmesin-1′-O-beta-D'galactopyranoside are present in the stem bark, exhibiting notable antifungal efficacy (Bonde et al., 2021).

To assess the biological relevance and potential of the plant extract, the antifungal prowess of *M. koenigii* (leaf ethanol extract with DMSO) was evaluated in vitro using the agar cup method against clinical fungal isolates, *Candida albicans* and *Aspergillus niger*. The assessment revealed that the leaf extract's antifungal activity against both strains exhibited no notable inhibition at concentrations of 25µl and 50µl but resulted in an 11mm zone of inhibition each at a concentration of 100µl. The present study indicated that the ethanol leaf extracts of *M. koenigii* exhibited higher activity against clinical bacterial pathogens like *E. coli* and *P. aeruginosa*. However, the antifungal activity was relatively modest compared to the bacterial activity. Literature corroborates that the antibacterial potential is linked to diverse chemical agents within the leaf extract, encompassing essential oils, flavonoids, terpenoids, and other bioactive compounds classified as active antimicrobial agents. These findings support, to a certain extent, the utilization of traditional medicinal plants in treating human and animal diseases and emphasize the efficacy of an ethnobotanical approach in exploring plants as potential sources of bioactive substances (Valsaraj et al., 2017). Notably, the aqueous extract tends to exhibit significant antibacterial efficacy, further affirming the traditional therapeutic claims associated with this plant (Perumalsamy, 2018).

Conclusively, Raaman's research underscores the variable phytochemical makeup of *M. koenigii* leaves, highlighting the presence or absence of specific components, yet consistently revealing substantial levels of significant phytochemical compounds.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Bacterial isolates used for study

The clinical isolates which were *Escherichia coli, Staphylococcus aureus* and *Klebsiella* were obtained from the culture the culture collection unit of Medical Microbiology Laborartory, University of Benin Teaching Hospital (UBTH). The bacterial stock cultures were maintained at 4^0C. All microbial stock cultures were freshened by sterile inoculation loop on nutrient agar plates under a suitable condition and temperature of 37^0C for 24hours. The following day, the streaked cultures were again subcultured on media plates and incubated at 37^0C for 24 hours.

3.2 Apparatus and Equipment

Test tubes, conical flask (500ml and 1000ml), Petri dish, measuring cylinder, Bunsen burner, pipette, inoculating loop, cotton wool, sensitivity disk, incubator and microscope.

3.3 Preparation and Sterilization of Materials

The media used was prepared according to the manufacturer's instructions. Sterilization of glassware and other autoclavable materials was performed at 121^0C for 15 minutes. Work surfaces was sterilized using 3% alcohol.

3.4 Identification And Confirmatory Test Of The Organisms

The bacteria isolates obtained from the culture collection unit of Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH) were identified based on their morphological, cultural and biochemical characteristics. Biochemical test included indole, citrate, oxidase, triple sugar iron, and Potassium hydroxide test.

3.4.1 Indole test

The indole test relies on the reaction between indole and p-dimethylaminobenzaldehyde in kovac reagent, yielding a red complex. Pure bacterial cultures were grown in sterile peptone broth at 37°C for 24 hours, and kovac reagent was added to the specimen concentration in the test tubes, resulting in the rapid appearance of a red coloration on the medium's surface, indicating the presence of indole (*Cheesbrough, 2006*).

3.4.2 Citrate test

For the citrate test, Simmon's citrate agar was used to determine the bacterium's ability to utilize citrate as its sole carbon source. Bacterial colonies were inoculated into Simmons citrate agar slopes and incubated at 37°C overnight, causing a color change from green to blue in the medium if citrate utilization occurred.

3.4.3 Oxidase test

The oxidase test involved placing two drops of 1% freshly prepared oxidase reagent (phenylenediamine) on a filter paper. Test organisms were smeared on the paper, with a positive result indicated by deep purple coloration within 5-30 seconds.

3.4.4 Triple Sugar Iron (TSI) Test

Triple Sugar Iron (TSI) test is a microbiological test that assesses a microorganism's ability to ferment sugars and produce hydrogen sulfide. TSI is inoculated by stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The tube is incubated at 35°-37°C in ambient air for 18 to 24 hours. Carbohydrate fermentation is evidenced by gas production and a change in pH indicator color from red to yellow. Bubbles or cracks in the agar medium indicates gas formation (CO2 and H2).

Potassium Hydroxide Test (KOH Test)

The purpose of the potassioum hydroxide test (KOH test) is to identify gram-negative bacteria. KOH dissolves the thin layer of peptidoglycan in the cell walls of gram-negative bacteria, but does not affect the cell walls of gram-positive bacteria. One drop of 3% KOH is applied on a microscopic slide and a loop is used to transfer a good amount of bacteria (cultivated for 24-48 h) to the drop of KOH. It is stirred carefully. The solution of gram negative-bacteria becomes viscous and forms a mucous thread in 30 seconds. For positive results, the solution with the bacteria (gram negative) will be viscous while for negative results, the solution with the bacteria (gram positive) will not be viscous.

3.5 .0 Preparation of Media Culture

To successful carry out our research, the clinical isolates were cultured in suitable medium. Three (3) Agar medium in total were utilized at different occasion in the course of this research experiment.

3.5.1 Mueller Hinton Agar

This is a microbiological growth medium that is commonly used for antibiotic susceptibility testing. In preparation, 38g was dissolved in 1000ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{0}C for 15 minutes. The medium was cooled to 45-50^{0}C and then dispensed aseptically into sterile Petri dishes in the laminar flow hood cabinet.

3.5.2 Nutrient Agar

Nutrient Agar is a general-purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and non-fastidious microorganisms. In preparation, 15g was dissolved in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{O}C for 15 minutes.

3.5.3 McConkey Agar

McConkey Agar is a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. . It is prepared by suspending 111 grams of Mannitol Salt Agar in 1000mls of distilled water then boiled to dissolve the medium completely. The solution is finally sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

3.6 Collection and identification of plant material

Fresh leaves of *Murraya koenigii* (curry leaves) were obtained from local sellers of vegetables and herb in New Benin market, Edo state, Nigeria. They were identified and confirmed by the

Plant Biology and Biotechnology Department (PBB) of the University of Benin (UNIBEN), Edo state, Nigeria.

3.7 Extraction of plant Materials

Procedure:

Fresh leaves of *Murraya koenigii* (curry leaves) were bought from New-Benin market, Edo state. The leaves were thoroughly washed, shade-dried and finely powered. They were thoroughly washed to remove dirt and dust, then dried in the shade to preserve its medicinal properties. The dried leaves were grinded using dry blender. The curry leaf powder was stored in an airtight container and used for further experiments.

The experiment was carried out in three solvents namely; ethanol, methanol and aqueous. 1g of plant source was dissolved in 25 ml. Solvent at a concentration of 70 % (ethanol and methanol), then the three prepared solutions were stored for 24 hours at room temperature in a closed tubes. After 24 hours, this solution was centrifuged and filtered using Wattman filter paper and then kept in an airtight bottles at 4°C for further experiment. This fine powder was analyzed for its phytochemicals constituents.

3.8 PHYTOCHEMICAL ANALYSIS

The leaf powder of the plant examined was dissolved in various solvents and the following phytochemical preliminary tests were carried out as follow:

3.8.1 Alkaloids [Mayer's test]:

1.36gm of mercuric chloride were dissolved separately in 60ml and 5gm of potassium iodide and concentrated with 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml with distilled water. The presence of alkaloids is shown by the formation of a white or pale precipitate when a few drops of reagent are added to 1ml of acidic aqueous solution of samples.

3.8.2 Flavonoids:

5-10 drops of diluted HCl and small amount of Zn or Mg were added in a test tube containing 0.5ml of alcoholic extract of the samples and the solution was boiled for few minutes. Observation of a reddish pink or dirty brown color indicates the presence of flavonoids.

3.8.3 Glycosides:

A small amount of alcoholic extract of samples was dissolved in 1ml of water and then an aqueous solution of sodium hydroxide was added. The presence of glycosides is indicated by the appearance of a yellow color in the sample.

3.8.4 Steroids [Salkowski's test]:

Dissolving about 100mg of dried extract in 2ml of chloroform and adding sulfuric acid carefully results in the formation of a lower layer. A reddish-brown colour at the interface indicates the presence of a steroid ring.

3.8.5 Cardiac glycosides [Keller killiani's test]:

In 1ml of glacial acetic acid containing, 100mg of extract was dissolved in it. Add a drop of ferric chloride solution together with 1ml of concentrated sulphuric acid. The formation of a brown ring at the interface suggests the presence of deoxysugar, which is a characteristics of cardenolides.

3.8.6 Saponins:

A drop of sodium bicarbonate was placed in a test tube containing about 50ml of the aqueous extract of the sample, then shaken vigorously and allowed to stand for 3 minutes. The formation of a honeycomb froth indicates the presence of saponins.

3.8.7 Resins:

To 2ml of chloroform or ethanolic extract, we add 5 - 10ml of acetic anhydrite and dissolve it with moderate heating. After cooling, 0.5ml of H_2SO_4 was added and the formation of a light purple colour was seen. This indicates the presence of resins.

3.8.8 Phenols [Ferric Chloride Test]:

To 1ml of sample alcoholic solution, 2ml of distilled water was added, followed by a few drops of a 10% aqueous ferric chloride solution. The presence of phenols is indicated by the appearance of blue or green color.

3.8.9 Tannins [Lead acetate test]:

A few drops of 1% of lead-acetate solution were placed in a test tube containing about 5ml of aqueous extract. The presence of tannins is indicated by the appearance of a yellow or red precipitate.

3.8.10 Terpenoid:

To 1mg of extract add 2ml of chloroform and 1ml of concentrated of H_2SO_4. The observation of a reddish-brown color indicates the presence of terpenoids.

3.8.11 Test for Quinone:

A few drops of concentrated hydrochloric acid were added to 1ml of extract. A yellowish-brown colour was observed, indicating the presence of quinone.

3.8.12 Test for Proteins Ninhydrin Test (Acetone):

Ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed to develop a purple colour.

3.9 ANTIMICROBIAL ACTIVITY

Antimicrobial activity was carried out against three selected pathogens (such as *Escherichia coli, Staphylococcus aureus and Klebsiella.* Using Kirby-Bauer test also known as disk diffusion test. A sterile inoculating loop is dipped into the bacterial suspension and streaked evenly across the surface of a Mueller Hinton agar plate. With the aid of sterile forceps, the antibiotics disks is placed evenly on the agar surface and pressed gently to ensure contact with the agar. The plate is incubated at 35-37^{0}C for 24 hours. This allows the bacteria to grow and form a lawn on the agar. After about 24hours, the disk was examined for zones of inhibition (clear zone) around each antibiotics disk. It was then measured using meter rule.

Zone of inhibition such as the minimum and maximum antimicrobial concentration of each extracts were determined.

Antibiotic sensitivity test was carried out using standard antibiotics sensitivity disk. The antimicrobial effects of the curry leaves extracts was compared to standard antibiotics such as Tarivid, Augmentin, Amoxillicin, Pefloxacin, ampiclox, Streptomycin, Septrin, Erythromycin, Sparifloxacin, Ciprofloxacin, and Chloramphenicol etc.

CHAPTER FOUR

RESULT

Isolation and confirmatory tests reveals the morphology and identity of the test organism.

Upon subjection of the isolates to Gram stain, both organisms identified as *Escherichia coli* and *Klebsiella* were confirmed to be gram negative while *Staphylococcus aureus* was confirmed to be gram positive organisms.

Table 1 shows the cultural characteristic and morphological identification of bacterial isolates collected from the University of Benin Teaching Hospital (UBTH). Data from this study showed that these three (3) pure cultures of bacterial isolates, *E. coli* of gastrointestinal origin, *Staphylococcus aureus* and *Klebsiella* were collected, characterized and identified.

Table 1: Cultural characteristic and morphological identification of bacterial isolates

Isolates	Test organism A	Test organism B	Test organism C
CULTURAL			
Color	Grayish white	Grayish white	Milky
Elevation	Convex	Dome-shaped	Convex
Size	1-3mm	2-3mm	2mm
MORPHOLOGICAL			
Gram test	Gram-negative	Gram-negative	Gram-positive
Arrangement	Rod	Rod	Chain
BIOCHEMICAL TEST			
Indole	+	-	-
Catalase	-	-	+
Simmon's citrate	-	+	-
Oxidase test	-	-	-
TSI Test	A/A	A/A	-
KOH Test	+	+	-
Probable isolate	*E. coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*

Phytochemical Analysis of the Curry Leaves Extracted In Different Solvents

Crude extracts (Aqueous, Methanol and Ethanol) of *M. koenigii* Linn were subjected to different phytochemical screening for alkaloid, carbohydrate, tannins, terepnoids, cardiac glycosides, flavonoids, phenols, phylobatannins, quinons, amino acids and protein. Presence of different phytochemicals in leaves of the curry leaves are showed in Table 2.

Table 2: Qualitative phytochemical analysis of the curry leaves.

Phytochemicals	**Aqueous**	**Methanol**	**Ethanol**
Alkaloids	+	+	+
Carbohydrate	+	+	+
Cardiac glycosides	-	+	+
Flavonoid	-	-	-
Phenols	+	-	+
Tannins	+	+	+
Terpenoid	+	+	+
Quinons	+	-	-
Saponins	-	-	-

Keys: - Absent
+ Present

Based on the three (3) solvent for extraction, the curry leaves showed the presence of Alkaloid, carbonhydrate, Tannins and terpenoid in all three solvents for extract.

Cardiac glycosides was present only in methanol and ethanol extract of the sample while saponins was absent from all sample extract of the curry leaves.

<u>Antimicrobial Activity of the Curry Leaves Extracts</u>

Based on the three solvents used for the plants extraction (methanol, water, and ethanol) used for the curry leaves extracts, this experiment reveals that all test isolates except Klebsiella pseudomonas were successfully inhibited at MICs of 100mg/ml, 50mg/ml, and 25mg/ml.

In comparing the inhibition zones of the three organisms on Muller Hinton agar using Disc diffusion method;

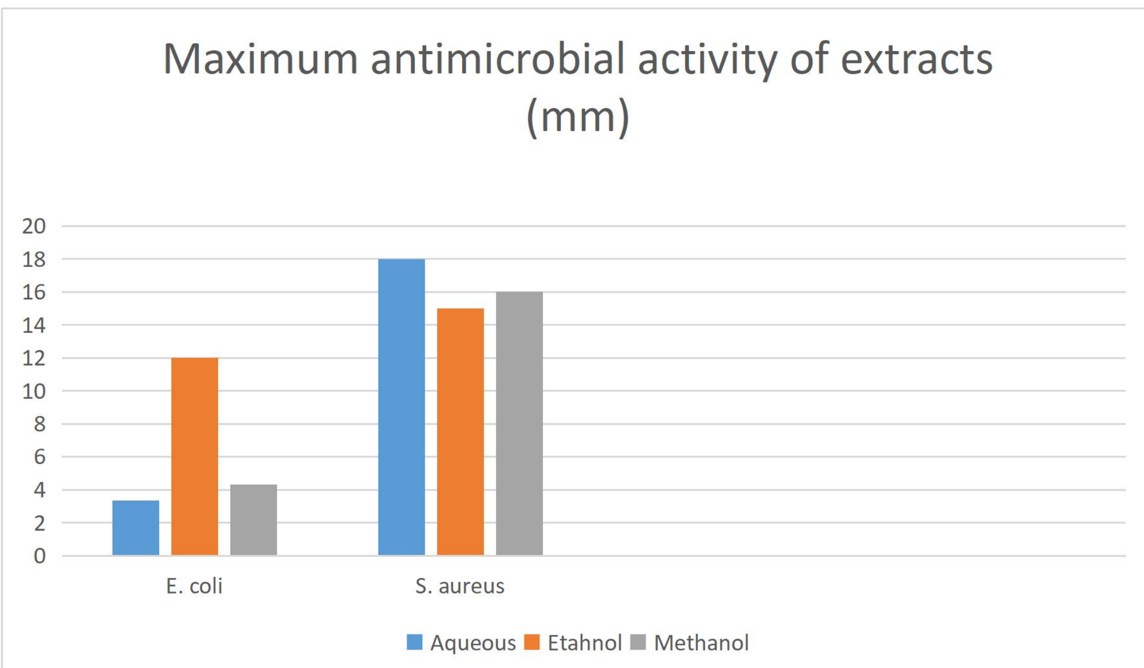
- From the solvent extract of the curry leaves, minimum antimicrobial activity was recorded against *E. coli* with aqueous extract at 25mg/ml and maximum antimicrobial activity was recorded with ethanol extract at 100mg/ml.
- Minimum antimicrobial activity was recorded against *Staphylococcus aureus* was recorded at a concentration of 25mg/ml of ethanol extract and maximum activity with aqueous extract at 100mg/ml.
- In the case of *Klebsiella pseudomonas*, all extracts failed to show antimicrobial activity.
- In summary, all solvent extracts of the curry leaves had minimum antimicrobial activity at 25% aqueous concentration of the curry leaves extract against *E. coli*, while the overall maximum antimicrobial activity was recorded at a 100% aqueous concentration *against Staphylococcus aureus*.

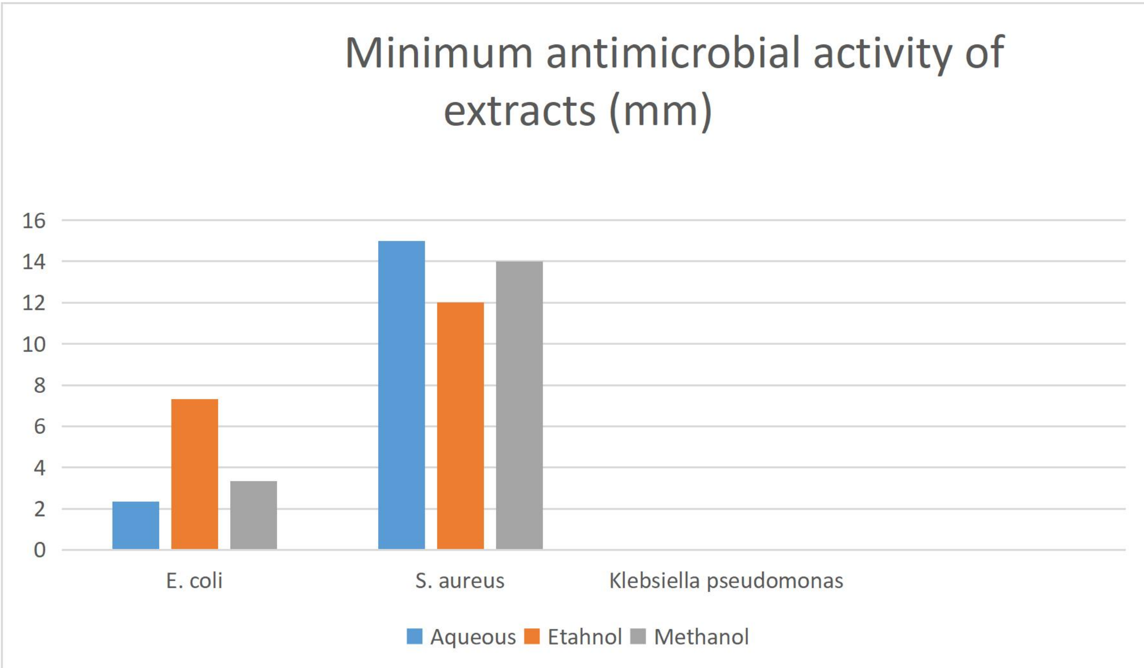
  These are summarized in table 3 below;

Table 3: Antimicrobial activity of the curry leaves extracts using Muller hinton showing the zone of inhibition in mm.

Isolates	**Concentration**	**Extracts**		
		Ethanol	**Methanol**	**Aqueous**
E. coli	100mg/ml	12.00	4.30	3.50
	50mg/ml	10.30	3.30	3.30

	25mg/ml	7.30	3.30	2.30
Staphylococcus aureus	100mg/ml	15.00	16.00	18.00
	50mg/ml	13.30	15.50	16.20
	25mg/ml	12.30	14.00	15.00
Klebsiella Pseudomonas	100mg/ml	0.00	0.00	0.00
	50mg/ml	0.00	0.00	0.00
	25mg/ml	0.00	0.00	0.00





<u>Susceptibility Testing</u>

Tables 4 showed the antibiotic susceptibility profile of the bacteria isolates. Comparison of zones of inhibition (mm) of the curry leaves methanol extract and standard antibiotics (mm).

Table 4 shows the results of the standard antibiotics sensitivity testing of the isolates carried out using sensitivity disc.

E. coli was susceptible to Sparifloxacin, Ciprofloxacin, Amoxillicin, Gentamycin, Pefloxacin, and Tarivid.

Staphylococcus aureus was susceptible to Pefloxacin, Gentamycin, Ampliclox, Zinnacef, Amoxillicin, Rocephin, Ciprofloxacin, Streptomycin, Septrin, and Erythromycin.

While *Klebsiella pseudomonas* showed negative result (no inhibition).

Table 4 (a): Standard antibiotics sensitivity test (mm)

GRAM NEGATIVE ISOLATE

Isolates	SP	CPX	AM	CN	PEF	OFX	S	SXT	CH	AU
E. coli	24 (S)	26 (S)	17 (S)	26 (S)	20 (S)	22 (S)	16 (I)	0 (R)	14 (I)	0 (R)
K. Pseudomonas	9 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)

Table 4 (b)

GRAM POSITIVE ISOLATE

Isolates	PEF	CN	APX	Z	AM	R	CFX	S	SXT	E
S. aureus	20 (S)	18 (S)	17 (S)	20 (S)	20 (S)	26 (S)	20 (S)	18 (S)	24 (S)	26 (S)

Resistance (R) = 0 -10mm

Intermediate (I) = 11-16mm

Sensitive (S) = 17mm and above

OFX- Tarivid	AUG- Augmentin	AM- Amoxillicin
PEF - Pefloxacin	CN- Gentamycin	APX- Ampliclox
Z- Zinnacef	R- Rocephin	CFX- Ciprofloxacin
S- Streptomycin	SXT- Septrin	E- Erythromycin
SP- Sparifloxacin	CPX- Ciprofloxacin	CH- Chloramphenicol

CHAPTER FIVE

5.0 DISCUSSION

This research project reveals the antimicrobial activity and potency of curry leaves extract. For the context of this research, this research was limited on bacterial isolates rather than fungal.

Results from my lab work shows that the ethanol extracts of the curry leaves was more active against the clinical bacterial pathogens viz. *E. coli* at 100% concentration while aqueous extracts of the curry leaves was more effective against *Staphylococcus aureus*.

In literature, it has been reported that the antibacterial activity is due to the presence of different chemical agents in the leaf extract including essential oils, flavonoids, terpenoids and other components. For example, Terpenes function as antimicrobial agents by weakening microorganism tissues and cell walls (Cox-georgian *et al*., 2019).

The results of this research supports to a great extent, the traditional use of plants such as curry leaves in human and animal disease therapy that are of pathogenic origin. This study also reveals the antimicrobial effect of curry leaves when used in food. From confirmatory results, aqueous extract generally exhibit high potency and antibacterial activity which seems to confirm the traditional therapeutic claims of this plant as well as reinforcing the idea of using an ethnobotanical approach to identify potential sources of bioactive compounds in plants. Curry leaves are utilized as medicinal plants due to the antimicrobial activity they display (potency to cure certain bacterial infection) and pharmacological and biochemical activities.

On the basis of the results obtained from this research project, we can safely infer that curry leaves are rich in phytochemical constituents even though the phytochemical screening of the sample (leaf extracts) had shown some variation in their phytochemical constituents, owing to the presence or absence of certain components. Inference of this result shows that aqueous extracts of the leaves had more phytochemical constituent. These phytochemical constituents are secondary metabolites produced by curry plant. They include; glycosides, phytosterols, alkaloids, oils, saponins, phenols and flavonoids and they exhibit the antibiotic properties responsible for their antimicrobial efficacy against selected pathogens.

These aligns with earlier findings of (Rashmi, Jyoti Bisht, and Naveen G, 2018), illustrating that *M. koenigii* contains secondary metabolites like phenols, essential oils, terpenoids, alkaloids, and flavonoids, which possess strong antibacterial properties as well as the fact that the antibacterial activity variation was based on extract concentration; with higher concentrations showing heightened effectiveness.

5.1 CONCLUSION

This report highlights the possible use of *M. koenigii* (curry leaves extracts) as a source of antimicrobial agents that can be used to prevent enteric diseases. From this research, I could infer that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied due to the type of solvent being used. Curry leaves contain a considerable quantity of phenol – flavonoid compounds which were considered to be the major drive for leaves' antimicrobial activities. With successful experiments conducted on the antimicrobial properties of curry leaves, this could justify its potential therapeutic use. This could also pave way for its use in the formulation of new and more potent anti-microbial drugs of natural origin. Also, curry leaves could be effectively used as a natural remedy in everyday meal, for the prevention of bacterial infections. Although, it is suggested that future and further research should address the application of curry leaves as natural remedy against infectious diseases.

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