In vitro Antimicrobial, Qualitative, and Quan\itative Analysis of the Leaves of _Azadirachta Indica and Morinda Lucida_ against selected Bacteria

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2022

Certification

This is to certify that Musodeeq Bello Oluwatosin with matriculation number LCU/PG/001311 carried out this research work titled "Antimicrobial, Qualitative, and Quantitative Analysis of the Leaves of *Azadirachta Indica and Morinda Lucida* against selected bacteria. " in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master's Degree (M.Sc.) in Medical Microbiology and that this has not been previously submitted.

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Dedication

I dedicated this project to God Almighty who is the source of Knowledge.

Acknowledgement

I want to thank those who have been most supportive of me throughout this effort. I want to sincerely thank my supervisor, Dr. B.A Bamkefa, for her unwavering support of the study. I want to give a special thank you to Dr. F. Adesina, the department head. I would especially like to thank Mrs. Adeyemi, the Head of the Biochemistry Technologist, for her unending assistance. I want to express my gratitude to my parents for their unwavering support and for being a continuous source of inspiration.

Abstract

Micro-organism have developed resistance against various antibacterial drugs, and to overcome this alarming situation medicinal plants are studied as the possible alternatives for the currently used antibiotics. Aqeous, ethyl acetate, and ethanolic extracts from the dried leaves of *Azadirachta indica and Morinda lucida* were tested against seven clinically important pathogens that cause various infections. The extracts had varying levels of effectiveness, but performed well as broad spectrum potential antimicrobials as all of the strains gave a promising zone of inhibition against the plant extracts as measured in the diameter of the zone of inhibition. The results showed that ethyl acetate extracts had the most efficacy against the selected microorganisms with a 71% potency rate for *Morinda lucida* 86% potency rate for *Azadirachta indica*. Aqueous extracts of *Morinda lucida* had a 43% potency rate against the bacteria while a 71% potency rate was recorded for aqueous extracts of *Azadirachta indica.* Ethanol extracts of the *morinda lucida* had a 14% potency rate, while ethanol extracts of *Azadirachta indica* had a 71% potency rate against the selected bacteria. Evaluation of antimicrobial properties of *Morinda lucida* and *Azadiracta indica* using different solvents as extracant in this work showed that the use of ethyl acetate as solvent a solvent of extraction give a better phyto- constituents against bacteria (gram positive & gram negative). Aqueous extract of the *Azadiracta indica* sample was 71% effective against the bacterial isolates. From this result, the presence of some phytoconstituents like chemicals like alkaloids, tannins, tannins, saponins, flavonoids and phenol in the sample, confers on the plant the antimicrobial properties. In the agreement with this report, they further submitted an independent report that antimicrobial potency of extract from these plant were traceable to the phytochemistry of their chemical constituents.

Keywords: medicinal plants, *Morinda lucida*, extracts, ethyl acetate, potency, antibiotics.

Word Count: 300 words.

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Chapter One

Introduction

1.1 Background to the Study

Because medicinal plants contain chemical components with therapeutic benefits, they have been utilized as cures for human diseases for ages, particularly those caused by bacteria, fungi, viruses, protozoans, and other pathogens[1]. While synthetic medicinal compounds continue to be the focus of research, natural materials are becoming increasingly important. Similarly, investigations have demonstrated that drug-resistant strains of numerous pathogenic bacteria have hampered antibiotic therapy, necessitating the development of novel, safe, inexpensive, and effective antibiotics[1]. Phytochemical screening of plants used in traditional medicine is becoming important, particularly in Africa, where medicinal plants have long been a part of the culture[2]. In an African setting, the study found that the usage of therapeutic plants is gaining traction in comparison to manufactured products[2]. The Bible and the Quran also both promote the use of herbs in health care and prevention. The function of herbs in disease management is also confirmed from an Islamic perspective, with Prophet Mohammed (PBUH) recommending several plants/fruits for disease treatment[3].

The existence of secondary metabolites such as tannins, saponins, steroids, flavonoids, protein, and reducing sugar in some of these native plants was discovered through phytochemical research, and their presence in this extract was found to be responsible for their antiplasmodial effect[4]. As a result, ethnomedicine screening of plants with antibacterial characteristics is an essential method in the treatment and control of diseases caused by pathogenic bacteria.

Drug resistance is one of the most difficult problems in the battle against harmful germs. Other strains of bacteria are becoming increasingly resistant to their synthetic medicines,

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therefore these difficulties are not confined to antibactrial resistance. Malaria resistance is a classic illustration of this problem. Chloroquine resistance, the cheapest and most extensively used antimalarial, is widespread across Africa (especially in the continent's southern and eastern regions)[4]. Resistance to sulfadoxine-pyrimethamine, which is widely used as a first-line and low-cost alternative to chloroquine, is also on the rise across East and Southern Africa. Many countries are changing their treatment policies as a result of these trends[4]. The greatest threat to malaria control is chemotherapy's loss of effectiveness. As a result, new information, goods, and techniques, particularly new medications, are urgently required to combat malaria[3]. Antimalarial properties have also been reported for *Azadirachta indica* and *Morinda lucida*.

Resistance to sulfadoxine-pyrimethamine, which is frequently used as a first-line and low-cost alternative to chloroquine, is also on the rise. Traditional approaches for treating diseases caused by bacteria could be a promising source of novel antibacterial chemicals. In Africa, traditional medicines are used by more than 80% of the population, and most households rely on this medicine based on plant extracts for disease treatment[2]. In fact, this continent's traditional medicine provides a significant source of antibiotic action. Africa's east and south Many governments have had to adjust their treatment programs as a result of these changes[4]. Chemotherapy's efficacy has diminished.

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1.2 Statement of the Problem

Pathogenic organisms are the leading cause of diseases in humans, and bacteria are one of its main classes. Synthetic drugs have been used to fight off infections and diseases, but they have unprecedented side effects. Moreover, pathogenic bacteria are increasingly becoming resistant to these treatments. This study builds up on previous researches to further explore natural products as a potential source of treatment.

1.3 **Justification of the Study**

Medical scientists, the government and individuals interested in improving health care or developing pyhamercy cooperative activities for human effort will benefit greatly from this research. The conclusions of this study will mandate steps that will increase demand for improved traditional Medician application in the local area. This study will also act as a resource for other academics and researchers who want to do more research in this topic in the future.

1.4 Aim and Objectives of the Study

The aim of this study is to investigate the antibacterial potentials of the extract of *Azadirachta indica (neem)* and *Morinda lucida leaves*, as well as conduct the qualitative and quantitative analysis of the leave extracts.

The objectives of this study are as follows:

i. evaluate the antibactericidal properties of the extracts of the leaves *Azadirachta indica* (neem) and *Morinda lucida* leaves.

ii. determine the phytochemical contents of *Azadirachta indica* (neem) and *Morinda lucida* leaf extracts in water, ethanol, and ethyl acetate

iii. determine the amount of phytochemical components present in the plant extract.

iv. provide a comparative evaluation of the leaf extracts' antibacterial activity.

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1.5 **Scope of the Study**

The antibacterial potential, as well as the qualitative and quantitative analyses of *Azadirachta indica* (neem) and *Morinda lucida* leaf extracts, is the focus of this research.

1.6 Limitations of the Study

i. Fund: This serves as a major constraint to the research work. Enough funds are required in getting materials for this work.

ii. Time: The research could have been broader of not for time constraints.

1.7 Operational definition of terms

Extracts: a preparation containing the active ingredient of a substance in concentrated form.

Antibacterial: a substance with antibacterial properties.

Phytochemical : any of various biologically active compounds found in plants.

Pathogenic: Causing or capable of causing disease

Resistance : the impeding or stopping effect exerted by one material thing on another

Efficacy : the ability to produce a desired or intended result

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Endnotes

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Chapter Two

Literature Review

2.1 *Azadirachta Indica* (Neem)

Many infectious, metabolic, and malignant illnesses are treated with neem components in Ayurveda, Unani, Homeopathy, and modern medicine[1]. In many countries, several types of preparations based on plants or their elements are quite popular in illness management. Based on the fact that neem (*Azadirachta indica*), a member of the *Meliaceae* family typically found in India, Pakistan, Bangladesh, and Nepal, has therapeutic implications in disease cure and formulation.

Through the augmentation of antioxidant activity, suppression of bacterial growth, and manipulation of genetic pathways, plant products or natural products play an essential role in disease prevention and therapy. Because of their low side effects and low cost, the medicinal uses of a variety of plants in disease management are still vigorously explored. It is widely acknowledged that allopathic medications are pricey and have a harmful effect on normal tissues and biological activity. The notion that many pharmacologically active medications are sourced from natural resources, including medicinal plants, is widely recognized [1, 2].

2.1.1 Botanical Description of Neem

The *Meliaceae* family includes the neem tree, which can be found in abundance in tropical and semitropical locations such as India, Bangladesh, Pakistan, and Nepal. It is a fast-growing tree that reaches a height of 20–23 m and has a straight trunk with a diameter of 4-5 ft. The leaves are compound and imparipinnate, having 5–15 leaflets per leaflet. It produces green drupes that ripen to a golden yellow color in the months of June–August[3]. The taxonomic classification of *Azadirachta indica* (neem) is as follows:

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Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*Indica*

2.1.2 Active Compounds of *Azadirachta indica* L. (Neem)

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Azadirachta indica has a complex of compounds, including Nimbin, nimbidin, nimbolide, and limonoids, which play a role in illness management through modulating genetic pathways and other activities. Quercetin and ß-sitosterol were the first polyphenolic flavonoids isolated from fresh neem leaves, and they were discovered to exhibit antifungal and antibacterial properties [4,5,6]. Antibacterial antifungal, and anti-inflammatory biological and pharmacological actions have been described [7,8]. Anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumor properties have been established by previous researchers, and a review described the many therapeutic roles of neem[11]. The role of neem and its active components in disease prevention and therapy via modification of numerous biological pathways [9, 10].

Because it is an abundant source of numerous sorts of components, *Azadirachta indica L.* (neem) has a therapeutic role in health management. Azadirachtin is the most active ingredient, followed by nimbolinin, Nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin,

salannin, and quercetin. Nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, and amino acid, 7-desacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, and nimbiol are all found in the leaves[12]. Polyphenolic flavonoids quercetin and ß-sitosterol were isolated from fresh neem leaves and were known to have antibacterial and antifungal activities, and seeds contain important compounds such as gedunin and azadirachtin [13.]

2.1.3 Mechanism of Action of Active Compounds

Neem (*Azadirachta indica*), a *Meliaceae* family member, has medicinal implications in the prevention and treatment of illnesses. However, the particular molecular mechanism that prevents disease is still unknown. *Azadirachta indica* is thought to have medicinal properties due to its high concentration of antioxidants and other beneficial chemicals such as azadirachtin, nimbolinin, Nimbin, nimbidin, nimbidol, salannin, and quercetin[14].

Possible mechanism of action of *Azadirachta indica* is presented as follows:

Plant components of the neem tree (*Azadirachta indica*) have an antibacterial effect by inhibiting microbial development and the potential for cell wall collapse. Azadirachtin, a complex tetranortriterpenoid limonoid found in seeds, is the main component responsible for insect antifeedant and poisonous actions[15]. The ethanol extract of neem leaves was found to have antibacterial action in vitro against both *Staphylococcus aureus* and MRSA, with the highest zones of inhibition observed at 100 percent concentration[16].

Neem possesses free radical scavenging properties due to its high antioxidant content. In that order, azadirachtin and nimbolide showed concentration-dependent antiradical scavenging and reductive potential: nimbolide> azadirachtin > nimbolide17. Ascorbate >nimbolide>azadirachtin >nimbolide [17.] By modulating cell signaling pathways,

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neem extract has been proven to be effective in the treatment of cancer. Neem impacts tumor suppressor genes such as p53 and pTEN, as well as angiogenesis (VEGF), transcription factors such as NF-B, and apoptosis (e.g., bcl2, bax). Neem also has anti-inflammatory properties because it inhibits the activity of pro-inflammatory enzymes like cyclooxygenase (COX) and lipoxygenase (LOX).

2.2 Therapeutic Implications of Neem and Its Various Ingredients in Health Management

Active constituents help to heal diseases by activating antioxidative enzymes, rupturing bacteria's cell walls, and acting as a chemopreventive via regulating cellular pathways. The pharmacological properties of neem are thoroughly examined (Figure 1).

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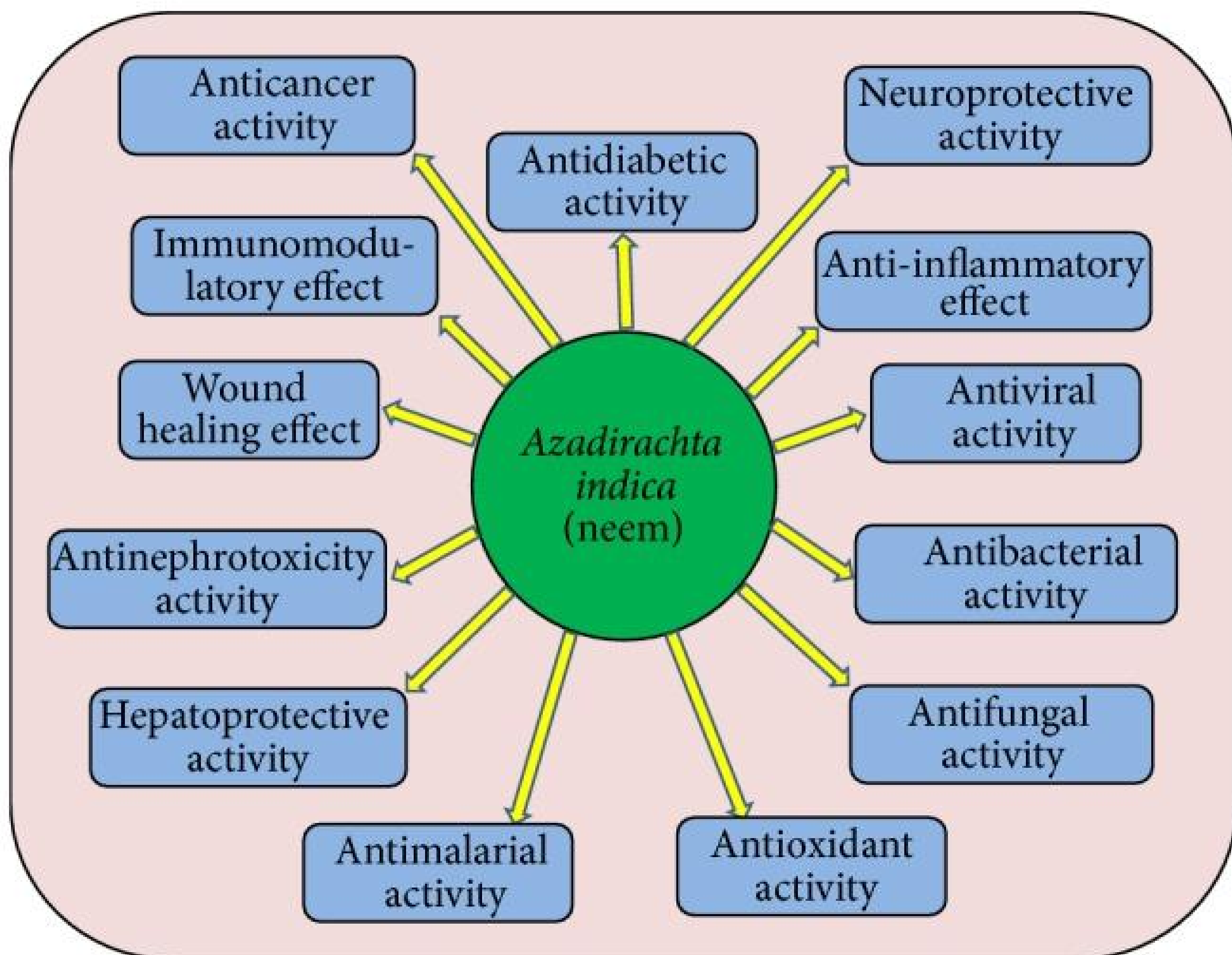

Figure 1.1: Pharmacological activities of Azadirachta indica L. neem in diseases management through the modulation of various activities.

Source: https://www.ncbi.gov

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2.2.1 Antioxidant Activity

One of the main causes of disease is free radicals, also known as reactive oxygen species. The neutralization of free radical activity, on the other hand, is a key step in disease prevention. Antioxidants stabilize/deactivate free radicals before they assault targets in biological cells[19] and also have a role in the activation of antioxidative enzymes that control free radical/reactive oxygen species damage. Antioxidant activity has been reported in medicinal plants[20]. Because of their high antioxidant content, plants' fruits, seeds, oil, leaves, bark, and roots play a significant role in disease prevention.

The antioxidant activity of *A. indica* leaf and bark extracts was investigated, and the results showed that all of the examined leaf and bark extracts/fractions of neem cultivated in the foothills have strong antioxidant characteristics[21]. Another major study looked at the antioxidant activity of leaves, fruits, flowers, and stem bark extracts from the Siamese neem tree, and the results showed that extracts from the leaves, flowers, and stem bark contain a lot of antioxidant potentials[22].

The following study was conducted to examine *in vitro* antioxidant activity in different crude extracts of *Azadirachta indica* (neem) leaves, as well as antioxidant capacity of different crude extracts: methanol extract > chloroform extract > butanol extract > ethyl acetate extract > hexane extract > methanol extract According to the current findings, neem chloroform crude extracts could be employed as a natural antioxidant[23].

Other findings revealed that azadirachtin and nimbolide, in the sequence nimbolide > azadirachtin > ascorbate, had concentration-dependent antiradical scavenging activity and reductive potential. Additionally, azadirachtin and nimbolide treatment reduced the formation of DMBA-induced HBP carcinomas by preventing procarcinogen activation and oxidative DNA damage, as well as upregulating antioxidant and carcinogen detoxification enzymes[24].

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The antioxidant activity of the flowers and seed oil of the neem plant *Azadirachta indica* A. Juss. was tested, and the results showed that the ethanolic extract of the flowers and seed oil at 200 g/mL produced the highest free radical scavenging activity, with 64.17 0.02 percent and 66.34 0.06 percent, respectively[25].

The study's findings revealed that root bark extract had a greater free radical scavenging effect, with 50 percent scavenging activity at 27.3 g/mL and overall antioxidant activity of 0.58 mM of standard ascorbic acid[25]. Other findings of the study showed that neem leaf and bark extracts/fractions cultivated in the subtropical foothills have substantial antioxidant activities[26].

The antioxidant activity of leaves, fruits, flowers, and stem bark extracts from the Siamese neem tree was tested, and the results revealed that aqueous leaf extract, flower, and stem bark ethanol extracts had higher free radical scavenging activity with 50 percent scavenging activity at 26.5, 27.9, and 30.6 micro g/mL, respectively. Furthermore, extracts had total antioxidant activity of 0.959, 0.988, and 1.064 mM of standard Trolox, respectively[27].

2.2.2 Anticancerous Activity

Cancer is a complex disease that affects people all around the world. Changes in molecular/genetic pathways play a role in cancer's growth and progression. The allopathic therapy module is effective on the one hand, but it has a negative impact on normal cells. Plants and their contents have previously been shown to prevent the formation of malignant cells via modulating cellular proliferation, apoptosis, tumor suppressor genes, and a variety of other molecular pathways[28]. Flavonoids and other compounds in neem help to prevent cancer by inhibiting the growth of cancer cells (Figure 2). A large number of epidemiological studies suggest that a high flavonoid consumption is linked to lower cancer risk[29].

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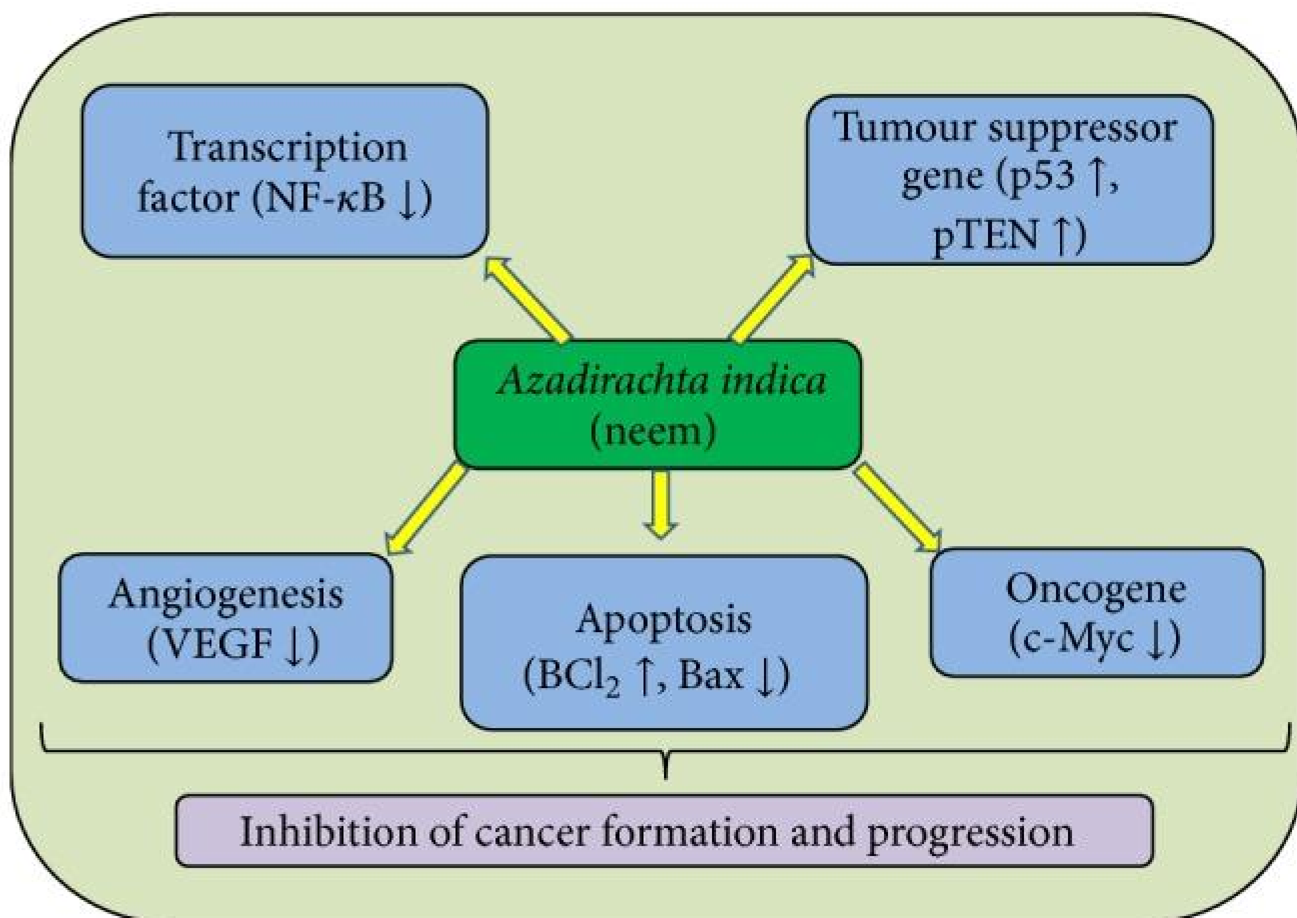

Figure 1.2: Anticancerous activities of *Azadirachta indica* L. neem through the modulation of various cell signaling pathways.

Source : https://www.ncbi.nlm.nih.gov

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Neem oil contains a variety of neem limonoids that prevent 7,12-dimethylbenz(a)anthracene from becoming mutagenic[30]. The cytotoxic effects of nimbolide found in leaves and flowers on human choriocarcinoma (BeWo) cells were investigated in a study, and the results showed that treatment with nimbolide resulted in dose- and time-dependent inhibition of BeWo cell growth, with IC50 values of 2.01 and 1.19 M for 7 and 24 hours, respectively[31]. The chemopreventive potential of the limonoids, azadirachtin, and nimbolide was investigated, and the results revealed that azadirachtin and nimbolide inhibited the development of DMBA-induced HBP carcinomas by influencing multiple mechanisms, including prevention

of procarcinogen activation and oxidative DNA damage, upregulation of antioxidant and carcinogen detoxification enzymes, and inhibition of tumor invasion and angiogenesis[32].

Azadirachta indica and its active chemicals have an important role in cancer prevention and progression. The actual chemical process at work in this vision is unknown. Neem and its components are thought to play a function in the modulation of numerous cell signaling pathways, according to research[33].Several compounds of *Azadirachta indica* activate tumor suppressor genes and inactivate the activity of several genes implicated in cancer formation and progression, including VEGF, NF-B, and PI3K/Akt. Neem has been shown to be a good tumor suppressor gene activator as well as a VEGF and phosphoinositol PI3K/Akt pathway inhibitor. Apoptosis, NF-B signaling suppression, and the cyclooxygenase pathway are all activated by this compound.

Azadirachta indica L. neem has anticancer properties via modulating multiple cell signaling pathways. Neem and its constituents play a role in the prevention of malignancies through the modulation of molecular pathways, which are described below[33].

2.2.3 Effect of Neem and Its Constituents on Tumour Suppressor Genes

p53 is a tumor suppressor gene that prevents aberrant cells from proliferating, preventing cancer from developing and progressing. The pro-apoptotic genes and proteins p53, Bcl-2-associated X protein (Bax), Bcl-2-associated death promoter protein (Bad) caspases, phosphatase and tensin homolog gene (pTEN), and c-Jun N-terminal kinase (JNK) were all elevated by the ethanolic fraction of neem leaf (EFNL) treatment[33]. In 7,12-dimethylbenz(a)anthracene-induced cancer cells, ethanolic neem leaf extract increased the expression of pro-apoptotic genes including caspase-8 and caspase-3 while suppressing the expression of Bcl-2 and mutant p53[34, 35].

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One of the most major contributors to the cytotoxicity of neem extracts is nimbolide, a tetranortriterpenoid limonoid[36]. I-FLICE, cIAP-1, cIAP-2, Bcl-2, Bcl-xL, survivin, and X-linked inhibitor of apoptosis protein were all downregulated by Nimbolide, while the pro-apoptotic proteins p53 and Bax were elevated.

In numerous types of primary and metastatic malignancies, pTEN function is frequently decreased due to mutations, deletions, or promoter methylation silencing[37, 38]. pTEN inactivation has been observed in a variety of tumors. A study found that treatment with the ethanolic fraction of neem leaf dramatically raised the expression of pTEN, which inhibits mammary tumorigenesis via inhibiting Akt[36].

2.2.4 Effect of Neem and Its Constituents on Apoptosis

The proteins Bcl2 and Bax are essential regulators of the apoptotic process. Any change in bcl2 and bax leads to tumor formation and progression[39]. Many tumors have been shown to have altered the expression of these genes[40]. The effect of the extract in an in vivo 4T1 breast cancer model in mice was investigated, and the results revealed that the CN 250 and CN 500 groups had a higher incidence of apoptosis than the cancer controls[41]. The extract was found to trigger cell death in prostate cancer cells (PC-3) by inducing apoptosis in another investigation[43].

Leaf extract downregulated Bcl-2 expression and upregulated Bim, caspase-8, and caspase-3 expression in the buccal pouch, indicating that it induces apoptosis in the target organ[35], and study results confirmed that leaf extract induced a dose-dependent reduction in chronic lymphocytic leukemia (CLL) cell viability, with significant apoptosis observed by 24 h at 0.06 percent (w/v) [43]. Neem's isolated molecule and main constituents have a wide variety of activities that influence many targets and also have a role in cancer cell death induction[44, 45].

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2.2.5 Effect of Neem and Its Constituents on Angiogenesis

Angiogenesis is a complicated process that provides blood to tissue and is necessary for tumor growth and metastasis. Angiogenesis is controlled by both activators and inhibitors. Antiangiogenic drugs that impede the creation of new blood vessels are a critical step in the inhibition/prevention of tumor growth. Because of their antiangiogenic properties, medicinal plants and their components help to inhibit tumor formation[46].

An important study found that treatment with the ethanolic fraction of neem leaf (EFNL) efficiently suppressed the production of proangiogenic genes, vascular endothelial growth factor A, and angiopoietin, implying that EFNL has antiangiogenic potential. Furthermore, contemporary research[33] suggests that the ethanolic fraction of neem leaf (EFNL) inhibits angiogenesis, which could explain why mammary tumor volume is reduced and new tumors are not developing. Another study looked at the antiangiogenic activity of extract of leaves (EENL) in human umbilical vein endothelial cells (HUVECs), and the results showed that EENL inhibited the VEGF-induced angiogenic response in vitro and in vivo, as well as suppressing HUVEC proliferation, invasion, and migration in vitro[46]. The results of a study on zebrafish embryos treated with various concentrations of water-soluble fractions of crude methanolic extract of neem root, imatinib (standard), and control concluded that water-soluble fractions of crude methanolic extract of neem root have the ability to inhibit angiogenesis[47].

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2.2.6 Effect of Neem on Oncogene

An oncogene is a mutant gene that plays a key function in tumor formation and progression. In a study of 4T1 breast cancer BALB/c mice, the effect of leaf extract on c-Myc oncogene expression was investigated, and the results revealed that the 500 mg/kg neem leaf extract

(C500) group showed significant suppression of c-Myc oncogene expression when compared to the cancer control group[48].

2.2.7 Effect of Neem as Anti-Inflammatory

Plants or isolated products of plants are used to treat or serve as anti-inflammatory agents. The anti-inflammatory effect of *A. indica* leaf extract at a dose of 200 mg/kg, p.o. in a cotton pellet granuloma assay in rats was validated in a study[49]. Other research found that neem leaf extract has a strong anti-inflammatory effect, but it is less effective than dexamethasone[50] and that nimbidin suppresses macrophage and neutrophil actions that are related to inflammation[51].

Earlier research revealed that bark and leaf extracts had immunomodulatory and anti-inflammatory properties, whereas oil seeds have antipyretic and anti-inflammatory properties[52, 53]. The analgesic action of neem seed oil was tested on albino rats, and the results showed that neem seed oil had a substantial analgesic impact at doses of 1 and 2 mL/kg and that the oil has dose-dependent analgesic activity[54].

Another study looked at the anti-inflammatory effects of neem seed oil (NSO) on albino rats with carrageenan-induced hind paw edema, and the results showed that NSO showed improved suppression of paw edema as the dose was increased from 0.25 mL to 2 mL/kg body weight. NSO demonstrated the greatest (53.14 percent) edema inhibition after the 4th hour of carrageenan injection at a dose of 2 mL/kg body weight[55]. The study concluded that mice were given a 100 mg kg-1 dose of *Azadirachta indica* fruit skin carbon tetrachloride extract (CTCE) and the isolated component azadiradione had considerable antinociceptive and anti-inflammatory effects[56].

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2.2.8 Hepatoprotective Effect

Medicinal plants and their constituents serve an important function in hepatoprotection without causing any side effects. The hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl_4) caused hepatotoxicity in rats was investigated, and histology and ultrastructure data revealed that pretreatment with azadirachtin-A reduced hepatocellular necrosis dose-dependently[57]. Furthermore, the study's findings suggest that pretreatment with azadirachtin-A at higher dose levels restores the rat liver to a reasonable degree of normalcy[59].

Another study looked at the protective effect of nimbolide, a neem active constituent, against carbon tetrachloride (CCl_4)-induced liver toxicity in rats, and the results showed that nimbolide has a hepatoprotective effect against CCl_4-induced liver damage with efficiency comparable to that of silymarin standard[60].

A study looked at the hepatoprotective effects of *Azadirachta indica* (AI) leaf extract on antitubercular drug-induced hepatotoxicity, and the results showed that the aqueous leaf extract significantly reduced changes in serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, as well as histological changes, when compared to the antit Other findings revealed that ethanolic and aqueous leaf extracts of *A. indica* had moderate efficacy against carbon tetrachloride-treated rats[61]. The hepatoprotective activity of methanolic and aqueous extracts of *Azadirachta indica* leaves was studied in rats, and the results showed that the plant has a lot of potentials to protect the liver[61].

The protective effect of neem extract on ethanol-induced gastric mucosal lesions in rats was investigated, and the results revealed that pretreatment with neem extract protected against ethanol-induced gastric mucosal damage[62].

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2.2.9 Wound Healing Effect

The wound-healing effect is influenced by a variety of plants and their contents. Excision and incision wound models in Sprague Dawley rats were used to evaluate the wound healing activity of extracts of leaves of *A. indica* and *T. cordifolia,* and the results revealed that extracts of both plants significantly promoted wound healing activity in both excision and incision wound models[63]. Furthermore, the tensile strength of the healing tissue of both plants treated groups was significantly higher than the control group in an incision wound[63]. Other findings revealed that *Azadirachta indica* leaf extracts boost wound healing activity by increasing inflammatory response and neovascularization[64].

2.2.10 Antidiabetic Activity

A study was conducted to test the 70 percent alcoholic neem root bark extract (NRE) in diabetes, and the results revealed that in an 800 mg/kg dose, neem root bark extract showed statistically significant outcomes[65]. Another study looked into the pharmacological hypoglycemic action of *Azadirachta indica* in diabetic rats, and the results showed that in a glucose tolerance test with neem extract 250 mg/kg, glucose levels were significantly lower than in the control group, and *Azadirachta indica* significantly reduced glucose levels at the 15th day in diabetic rats[66].

Studies utilizing *in vivo* diabetic mouse models, *A. indica,* and *B. spectabilis* chloroform, methanolic, and aqueous extracts revealed that *A. indica* chloroform extract and *B. spectabilis* aqueous, methanolic extracts significantly reduced intestinal glucosidase activity[67]. Another noteworthy study found that *Azadirachta indica* and *Andrographis paniculata* leaf extracts have strong antidiabetic action and could be used to treat diabetes mellitus[68].

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2.2.11 Antimicrobial Effect

Neem and its constituents impede the growth of a variety of microorganisms, including viruses, bacteria, and dangerous fungi. Individually, the role of neem in preventing microbial growth is stated as follows[69].

2.2.12 Antibacterial Activity

In a study comparing the antibacterial efficiency of herbal alternatives as endodontic irrigants to the traditional irrigant sodium hypochlorite, researchers discovered that leaf extracts and grape seed extracts displayed zones of inhibition, indicating that they have antimicrobial capabilities[70]. Leaf extracts also demonstrated much more inhibitory zones than 3 percent sodium hypochlorite[71].

The antibacterial activity of guava and neem extracts was tested against 21 strains of foodborne pathogens, and the results revealed that guava and neem extracts contain antibacterial components that could be effective in controlling foodborne pathogens and spoilage organisms[72].

Another study looked at the antibacterial activity of *Azadirachta indica* (neem) bark, leaf, seed, and fruit extracts on bacteria isolated from adult mouths, and the results showed that bark and leaf extracts had antibacterial activity against all of the bacteria tested [73]. Furthermore, only at greater doses did seed and fruit extracts display antibacterial action [73].

2.2.13 Antiviral Activity

At doses ranging from 50 to 100 g/mL, neem bark (NBE) extract effectively inhibited HSV-1 entrance into cells[74]. Furthermore, when the extract was preincubated with the virus but not with the target cells, blocking action was seen, implying that the neem bark had a direct anti-

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HSV-1 property[74]. Neem (*Azadirachta indica* A. Juss.) leaves extract (NCL-11) has been demonstrated to have virucidal activity against coxsackievirus virus B-4, as evidenced by virus inactivation and yield reduction assays, as well as interfering at an early stage of the virus's reproduction cycle[75].

2.2.14 Antifungal Activity

The efficacy of several neem leaf extracts on seed-borne fungus *Aspergillus* and *Rhizopus* was tested, and the results revealed that both alcoholic and water extracts significantly inhibited and regulated the growth of both fungal species. Furthermore, as compared to aqueous extract, alcoholic extract of neem leaf was most effective in inhibiting the growth of both fungal species[76]. Another discovery was that aqueous extracts of neem cake inhibited spore germination in three sporulating fungi, including *C. lunata*, *H. pennisetti*, and *C. gloeosporioidesf. sp. Mangiferae*, and that methanol and ethanol extracts of *Azadirachta indica* inhibited growth in *Aspergillus flavus,* and *Alternaria sol*[76].

Previous research has shown that aqueous extracts of various elements of neem, such as neem oil and its main principles, have antifungal properties[77, 78]. The antifungal activity of *Azadirachta indica* L. against *Alternaria solani* Sorauer was investigated, and the results revealed that the ethyl acetate fraction was most effective in inhibiting fungal growth, with a MIC of 0.19 mg, and that this fraction was also more effective than the fungicide (metalaxyl + mancozeb), which has a MIC of 0.78 mg[79].

2.2.15 Antimalarial Activity

Experiments using *Plasmodium berghei* infected albino mice revealed that neem leaf and stem bark extracts reduced parasitemia in infected mice by about 51–80 percent and 56–87

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percent, respectively[80], and other studies revealed that azadirachtin and other limonoids found in neem extracts are active on malaria vectors[81, 82].

Another study used a crude acetone/water (50/50) extract of leaves (IRAB) to test its activity against the asexual and sexual forms of the malaria parasite, *Plasmodium falciparum*, *in vitro*. The results showed that parasite numbers were less than half of what they were in separate 72-hour cultures of both asexual parasites and mature gametocytes treated with IRAB (0.5 micro g/mL)[83].

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2.3 *Morinda lucida*

In southwestern Nigeria, *Morinda lucida* is a nutrition factory that is easily available all year. It contains a high concentration of two powerful antioxidants, vitamins A and E, which may help to prevent degenerative diseases like atherosclerosis; vitamin K; and various secondary metabolites responsible for the plant's ethnomedicinal properties, such as alkaloids, tannins, saponins, flavonoids, and phenols[84]. Bioactive phytochemicals that act as an antibiotic, antiviral, antiplasmodial, and anti-parasitic; nutrient component with moderate qualities of proximate compounds- carbohydrates, protein, fat, fiber, ash, and high moisture content; nutrient component with moderate qualities of proximate compounds- carbohydrates, protein, fat, fiber, ash, and high moisture content[85]. The plant is high in phytochemical constituents as well as nutritional components. The leaves are abundant in vitamin K, which aids in the formation of strong bones. It has trypanocidal and aortic vasorelaxant properties, according to reports. Anticancer, hepatoprotective, cytotoxic, and genotoxic, anti-spermatogenic, hypoglycemic, and anti-diabetic actions have been described for the leaf and stem bark[86].

All forms of infertility in women can be properly treated and improved using the leaves. Locally, the brimstone tree is used to cure irregular menstruation, sleeplessness, and jaundice, as well as wound infections, abscesses, and chancre[86]. If eaten three times daily, the

decoction is said to be anti-diarrheal. Yellow fever, malaria, trypanosomiasis, and feverish condition after childbirth are all known to be treated with decoctions and infusions of plasters made from root bark and leaves[87]. Diabetes, hypertension, cerebral congestion, diarrhea, stomach discomfort, ulcers, leprosy, and gonorrhea are some of the conditions that the herbs are used to cure[86]. *Morinda lucida* has been shown to contain a steroid, making it useful against cerebral malaria and validating its anti-plasmodial properties[88]. Except for the ability to stimulate the liberation of insulin already produced by beta-cells, *Morinda lucida* is extrapancreatic in nature[84]. One diabetic patient's blood sugar was shown to be lower after using *Morinda lucida*. *Morindalucida*'s toxicity is mostly unknown. However, according to one study, huge amounts of the extract can be given without causing harm[89].

2.3.1 Biological Source

Botanical Name: *Morinda lucida Benth*

Family Name: Pyroiodea

Common Name: brimstone tree, Morinda, Indian Mulberry, Hog tree apple, Oruwo.

Part Used: Leaves, barks, and roots[90].

2.3.2 Nutritional Value

Morinda lucida is a tropical rainforest tree that belongs to the kingdom Plantae and is one of the 80 species in the genus Morinda of the pyridyl family[91]. The brimstone tree gets its name from its yellow wood. *Morinda lucida* is a medium-sized evergreen shrub with a crooked or twisted bole and branches; bark is smooth and scaly, grey to brown, and stipules are oblong or triangular, 1-7mm long, and fall early[92]. Leaves are opposite, simple, and entire; stipulates are ovate or triangular, 1-7mm long, and fall early; petiole is up to 15cm long; the blade is

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elliptical, 6-18cm x 2-9cm, base rounded, cuneate, apex acute to accumulate, shiny above, with tufts of hairs in vein axils beneath and some hairs on the midrib; petiole is up to 15 A stalked head 4-7mm in diameter, 1-3 at the node opposite a single leaf, and a peduncle up to 8cm long bearing a stalked cup-shaped gland at the base of the inflorescence[93, 94].

Ten anthraquinones, alkaloids, tannins, flavonoids, saponins, glucosides, and triterpenoids are among the phytochemicals found in *Morinda lucida*. *Morinda lucida* has nutritional properties; these nutrients are necessary for the human body's physiological functioning. Carbohydrates, proteins, lipids, minerals, vitamins, and even dietary fiber are examples of such nutrients[95]. These all play a significant part in meeting human energy and life-process needs.

2.4 Pharmacology Activity of Each Division of *Morinda lucida*

The entire *Morinda lucida* plant has its own set of pharmacological properties[95].

2.4.1 Leaves

Morinda lucida leaves provide numerous advantages. Young leaves of *Morinda lucida* were blended with leaves of *Hymenoptera* species in an area of Kasongo in the north-eastern part of the country to make a pale green dye that was used in basket weaving[96]. Different findings imply that *Morinda lucida* leaves contain anthraquinone, which makes it a protective laxative[93]. Due to the presence of steroids, alkaloids, and tannins, the leaves are also potential anti-malaria and anti-diarrhea medications. For similar reasons, the leaves can also be used in the development of therapy for the treatment of heart disease, and diarrhea, among other maladies [93].

The leaves have also displayed potential as an analgesic agent as the therapy made from the leaves are used in relieving pain. For the treatment of coughs, enlarged spleen, colic, nausea, and fever (yellow fever, malaria, trypanosomiasis, and feverish condition after childbirth), the

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leaves are cooked and applied to the belly or chest[94]. Dysentery, diarrhea, dizziness, and headache are all treated with the leaves.

Additional benefits of *Morinda lucida* leaves includetreatment of infertility hypertension, and cerebral complication. The leaf of the plant also has usage as an anticancer, hepatoprotective, cytotoxic and genotoxic, anti-spermatogenic agent. The leaves are also useful in the treatment of hypoglycemia and dibetes, gonorrhea, leprosy, and stomach ache[94].

2.4.2 Wood and Stem Bark

Morinda lucida produces yellow to crimson colors from its wood. The root bark is used to color bright red textiles in Nigeria and Gabon. The Ashanti people of Ghana dye cotton cloths red with the root bark of *Morinda lucida*[95] on moments of national sadness or the death of a chief. Officials and the deceased's family wear these clothes, known as Kobane, as mourning attire.

A mild decoction of stem bark is used to treat severe jaundice, which is commonly accompanied by hemoglobinuria and hematuria[96]. Treatment with *Morinda lucida* stem bark also cure vomiting, diarrhea, and diuresis, with a cure confirmed by the absence of yellow coloring in the urine[97]. The stem bark extract has been proven to have a potent but short-acting antihypertensive effect, and it is now recommended for the prevention and treatment of hypertension and its cerebral complications[94].

The wood of the *Morinda lucida* is particularly good for creating charcoal[94]. The wood *of Morinda lucida* are also in construction, mining props, furniture, boats, poles, and fuelwood. The bitter-tasting roots are used as a flavoring for food and alcoholic beverages[97], while the wood is also used as chewing sticks.

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2.4.3 Root

In the DR Congo's Kasal province, the root is the most important traditional source of yellow dye for textiles. It can be used without the addition of a mordant. It is also used to indigo vats in Cote d'Ivoire to aid in the fermentation and reduction processes required for indigo dyeing and to get a darker blue color. It is frequently mixed with *sabacomorensis* leaf twinges in this method[94].

The roots of the plant is used in the treatment of kidney and liver dysfunction. Liver and kidney dysfunction may manifest as weakness, fatigue, pain, and coldness in the waist and leg, or as persistent rheumatoid joint pain, weakness, joint pain, coldness, tiredness, and pale tongue fur. The root of *Morinda lucida* has been shown to be useful in the treatment of liver and renal disease[95].

The roots also show promise in boosting immunity and disease resistance capacity. The *Morinda lucida* root possess polysaccharide with adrenocorticotropic hormone that has a role of in the build up of strength and can enhance human immunity as well as reduce disease[94]. The plant's root is also known for its ability to increase sexual capacity. *Morinda lucida* root extract has a long history of being used to improve sex capacity in Chinese and Far Eastern civilizations. It is commonly used in male and female sexual function to improve strength, improve weak impotence and deficiency premature ejaculation, women uterus cold and infertility, and menstrual not sex frigidity reconcile [93, 94]. The root is also used in the treatment of incontinence, chronic rheumatism, fatigue, chronic inflammation of the nerve, menstrual disorders, hernia, upper back pain, depression, muscular and skeletal atrophy [93]

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2.5 Medicinal Value

a. Trypanocidal Activity

The effect of *Morinda lucida* dried leaves methanol extract on *Trypanosoma brucei* infected rats. The results revealed that intraperitoneal injection of the extract considerably reduced parasitemia in mice after infection with *Trypanosoma brucei*, with 1000mg/kg intraperitoneal injection producing the highest impact[94]. However, they found that treatment with *Morinda lucida* leaf extract started simultaneously with Trypanosome inoculation yielded the highest trypanocidal action[94].

b. Hypoglycemia

The anthraquinones in *Morinda lucida* (brimstone tree) leaves bind hyperglycemia toxins in the body, keeping them away from normal blood sugar levels. These nutrients work together to keep blood sugar levels in check[96].

c. Antimalarial

Because of a study that demonstrated *Morinda lucida* (brimstone tree) had a MIC of 0.6mg/ml, the leaves are used as an antimalarial. *Morindalucida*'s anti-plasmodial activity is detected primarily in the N-hexane and chloroform fractions[97]. The N-hexane and chloroform extracts of *Morinda lucida*, on the other hand, have higher antimalarial activity. Aqueous extract of *Alstoniaboonei* stem bark, *Mangifera indica* leaves, *Carica papaya*fell dried leaves, *Parkia biglobosa* or *Parkia clappertoniana* stem bark, *Morinda lucida* leaves, *Cymbopogon citratus* leaves, and *Cassia podocarpa*leaves[97]. This is helpful in the treatment of malaria infections caused by the parasites *Plasmodium falciparum* and *Plasmodium berghei*. Aqueous extract of *Ocimumgratissimum* leaf, *Azadirachta indica* leaf and bark, *Morinda lucida* leaf and bark, and *Enantiachloranta* bark. This is beneficial in the treatment of malaria infections

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caused by *Plasmodium coeli*. Ethanol extracts of *Cryptolepissanguinolenta* root bark, *Euphorbia hirta* entire plant, *Morinda lucida* leaves, and *Phyllantusniruri* whole plant[97]. This is helpful in the treatment of malaria infections caused by the parasites *P. falciparum* and *P. berghei.*

d. Antifungal Activity

Ten anthraquinones obtained from a dichloromethane preparation of *Morinda lucida* roots have antifungal action. Four of these anthraquinones were found to be efficacious against *Cladosporium cucumerinum* and *Candida albicans.* The most effective antifungal anthraquinone was discovered to be alizarin -1- methyl ether, which showed efficacy against *Aspergillus fumigates* and *Trichophyton mentagrophytes* at MIC doses of 100 and 50g/ml, respectively[98].

e. Gastrointestinal Activity

The effect of a methanol extract of the leaves on gastric emptying and intestinal motility in rats and mice was tested, as well as the effect of the extract on an acetylsalicylic acid-induced ulcer in rats. The extract improved gastric emptying time in rats and intestinal motility in mice, according to the findings[96]. Although the extract did not cause stomach ulcers in rats, it did not protect them from acetylsalicylic acid-induced ulcers[99].

f. Impact Of the Leaf And Bark Aqueous Extracts on Cell Populations In Various Organs Of Mice

The effect of extracts of some anti-malaria medicinal herbs, including *Morinda lucida* leaves and bark aqueous extracts, on cell populations in various organs of mice was compared to the effect of chloroquine-treated mice[98]. It was discovered that mice given plant extract had chemo suppressive efficacy against early parasitemia. However, this did not result in their

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survival[96]. Before the animal died, the total number of nucleated cells in the liver, spleen and peripheral circulation of malaria-infected mice grew dramatically. All infected mice treated with chloroquine, on the other hand, survived, and the number of nucleated cells in both infected and uninfected mice was reduced[98].

g. Anti-Diarrhea

Tannins, flavonoids, alkaloids, glycosides, saponins, and anthraquinone have been discovered in the leaves of *Morinda lucida*[99]. Tannins in medicinal plants are known to denature protein to generate protein tennates, which is thought to increase the intestinal mucosa's resistance to chemical change, resulting in less diarrhea hypersecretion[100].

h. Antioxidant Activity

Antioxidant activity has been demonstrated in many phytochemicals with phenolic moieties. The antioxidant activity of *Morinda lucida* stem bark extract was assessed by its ability to prevent oxidation of B – Cardene – linoleic acid emulsion experiences an oxidation pattern in which – carotene shields linoleic acid from oxidation[99].

I. Allergic and Side Effect

According to studies, *Morinda lucida* has no known negative effects, but side effects are conceivable but not usually shown when the body reacts to medications. Some of the side effects may be uncommon, yet they can be dangerous[101]. Although it is widely documented that *Morinda lucida* leaf extract offers a variety of therapeutic benefits with no known side effects in users, the responses of numerous organs in humans, particularly the liver and kidney, to ingestion of this extract are mainly unknown[102].

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2.6 Preliminary Research

The alkaloids anthraquinone and anthraquinols are the main ingredients of *Morinda lucida* extracts. The red colorants 1-methyl ether-alizarin, rubiadin and derivatives, *lucida*in, soraanjidiol, damnacanthal, nordamnacanthal, morindin, munjistin, and purpuroxanthin have all been isolated from the wood and bark of *Morinda lucida*[100]. The stem yielded two chemicals, which were isolated and described. Tannins, flavonoids, and saponosides have all been identified in addition to anthraquinone. Anthraquinone and oruwacin were isolated from the roots of *Morinda lucida*. The leaves yielded two recognized triterpenic acids (ursolic and oleanolic acids). The stem bark was used to extract three chemicals (digitolutein, rubiadinn 1-methyl ether, and damnacanthal) [102].

An ethnomedicinal study was undertaken at Akin – tafo, in Ghana's Eastern region, through an informal conversation with a herbalist. Saponins, anthraquinone, cardenolides, alkaloids, sterols, and tannins were found in preliminary phytochemical analyses on the leaves of *Morinda lucida*[100]. In the laboratory, a crude extract from *Morinda lucida* was analyzed using thin-layer chromatography and column chromatography[103]. A solvent solution using hexane and ethyl acetate (2%) as the mobile phase and a stationary phase of silica gel was used to separate nine components from *Morinda lucida*. The agar well diffusion method was used to test the crude leaf extract of *Morinda lucida* for inhibitory action on *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhii, Enterobacter Cloaval, Proteus mirabilis and Staphylococcus aureus.in vitro*. At a dosage of 10 mg/ml, *Morinda lucida* demonstrated an inhibitory effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* but no activity against *Salmonella typhii*[103].

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2.7 Overview of Phytochemical Analysis

The extraction, screening, and identification of medicinally active chemicals present in plants are referred to as phytochemical analysis. Flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds are some of the bioactive molecules derived from plants. Qualitative and quantitative phytochemical analysis are the two basic forms of phytochemical analysis.

2.7.1 Qualitative Analysis

The term qualitative analysis refers to the process of identifying and grouping items in a sample. The presence of specific bioactive components in plants confers therapeutic efficacy. Different qualitative phytochemical investigations are known that allow the determination of chemical groups or compounds in aqueous extracts from various plants using standard analytical techniques. All of the color reactions can only tell you whether a chemical group is present or not, not how much of it is present in different aqueous extracts.

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2.8 Variation

Genus *Allium* belongs to the family *Alliaceae* subf. Allioideae. It is a large genus, comprising more than 800 species[84] and is widely distributed 105 in the Northern hemisphere. A phylogenetic analysis based on internal transcribed spacer (ITS) sequences showed that *Azadirachta indica (Neem) and Morinda lucida*, part of section Neem, was quite strictly related to A. vavilovii, a position confirmed by chloroplast sequences. The ancestral karyotype of genus *Allium* seems to be p = 8. *A. indica* has 2n = 16 chromosomes (data recorded from many locations in the dat abase Index to Plant Chromosome Number (IPCN). However, it is interesting to observe that the variety *A. indica var. viviparum* Alef. has been reported to have 2n = 24, hence it would be a triploid. Moreover, it observed large insertion in some accessions (and not in others!) of onion due to transposable elements.

Other variation was detected on the base of volatile compounds emission (flavor) with an electronic nose system[87]. In fact, intraspecific variation in *A. indica* may have been overlooked, as in most investigations no mention is made about the characterization of the onion cultivar used in antibacterialological testing, apart from a few reference to cv. *A. indica* (StuttgarterRiesen). Observed characters for assessing the antibacterial/ antibacterial effects in the presence of certain external stimuli, the cellular progress can be blocked in one of the phases of the cell cycle or cell division, and their action is called mitoinhibition. Mitogens act to overcome intracellular braking mechanisms that block cell cycle progression, and their action is called mitostimulatory. Any deviation from the orderly and directed progression of the cell cycle, and respectively, of mitosis and cytokinesis, is reflected in a state of antibacterial activities and antibacterial activities. These are evaluated by the mitotic index (MI; a measurement to determine the percentage of cells undergoing mitosis), percentage of cells in each mitosis phase (prophase, metaphase, anaphase and telophase index), as well as a series of clastogenic, aneugenic and turbagenic changes. Common clastogenic effects/aberrations include chromosome and/or chromatid fragments, interchromatid or subchromatid connections, nucleoplasmic bridges, heteromorphic chromosomes, dicentric or ring chromosomes, and micronuclei (MNs). Bimitosis and asynchrony of the cell cycle could also be added .Interchromatid or subchromatid connections, known as chromosomal bridges, are chromosomal structural changes that may result from exchanges between homologous or non-homologous chromosomes, and may be the consequence of dicentric chromosome formation or poor activity of replication enzymes. Nucleoplasmic bridges originate from dicentric chromosomes or occur as a result of a faulty longitudinal breakdown of sister chromatids during anaphase.

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The formation of ring chromosomes is explained by the production of two simultaneous breaks in the same chromosome and the subsequent union of non-centromeric fragments. Chromosome breakage, followed by rejoining of proximal chromatid breaks, leads to the formation of dicentric chromosomes. MNs, indicators of chromosomal antibacterial activities and instability, are formed from one or more chromosomes. Cell cycle asynchrony, including internuclear asynchrony, can be observed in large multinucleate cells in which nuclei coexist with early phases of mitosis, inter-chromosomal asynchrony manifested by an uneven chromosome condensation throughout the mitosis stages, and intra-chromosomal asynchrony which is manifested by a gradient condensation of chromatin. When the binucleate cell divides, the two nuclei enter mitosis synchronously which is called bimitosis[110].

Turbagenic changes include laggards (delayed chromosome movement at poles), vagrants or forward chromosomes (precocious movement of chromosome to mitotic spindle poles), and star-like polar anaphase. It is believed that the formation of lagging chromosomes (laggards) is due to inhibition of tubulin polymerization or cytoskeletal proteins. Aneugenic changes include sticky chromosomes, C-mitosis, and nuclear buds. The change in the ratio between the amount of histones and other proteins that ensure the optimal organization of nuclear chromatin may increase its adhesiveness, often leading to the formation of atypical metaphases and anaphases, chromosomal bridges in anaphase and telophase, and finally inhibition of cytokinesis and the formation of binucleated cells. Nuclear buds are considered as markers of polyploidization events and gene amplification, and their formation leads to the expulsion of excess genetic material from aneuploid cells.[107] Binucleated cells may be the consequence of an aberrant division of the spindle in early anaphase or the inhibition of cytokinesis after telophase. Giant cells may be polyploid cells that have occurred through endoreplication or endomitosis.

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C-mitosis, also called stathmokinesis is the mitosis in which the poisoning effect of colchicine blocks the progression of the metaphase cell in the anaphase, and consequently the cell becomes polyploid. *Azadirachta indica (Neem) and Morinda lucida L* . has a diploid (2n = 2x = 16) genome with monocentric chromosomes, with basic 220 chromosome number x = 8. The chromosomes are relatively large and so appropriate for the detection of karyomorphological changes. The chromosomes are metacentric (1, 4, 7 couple), submetacentric (2, 3, 5, 8 couple), and subtelocentric (6 couple). Karyotypes 225 can provide information about cytology, cytosystematics and antibacterial relationships, evolutionary origins of species and, as with the AT, genomic aberration. *A. indica* (StuttgarterRiesen) is suitable for the detection of antibacterial agents effects that are manifested in the form of clastogenic and aneugenic effects[103]. A classification model for the *A. indica* karyotype consists in a symmetric/asymmetric index S/ AI = 1.7500.

The monosomy karyotype without subtelocentric chromosomes has a symmetric/ asymmetric index of S/AI = 1.6666 and the trisomy karyotype with a subtelocentric chromosome has a symmetric/asymmetric index of S/AI = 1.8235. All investigated ploidy series in *A. indica* showed symmetric or between symmetric and asymmetric karyo- types (S/AI = 1.6666–2.8571). Investigated ploidy series in genus *Allium L*. showed a full symmetric to asymmetric karyotype (S/AI = 1.0000–3.5714). The symmetry/asymmetry index (S/AI) was applied to 579 taxa of genus *Allium* as an example. The formula includes chromosomal number, chromosomal type and centromeric position. The general formula is described with the different use of chromosome type.

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2.9 Single Nucleotide Polymorphism (SNP)

Single nucleotide polymorphism (SNP) is the mostsimplest form of DNA variation in *A. indica,*which could be transition or transversion type and may occur in entire length of a genome at a frequency of about one in 1,000 bp. The alteration or changes may be the reason for the diversity among organisms,[175,176.] genome evolution, the most common familial traits such as curly hair, interindividual differences in drug response. Complex and common diseases such as diabetes, obesity, hypertension,and psychiatric disorders and in may help in breeding a plant that will meet the taste of the breeder, such as good yield, resistance to diseases and pests.

SNPs may change the encoded amino acids (nonsynonymous) or can be silent (synonymous) or simply occur in the non codingregions.They may influence promoter activity (geneexpression), Messenger RNA (mRNA) conformation (stability), and subcellular localization of mRNAs and/or proteins and hence may produce disease. Therefore, identification of numerous variations in genes and analysis of their effects may lead to a better understanding of their impact on gene function and health of an individual. This improved knowledge may provide a starting point for thedevelopment of new, useful SNP markers for medical testing and a safer individualized medication to treat the most common devastating disorders. This will revolutionize the medical field in the future. To illustrate the effect of SNPs on gene function and phenotype, this minireview focuses on evidences revealing the impact of SNPs on the development and progression of three human eye disorders (Norrie disease, familial exudative vitreoretinopathy,and retinopathy of prematurity) that have overlapping clinical manifestations[177].

A single-nucleotide polymorphism (SNP), or single-nucleotide variation (SNV), is one of the mostcommon heritable variations. A SNP is a DNA sequence polymorphism caused by the

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alteration of a single nucleotide in a specific position at the genomic level[178]. The single-nucleotide base alteration can be a transition, transversion, insertion, or deletion. In the genome, widely distributed and highly conserved SNPs can be mapped and modeled as genetic markers with high resolution[179]. Furthermore, the amino acid sequence of a protein can be modified by sequence variations in the coding regions, which sometimes influence the functional or structural features of relevant proteins. SNPs are valuable indicators of clinical diagnosis and prognosis, and can be applied to investigate inter-individual differences in aspects such as antimicrobial resistance, theevolution of molecular epidemiology.

Studies focusing on SNP analysis can be broadly divided into two categories: first, the detection of unknown SNPs, which is primarily used to increase the marker density of genetic maps and search for genetic markers of targeted characteristics; and second, the screening for known SNPs in a population, which involves genotyping SNPs at certainknown positions in a sample[180,181]. The present review mainly focuses on mainstream detectionmethods and the significance of the practical application of SNPs to *Azadirachta indica (Neem) and Morinda lucida* research.

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2.9.1. Single Nucleotide Polymorphism (SNP) of *Azadirachta indica (Neem) and Morinda lucida*

Studies of genetic diversity in onion have been hampered by a lack of portable codominant molecular markers[182]. Although a variety of molecular marker methods have been successfully used to resolve questions of genetic diversity and relatedness to species level in *Allium*[183] identifying robust and informative markers within *A. indica* has proved much more challenging. Dominant randomly applied polymorphic DNA markers have been used in limited studies of *A. indica* diversity[184,185] but a more detailed evaluation by[186] showed that

identification of reliable, heritable polymorphisms is very challenging in onion. Isozyme[187,188] and restriction fragment length polymorphism (RFLP)[187] markers have been used but are limited, respectively, by low polymorphism[188] and the unusually large genome size typical of *Allium* species[187].

It was also reported by[187] that development of a set of genomic dinucleotide simple sequence repeat (SSR) markers from onion. As a result of complex amplification requirements, these markers have not proved sufficiently portable to enable wider use in mapping and diversity studies, although[187] reported optimization and chromosomal allocation of a subset of these. More recently, SSR and single-nucleotide polymorphism (SNP) markers have been developed from onion expressed sequence tag (EST) resources[188,189] and proved to be readily reproducible for mapping (and cultivar discrimination.

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2.9.2. Detection Methods for Single Nucleotide Polymorphisms

Although single-nucleotide-relevant alterations are more difficult to detect than numerous ones, multiple methods can be performed to identify mutations/SNPs in genomes. Specifically, distinguishing single nucleotide alterations with high sensitivity and specificity is important for evaluating these methods[190]. Common methods for detecting SNPs include DNA genome sequencing methods (e.g., whole-genome sequencing (WGS) and targeted gene sequencing),[191]and PCR-based methods (especially using real-time PCR platforms[191] and additional detection methods include mass spectrometry SNaPshot microchip methods and denaturing high-performance liquid chromatography (DHPLC)[192,193,194].

2.9.3. PCR-Based SNP Detection Methods

It is difficult to envisage current molecular biology research without PCR. PCR-based methods, especially real-time PCR, are widely applied to detect SNPs and mutations. In real-time PCR instruments, PCR-amplified products undergo gel electrophoresis followed by DNA-binding fluorescent dye staining to confirm the end products. RT-PCR can monitor the DNA amplification of each cycle, allowing a visual readout of PCR amplification kinetics, and provides platforms to measure the quality and quantity of PCR products. Generally, PCR-based methods for the detection of SNPs/mutations can be divided into two types:

1. primers matched with substituted nucleotides or oligonucleotides, to clamp or block the non-targeted template, are used to match mutant-allele-directed specific or polymorphic analysis; or2. melting curve analysis, using hybridization probes, hydrolysis probes, or double-stranded DNA-binding fluorescent dyes, combined with real-time PCR techniques [195].

Allele-specific PCR (ASPCR) is a sequence-specific amplification method using PCR, and is also known as mismatch amplification mutation assay. In this method, an amplification-refractory mutation system can be used to detect known SNPs/mutations. Primers or probes used in ASPCR are specific for the SNP/mutation under detection, and a PCR amplicon is needed to identify this SNP/mutation. The design of appropriate probes/primers is a crucial step of ASPCR.[196] TaqMan probe-based ASPCR for SNP detection has been commercialized for many years, and serves as a basic method to detect SNPs/mutations. This method is appropriate for low-throughput applications[197]. It is based on the energy transfer of fluorescence, in which the proximity of the indicator and quencher dyes in the intact probes reduces the indicator dye fluorescence. When the probes are mismatched with the template, the difference in annealing temperature varies, influencing the degradation of the probe.

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These changes are reflected in fluorescence, suggesting the detection of homozygous or heterozygous conditions with mutant or wild-type alleles.[198]

TaqMan probe-based ASPCR results are influenced by the activity of the probe used to detect the PCR products, while the probe degradation amount is affected by the concentration of the probe, the initial number of target molecules, and the number of cycles. Melting curve analysis, especially high-resolution melting curve (HRM) analysis, is also widely used to detect SNPs/mutations. HRM analysis can be performed to detect both known and unknown SNPs. Based on variations in the fluorescence of DNA-binding dye, through the conversion of double-stranded DNA to single-stranded DNA with a change in temperature, HRM analysis can detect the existence of SNPs/mutations and confirm the nucleotide substitution type.[199, 200] Melting temperature (T_m) is an elementary thermodynamic characteristic of DNA that can be altered by differences in nucleotide sequence, GC content, and amplicon length. A change in fluorescence indicates a shift in T_m that is visible in the melting curve analysis.[201, 202] Four classes of SNPs can be differentiated by HRM analysis, because these four kinds of SNPs have different T_m changes.

G/A and C/T base exchanges comprise the first class of SNP, while G/T and C/A form class two. Classes three and four consist of C/G and A/T base exchanges, respectively. Among the four classes of SNPs, class one and two base exchanges can be clearly genotyped by HRM because of their high T_m differences, of 0.5°C. Class three base exchange induces a T_m difference of 0.4°C, while a difference of 0.3°C is produced by class four base exchange. Digital PCR and co-amplification at lower denaturing temperature PCR (COLD-PCR) are important for detecting SNPs/mutations. In digital PCR (also called realistic single-molecule PCR), a DNA template is typically diluted in 96-well plates, where two wells cover one

template molecule, on average. Thereafter, this diluted template is later used for PCR amplification in nested PCR. To distinguish wild-type or mutant sequences, two molecular beacons are added to the reaction mixture before PCR amplification, and the two beacons are labeled with different fluorescent dyes. Without cloning the PCR products in advance, digital PCR is able to determine whether the variants are present in each allele or in only one allele, which is different from other methods[203]. Nevertheless, digital PCR is limited in its capacity to analyze wells, which impacts the sensitivity of this method. In contrast, the COLD-PCR method is named for its ability to enhance the amplification efficiency of one template by optimizing the denaturing temperature. In COLD-PCR, a critical denaturation temperature (T_c, at which mutation-containing DNA is preferentially melted over wild type) should be used that is lower than the melting temperature (T_m).

Furthermore, wild-type and mutant allele heteroduplexes are preferentially denatured over wild-type homoduplexes, enabling the heteroduplexes to be amplified several times more than the homoduplexes. There are a number of modified COLD-PCR methods, including improved and complete enrichment COLD-PCR and temperature-tolerant COLD-PCR, which have different approaches and should be differentially applied depending on the case [204].

2.10 Phylogenetics

Plants require at least 17 elements to complete their life cycles, including the Antibacterial activities Cu, Zn and Ni. Plants also accumulate nonessential metals, such as Pb and Cd, when they are present in the environment. Most metals contained within plant tissues are acquired from the soil solution, and plants have evolved elaborate rooting systems and transport mechanisms to mediate this process. Plants display a wide range of adaptations to

soils with contrasting metal contents. Such adaptations have occurred throughout evolution. For example, the phenotype of shoot metal hyperaccumulation is an extreme plant response to soils with elevated metal content. Often there is a single species exhibiting hyperaccumulation within a genus suggesting that the trait of hyperaccumulation has evolved independently at a taxonomic level below the genus many times. Similarly, the calciotrophicphenotype, which also impacts on the shoot content of other metals, has evolved independently many times.[205] Such evidence indicates that some differences in shoot metal content can be attributed to recent evolutionary processes.

By contrast, hyperaccumulation appears to have evolved most frequently in certain plant groups and the shoot Ca and Mg contents of dicotyledons (eudicots) are much greater than those of monocotyledons (magnoliids). This implies that differences in shoot metal content can also be attributed to ancient evolutionary processes. The influence of phylogeny on shoot metal content has not previously been quantified. In this paper the variation in shoot metal content has been partitioned into different classification levels. In addition, the hypothesis was tested that, independent of phylogeny, traits impacting on the accumulation of Cd, Cr, Cu, Ni, Pb and Zn in plant shoots are associated [204].

2.11 Review of Previous Empirical Works

A group of researchers compared different types of vegetables and *Azadirachta indica (Neem) and Morinda lucida* ,chromosomal aberrations resulted from heavy metal accumulation[206]. Industrial effluents were used to grow different varieties of vegetables and *Allium cepa* to determine the bioaccumulation of various toxic metals and consequent genetic changes in contaminated food crops. To test this hypothesis and extent of genetic modifications, *Azadirachta indica (Neem) and Morinda lucida*test was performed to food crops viz. tomato (*Lycopersicum esculentum*) and chili (*Capsicum annum*) as *Azadirachtaindica (Neem) and*

*Morinda lucida*test is a useful tool to assess genetic variations in plants. Prior to *A. indica* test, the plants were exposed to various metal concentrations 125–1000 mg/L in the synthetic wastewater. The extracts of harvested plants were used to grow the root of *A. indica* following its standard method.

The root tips were fixed, stained and examined under compound microscope (almost 300–400 dividing cells) to check the extent of chromosomal variations during various stages of mitosis. The results revealed various chromosomal abnormalities including laggards, stickiness, vagrant chromosomes, binucleated cells, nuclear lesions, giant cells and c-mitosis at different level of treatment. On the whole, aberrations were increasing with the increasing doses along the positive control. In comparison, chili crop had higher level of aberrations depicting the higher chromosomal changes. Lower mitotic index (MI) with increasing level of doses was also describing the hampered cell division due to increased metal stress.

The study showed that cell division was ceased with increasing metal stress, thus, increasing the rate of cell aberrations. The study assessed the effects of this effluent on the test organisms, mitotic index and different abnormalities were observed but failed to investigate the effects on the DNA sequence from which single nucleotide polymorphism (SNPs), SNPs frequence distance of occurrence along the DNA sequence and phylogenic tree.

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A group of researchersconsidered effects of organic and inorganic pollutants ladened with tannery liquid effluents on, antibacterial activities from a common effluent treatment plant (CETP) in Unnao district, India using *Vigna radiata* and *Azadirachta indica (Neem) and Morinda lucida*[207].The leather industry is seen as one of the major sources of environmental pollution in India. The liquid effluent (wastewater) generated by leather industries contains

very high pollution parameters as a result of mixture of both complex organic and inorganic pollutants even after the treatment at a common effluent treatment plant (CETP) and disturbs the ecological flora and fauna.

The nature, characteristics and toxicity of CETP treated wastewater is yet to be fully elucidated. Thus, this study aims to characterize and evaluate the toxicity of CETP treated tannery liquid effluents (wastewater) collected from the Unnao district of Uttar Pradesh, India. In addition to measuring the physico-chemical parameters, the residual organic pollutants was identified by GC-MS analysis and , antibacterial activities of the treated wastewater was evaluated using *Vigna radiata L*. and *Azadirachta indica (Neem) and Morinda lucida L.* Results showed that the treated liquid effluent (wastewater) contained very high pollutant.

The study revealed treated liquid effluent from leather industry in India contained some toxic substances which disturbed cell division processes in the plants used and as such some chromosome aberrations were observed but did not consider the level of damage done along the DNA sequence from which the level of SNPs, types, SNPs fequencedistance of occurrencealong DNA sequence, the relationships between test organism with control (phylogenetic tree).

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Previous work on the cytological effects of Chromium on root tip cells of *Azadirachta indica (Neem) and Morinda lucida L.* was based on the antibacterial effects of chromium a heavy metal on meristematic cells of root tips in *Azadirachta indica (Neem) and Morinda lucida L.* There was decreased in cell division rate and deviations from the normal mitosis in the result of chromosome mutations were registered[208]. To compare cell division rate in determining the mitotic index, we examined a total of 2040 cells for the control No1 and 1233 cells for the

test sample No2. The antibacterial analysis showed the absence of any mutations in the control and the presence of 2.231% in the test sample No 2. The highest frequency (1.79%) was shown by micronuclei. Using anaphase analysis, we established anaphase and telophase bridges, as well as chromosome fragments with frequencies of 0.11 % and 0.097%, respectively. Fragment chromosomes with frequencies of 0.024% in the anaphase and 0.04% in the telophase were also observed. In our opinion, the high micronuclei frequency observed in experimental sample was induced either by the lagging of whole chromosomes or the immobility of large acentric fragments.

The study was based on the cytological analysis only from which mitotic index and chromosome aberrations on the root tips of *Azadirachta indica (Neem) and Morinda lucida*were consideredwithouttaken antibacterial analysis into consideration.

A research assessed antibacterial effect of Antibacterial activities Cr, Cu, Pb and Zn using *Azadirachta indica (Neem) and Morinda lucida L.*Some higher plants are recognized as appropriate genetic attributes to detect heavy metal based environmental mutagens and are used in monitoring studies[209]. *Azadirachta indica (Neem) and Morinda lucida* (onion) has been used to determine DNA damages like chromosome aberrations and abnormalities in the mitotic cycle. The study was aimed at analyzing the antibacterial effects of chromium, copper, lead and zinc in *A. indica* root tip squash mitotic cell divisions. Mitotic indices and chromosomal abnormalities were calculated.

It was observed that these Antibacterial activities induced different types of chromosomal abnormalities comprising of Chromosome break, Chromosome bridge, C-mitosis, Vagrant, Delayed Anaphase and Vagrant, Chromosome Loss, Polyploidy and Chromosome Bridge,

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Chromosome Loss and Loculated Nucles, Stickiness, Multipolarity and Polyploid prophase along with the increasing doses. The effect of chromium and lead at 20 mg/100 ml concentration was found to be more toxic rather than copper and zinc to the root meristem of *A. indica.* The ranking of antibacterial potentials was in the descending order: lead > chromium > copper > zinc.

The study mainly looked into antibacterial and a section of antibacterial effects of Antibacterial activities Cr, Cu, Pb and Zn on the root tips of *Azadirachta indica (Neem) and Morinda lucida L*. and did not considersingle nucleotide polymorphism (SNPs), the SNPs frequence distance of occurrence along the DNA sequence and phylogenetic tree.

A group of scientist analysed the antibacterial effects of silver nanoparticles on meristematic cells of *Azadirachta indica (Neem) and Morinda lucida*roots[210]: A close analysis of particle size dependence.Silver nanoparticles (AgNPs) in commercial products has increased significantly in recent years due to its uses. Hence, more researches has to be launched in finding the toxic effects of the AgNPs as released into the environment. The paper reports an investigation on the antibacterial and antibacterial potential of the AgNPs on root cells of *Azadirachta indica (Neem) and Morinda lucida*.

Germination (GI), root elongation (REI), mitotic (MI), nuclear abnormality (NAI), and micronucleus index (MNI) were determined for seeds exposed to various AgNPs diameters (10, 20, 51, and 73 nm) as well as to the silver bulk (AgBulk) (micrometer-size particles) at the concentration of 100 mg·L−1. Transmission electron microscopy (TEM) provided the particle size distribution, while dynamic light scattering (DLS) was used to get the hydrodynamic size, polydispersity index, and zeta potential of the AgNPs. Laser-induced

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breakdown spectroscopy (LIBS) and inductively coupled plasma/optical emission spectrometry (ICP OES) were applied for quantifying the AgNPs content uptake by roots. Silver dissolution was determined by dialysis experiment. Results showed that the AgNPs penetrated the roots, affectingMI, GI,NAI, and MNI in meristematic cells. Changes in these indicatorswere AgNPs diameter-dependent so that antibacterial and antibacterial effects in *Neem* increased with the reduction of the particle diameter. The results also revealed that the AgNPs were the main responsible for the antibacterial activities since negligible silver dissolution was observed.

Their study only gave an insight on antibacterial and a section of antibacterial activities but did not consider others which deal with SNPs along DNA genome from which damages to the DNA sequence will be revealed and the genetic distance relationship between test organism and control.

A study by a group of scientists[211]examined the potential effects of cadmium and zinc antibacterial in the meristematic tissues of basil (*Ocimumbasilicum*L.)The antibacterial study on the meristematic tissues of basil (*Ocimumbasilicum*L.) aimed at evaluating some antibacterial effects induced by two Antibacterial activities (cadmium – Cdand zinc - Zn)applied in three different concentrations: 10, 50 and 100 ppm. Antibacterial tests reveal a decrease of the mitotic index and the occurrence of various chromosomal aberrations following heavy metal treatments. The cell division was significantly affected, especially in the case of Cd treatment, which showed the highest degree of toxicity in all variants compared to control variant. Instead, Zn has a lower degree of toxicity but only at concentrations of 50 ppm and 100 ppm.

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Types of chromosomal aberrations were relatively varied, being randomly distributed and concentration dependent, for both Cd andZn. Cells with large nucleus and disorganized-looking; interphases with pyknotic nucleus; cells with laggard chromosomes, pyknotic and sticky chromosomes, as well as cells with telophase bridge were observed. The results reveal that Cd (at all tested concentrations) and Zn in concentrations higher than 10 ppm exhibited significant antibacterial potential to *Ocimum basilicum* L. as a result of the effects reported in cell divisions of the meristematictissues.

This study only analysed the effects of cadmium and zinc on *Ocimiumbasilicum L* at the antibacterial level, but did not consider antibacterial effects, which should be done to reveal other areas like single nucleotide polymorphism (SNPs), SNPs frequencedistance of occurrence and even phylogenetic tree.

A research observed some chromosome abnormalities when root tips of *Azadirachta indica (Neem) and Morinda lucida* was induced by treated textile effluents: Spatial and Temporal Variations. When liquid effluents are appropriately treated, it is expected to have significantly reduce the toxicity of effluents before they are released to the natural environment[212]. Present study was aimed to assess the spatial and temporal variations of the physical and chemical water quality parameters of a natural water body receiving treated textile effluents and to assess the chromosomal abnormalities induced by the treated textile effluents. Four sampling sites (A: effluent discharge point; B: 100m downstream from site A along the tributary; C: 200m downstream from site A along the tributary; D: 100m upstream from site A along the tributary) were selected associated to a tributary that received treated textile effluent. The physical and chemical water quality parameters were measured in the composite water samples collected from the study sites, and *Azadirachta indica (Neem) and Morinda lucida*

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bioassay was conducted using aged tap water as the control. Sampling was conducted in both rainy and dry seasons. The conductivity, TDS, COD, and colour intensity of the water samples collected from the study sites were significantly higher during the dry season compared to those in the rainy season.

Azadirachta indica (Neem) and Morinda lucida root meristematic cells exposed to water samples from sites A, B, and C showed a significantly high interphase and prophase indices compared to those exposed to aged tap water and upstream site during both rainy and dry seasons. Mitotic index of the root tip cells of *Azadirachta indica (Neem) and Morinda lucida* bulbs exposed to the water samples collected from the effluent discharge point (site A) and from the 100m downstream site from site A (site B) was significantly lower than that of the other sites in both rainy and dry seasons. However, the mitotic index of the root tip cells of *Azadirachta indica (Neem) and Morinda lucida* bulbs exposed to the water samples from the upstream site was not significantly different from that of the control treatment during both sampling seasons.

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The bioassay indicated that the mitotic index and phase index of the root meristematic cells of *Azadirachta indica (Neem) and Morinda lucida* can be affected by the treated textile effluents released to the water body and the occurrence of C metaphase, chromosomal adherence, bridges, disturbed anaphase, vagrant chromosomes, and chromosomal breaks indicated that the treated textile effluent receiving tributary can possibly contain antibacterial and mutagenic compounds which can induce chromosomal abnormalities.

The study indicated that the effluent contained certain toxic substances even after treatment because analyses carried out revealed mitotic index and phase index of the root meristematic

cells were been disturbed and the occurrence of C metaphase, chromosomal adherence, bridges, disturbed anaphase, vagrant chromosomes but did not look into damages done along DNA genome (SNPs), its distance frequence of occurrence and genetic distance of relationship between test organism and control.

A group of Nigerian scientists[213] assessed the toxicity of leachates from Olusosun and Igando using African Catfish (*Clariasgariepinus*). Olusosun and Igando landfill leacahates were investigated for their toxicity using Clariasgariepinus as the bioindicator species. Physical and chemical analyses of leachates showed that pH was generally basic (11.46; 10.15 respectively). The electrical conductivity, total dissolved solids, total hardness, chloride, nitrate and sulphate for Olusosun and Solous landfills leachates were found to have exceeded the WHO limits for drinking water [213]. Antibacterial activities analyses showed that the concentration of nickel (75.5 ± 6.9 to 269.7 ± 1.33 µg/L), lead (74.7± 9.8 to 259.83 ± 8.3 µg/L), zinc (20 ± 7.5 to 198.2 ± 5.5 µg/L), cobalt (< detection limit to 12.1 ± 0.7 µg/L), cadmium (4.3 ± 0.6 to 4.7 ±0.2 µg/L) and copper (29.5 ± 1.3 to 220.3 ± 1.9 µg/L) were above the WHO (2007) recommended limits.

Toxicity assessments carried out showed that the treatment level of 7.5 % was the least toxic to the fingerlings. The haematological results showed that Packed cell volume, Red Blood Cell count (1.5 12 12 9 9×10 /L, 1.85 x 10 /L), Haemoglobin (6.5 g/dL, 7 g/dL), White Blood Cell (10.95 ×10/L, 13.5 x 10/L), Heterophils (28.5 %, 38 %), Lymphocytes (70.5%, 62%), Eosinophils (0.5 %, 00 %), Basophils (0.5 %, 01 %) and Monocytes (00 % 00%) for Olusosun and Solous landfills leachates respectively were higher than in the control fish not exposed to leachate samples. Histopathological analyses of the fishes that survived the leachate exposures showed damage to the gills and liver of the fishes.

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The analysis carried out showed leacheates obtained from both Olusosun and Igando landfill contained some chemical substances which affected the targeted organs of the organism but the study did not take into consideration effects on the DNA sequence.

Another group of scientists [214] carried out analysis on antibacterial activities effects of borehole water sources from densely populated of local government area of Lagos state using*Azadirachta indica (Neem) and Morinda lucida*test. In recent time, increased population in some areas in Lagos state, places like Ikotun, Ikeja and Alimosho local government areas is becoming alarming as this is expected to create pressure on the facilities needed for the basic day-to-day activities of inhabitants to these areas. The antibacterial and antibacterial potentials of borehole water in these areas were analysed using the *Azadirachta indica (Neem) and Morinda lucida*test. Water samples were collected from three points in each local government. Ikotun (Governor's road, Arida area and Igando Road), Ikeja (Balogun area, Oba Akran area and Opebi road) and Alimosho (Ponle area, Williams layout and Alaguntan area) water. The result showed that test water mitotic index decreased significantly ($p<0.05$) from control [214]. The water samples were characterized by a number of chromosomal aberrations notably bridges, fragments, sticky chromosomes, disoriented chromosomes, and binucleated cells in significant amounts and these were more pronounced in water samples obtained from Ikotun local government (Governor's road, Arida area and Igando Road). The findings in this study are of public health relevance as access to safe water is a fundamental human need and therefore, a basic human right.

Their study only gave insight into decrease that occurred in mitotic index as against the control and some chromosome abnormalities that were observed notably bridges, fragments,

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sticky chromosomes, disoriented chromosomes, and binucleated cells in significant amounts but did not investigate damages which may occur along the DNA genome such as SNPs, its frequence distance of occurrence and genetic distance of relationship between test organism and control.

Another research[215] reviewed antibacterial activities and mutagenicity of solid waste leachates. Solid waste production is inevitable and its unsanitary disposal in the environment is of public and environmental health concern. Leachate, generated due to the infiltration of water precipitation through the waste mass and the wastes biodegradation, is a mixture of dissolved organic matter, inorganic macro-components, Antibacterial activities, xenobiotic organic compounds and microorganisms[215]. Several studies have reported the acute toxicity of leachate using different end points, while evidences are accumulating on their potentials to induce genetic damage. In this wise, different short-term in vivo and in vitro bioassays are being utilized in the evaluations of antibacterial activities and mutagenicity of leachates; and the possible mechanisms of genetic damage. This paper reviews reports on leachate-induced genetic damage. There is need for a shift from waste disposal to sustainable waste management. Awareness on possible health impacts or consequences of exposure to solid waste should also be created through health education.

This study has showed both in vivo and in vitro effects, results indicated the presence of toxic substances in the leachate. Solid effluent would have been digested with concentrated acids then, neutralize with CaO to have pH of between 6.8 –7.2.This is necessary to have maximum concentration of Antibacterial activities ions presence in the leachate. The mutagenicity and a part of antibacterial were considered neglecting SNPs, its frequence distance of occurrence, functional parts of DNA genome when compare with the control and its phylogenetic tree.

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A research[216] investigated antibacterial activities of some chemicals on onion (*Azadirachta indica (Neem) and Morinda lucida L.*) and grass pea (*Lathyrussativus L.*). Chemicals that have adverse effects on normal cell division are called antibacterial chemicals. Root Length, mitotic index and cytological studies of root tips in the presence of different antibacterial agents were experimented on onion (*Azadirachta indica (Neem) and Morinda lucida L.*) and Grass pea (*Lathyrus sativus L.*). Different chromosomal abnormalities were observed from the root tip cytology[216]. The root Length decreases most in higher concentration of the chemical with respect to control[216]. The most frequent abnormalities are bridges, vagrant and stickiness of chromosome. Grass pea shows two additional abnormalities of pyknosis and karyorrhexis. The results show that the chemicals have toxic effects on both the samples. This study proves that besides onion, grass pea can also be used as effectively for cyto-antibacterial assessments for environmental toxic agents.

Aveek's study which was based on effects of certain chemical of the two angiosperms used altered the cycle of cell divisions is an indication that the chemical is toxic but would have gone further to consider its effects on the genome of the DNA.

Another research by a group of scientist[117]determined the antibacterial and phytotoxic potentials of "Chi Limited" industrial effluent on *Azadirachta indica (Neem) and Morinda lucida*and *Vigna unguiculata.* Chi limited industrial effluents were investigated using *Azadirachta indica (Neem) and Morinda lucida*and *Vigna unguiculata*. Physicochemical properties, Antibacterial activities and pH of the industrial effluent were determined in accordance with standard method. Onions root growth inhibition test was used to assess the antibacterial activities of the effluent. Onion bulbs were exposed to 0%(control), 4%, 8%,

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12% and 16% concentrations of the effluent samples in the dark for 72 hours before measuring the root lengths of the onion bulbs. Also, the seeds of *Vigna unguiculata* were planted in Petri dishes in the laboratory at concentrations of 0%(control), 5%, 10%, 15% and 20% for more than six days before measuring the root length, shoot length and determining the number of germinating seeds in each concentration of the effluent.

A. indica root tips exposed to effluent concentrations ranging from 4% to 16% v/v showed a significant reduction in mitotic index (MI) from 39% to 33% compared to control which is 77%, indicating effluent induced antibacterial activities. Statistical analysis using analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the mean root lengths of *A. indica* exposed to different concentrations of Chi limited industrial effluent at both day six and twelve. Also, the effects of concentrations and varieties on the shoot lengths of *Vigna unguiculata* are statistically different compared to root lengths which are not significantly different at $P< 0.05$.

The study gave insight into physicochemical properties, pH of the industrial effluent, took measurement of the root lengths of both plants, considered the mitotic index and compared with the control. There were disparities in the analyses carried out when compared with the control but did not consider antibacterial effects of this effluent which would have given more insight into the level of damages that occurred along DNA genome and functional parts of DNA genome when compare with the control.

Catalin Aurelian Rosculete, Elena Bonciu, Elena Rosculete, and LiviuAurel Olaru[218] worked on the environmental pollution potential of selected herbicides (quizalofop-p-ethyl and cycloxydim), using *Azadirachta indica (Neem) and Morinda lucida* test to determine the antibacterial and antibacterial effects. *Azadirachta indica (Neem) and Morinda lucida*(onion, 2n = 16), one of the most common plant indicators of environmental pollution.

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The working method consisted of obtaining the meristematic roots of *Azadirachta indica (Neem) and Morinda lucida*and their treatment with herbicides at three different concentrations (0.5%, 1%, and 1.5%) for each herbicide for 24 h, for comparison with an untreated control. The results obtained from the cytological study indicated a strong antibacterial and antibacterial effect for both herbicides, but especially for quizalofop-p-ethyl, where the mitotic index decreased from 30.2% (control) to 9.6% for the variant treated with 1.5% herbicide. In this case, a strong mitodepressive effect was shown by a highly significant percentage (35.4%) of chromosomal aberrations and nuclear alterations: stickiness, fragments, C-mitosis, lobulated nucleus, micronuclei, and nuclear erosion. The mitodepressive effect as well as the percentage of chromosomal aberrations increased with a higher herbicide concentration. The obtained results suggest the strong potential for pollution of the two herbicides, particularly at concentrations higher than 0.5%; therefore, we recommend caution in their use to avoid undesirable effects on the environment.

Their study revealed different analyses carried out to determine the antibacterial and antibacterial effects. The results obtained from antibacterial and antibacterial effects for both herbicides is

strong, especially for quizalofop-p-ethyl, where the mitotic index decreased and chromosomal aberrations like nuclear alterations: stickiness, fragments, C-mitosis, lobulated nucleus, micronuclei and nuclear erosion were observed. But the study did not take into consideration other analysis like SNPs, its frequence distance of occurrence and functional parts of DNA genome when compare with the control from which more detail of the damage could be revealed.

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Another study[219] assessed ecological effects on the leachate from Amilegbe dumpsite, Ilorin, Nigeria using *Clariasgariepinus* (Burchell 1822) and *Azadirachta indica (Neem) and Morinda lucida*. Indiscriminate discharge of solid waste materials from anthropogenic activities has become a major environmental problem in Nigeria. Leachate samples were collected from Amilegbe dumpsite in Ilorin. The physico-chemical qualities of the leachate as well as Antibacterial activities content were analysed using standard methods. Different concentrations (3.625 %, 6.25 %, 12.5 %, 25.0 %, 50.0 % and 100.0 % (v/v leachate/distilled water)) of leachate samples were prepared. The 96–h LC50 of leachate samples was determined for *Clariasgariepinus* fingerlings using Probit method[219].

*Azadirachta indica (Neem) and Morinda lucida*bulbs were also exposed to the different concentrations of the leachate and the root length inhibition and chromosomal aberration were investigated. The results showed certain sample-constituents of the leachate (e.g. pH, BOD, COD, Antibacterial activities) to be at concentrations beyond the maximum permissible limits set by National Environmental Standards and Regulatory Enforcement Agency NESREA. The 96–h LC50 of leachate to *Clariasgariepinus* fingerlings was 20.26%. Prior to the mortalities at various concentrations, symptoms of toxicity such as rapid and erratic swimming, uncoordinated movement and prolonged gaping of jaws were observed. These observations as well as mortality records were concentration–dependent. The root lengths' mean of *A. indica* exposed to different concentrations of the Amilegbe leachate when compared to the control, were statistically significantly different ($p<0.05$) with concentration dependent. The leachate concentrations were observed to induce different chromosomal aberrations with mitotic indices decreasing as the concentration rises. Leachates from the dumpsite have detrimental effects on both *Clariasgariepinus* and *Azadirachta indica (Neem) and Morinda lucida*.

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Their study revealed different analyses been carried out which include the physicochemical properties, Antibacterial activities concentrations, pH, BOD, COD, others are antibacterial and antibacterial on the roots of *Azadirachta indica (Neem) and Morinda lucida* .Solid effluent would have been digested with concentrated acids then, neutralize with CaO to have pH of between 6.8 – 7.2, this is necessary to have maximum concentration of Antibacterial activities presence in the leachate, study did not also considered SNPs, its distance frequence of occurrence and relationships between test organism and the control (phylogenetic tree).

A group of researchers[220]evaluated for the standardization of the *Azadirachta indica (Neem) and Morinda lucida*test as antibacterial activitiesassay.A general report on the use of the *Allium* test as antibacterialological and antibacterialological assay is proposed, with particular emphasis about the standardization of the test in several common applications. The intraspecific variation in *Azadirachta indica (Neem) and Morinda lucida*has been until now overlooked, as in most investigations no mention is made about the origin and denomination of the onion cultivar used in antibacterialological studies[220]. A standardization of the used material in 20 all studies would allow a better generalization of the results, since we cannot be absolutely sure that all cultivars will give the same answer in response to a given antibacterial agent.

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A more frequent use of transmission electron microscopy (TEM)AQ1 investigation is proposed. Even if it is relatively time consuming and not available in all laboratories, it may help to better understand the mechanism of antibacterial activities, since many morphological characters used in data 25 collection may appear to be morphologically similar but may have arisen from very different processes. In fact, some data can be observed only with TEM.

About statistical testing, tests other than chi-squared may be used in case of a lower amount of data. The most commonly used statistical tests are the parametric tests ANOVA and Student's t, and the non-parametric tests Kruskal–Wallis and Mann–Whitney U, for analysis of variance. Tests should be used also 30 to assess the minimal sample dimension for obtaining significance, since data collection (microscope observation) appears to be one of the main bottle necks of the test. Also the use of the *Allium* test for testing liposomes and other nanovectors for drug delivery is proposed, in order to assess the antibacterial activities of these types of medium and the possible increase in antibacterial activities of the associated drug.

Their study considered standardization of *Azadirachta indica (Neem) and Morinda lucida*test and employed most of the latest techniques of experimentations but did not invove DNA sequence for determination of SNPs, its frequence distance of occurrence, functional parts of DNA genome and its phylogenetics.

A research[221]investigated the antibacterial of Photographic effluent using *Azadirachta indica (Neem) and Morinda lucida*Assay.The increased need to keep graphic records of events has led to the use of photography in investigation and surveys and hence increased photographic activities. Toxicological survey of photographic effluents has received little attention in Nigeria, though the constituents have been shown to be very toxic. This study investigated the antibacterial effects of photographic effluents from selected locations in Lagos, Nigeria using *Azadirachta indica (Neem) and Morinda lucida*assay, viability test, root length measurements and cytological studies. There was inhibition of root length development in addition to several chromosomal aberrations observed in the root of *Azadirachta indica (Neem) and Morinda lucida*exposed to the effluents[221]. Furthermore, there were statistical

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differences among the aberrations produced by the different concentrations of the effluent ($p<0.05$; $p<0.01$; $p<0.001$). It was inferred that photographic effluents have antibacterial and antibacterial effects. Thus, there is need for biological detoxification of photographic effluents before disposal into the environment to remove or reduce the pollutant load in them.

This study has showed effects toxic substances present in activities of photography, results indicated that the substances are capable of causing aberration in the DNA sequence. But thy study only considered sections this effects neglecting part of antibacterial activities these include SNPs, its frequence distance of occurrence, functional parts of DNA genome when compare with the control and its phylogenetic tree.

Another group of Nigerian researchers[222]assessed DNA Integrity of Onion (*Azadirachta indica (Neem) and Morinda lucida L.*) Root Cells Exposed to Ballast Water. Contaminated ballast water can pollute fresh waters. Regular sampling followed up by detailed analysis appears to ensure the proper monitoring that shipping activities do not encourage its indiscriminate discharge on the environment. In this study, the random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in *Azadirachta indica (Neem) and Morinda lucida*L. roots exposed to ballast water at different concentrations [0.5, 1, 5 and 10% (v/v)]. Compared to the control (tap water), the DNA obtained from the onion roots exposed to the wastewater caused greater changes in the RAPD patterns. This was discernible with appearance/disappearance of bands in the treated plants. A total of 116 RAPD bands were obtained using five oligonucleotide primers and 61 (52.5%) of these showed polymorphism.

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Onion bulbs exposed to the ballast water caused 17 new bands to appear and 18 to disappear; the loss and gain of bands decreasing with the raise of wastewater concentration. The genetic distances shown on the dendrogram revealed that antibacterial activities of the wastewater was concentration-dependent. The data obtained from the RAPD fingerprinting imply that proper treatment should be given to ballast water to prevent, minimize, and ultimately eliminate the risks associated with its discharge into the environment as the wastewater is capable of inducing antibacterial effects.

Their study results indicated the presence of toxic substances in the ballast water with full scale of analyses but would have been better if universal primer (RBCL) instead of the primers used, the study did not mention the frequence distance of SNPs occurrence, said nothing about functional parts of DNA genome and no mention of phylogenetic tree.

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2.12 Synthesis of gaps Identified

The studies reviewed can be grouped into three: those that only considered the concentration of Antibacterial activities from their samples, those that used *Azadirachta indica (Neem) and Morinda lucida as* their test organism and lastly, those that used *Azadirachta indica (Neem) and Morinda lucida*together with other angiosperm. The last two groups can be sub-divided into: those that considered concentrations and antibacterial effects on their test organism(s) and those that considered concentrations, antibacterial and antibacterial effects on their test organisms. Some of the studies involved the use of liquid effluents while some used solid effluents.

From the review, the following dissimilarities with the current study are highlited:

i. Determination of only the concentration without it effects on organisms may not be too good for research.
ii. In other to have maximum concentration of Antibacterial activities, those with solid effluent would have digested with acids (HNO_3 and HCl) in ratio 3:1, then, neutralize it CaO to have a pH of between 6.8 – 7.2.

i. In almost all the papers reviewed except one, it was both antibacterial and antibacterial effects but despite mentioning them only determination of Antibacterial activities concentrations, mitotic index and chromosomes aberrations were considered which are mainly of antibacterial.
ii. None of the studies made mention of Single nucleotide polymorphism (SNP), its frequence distance of occurrence and the functional parts of DNA genome in which their could be removal or addition when compare with the control. Addition of new parts may result to evolution. The other part of genoxicity which was mentioned only in one of the studies is the genetic distance(s) between the control and test organisms sequences(phylogenetic tree).
iii. The use of universal primer (RBCL) is universal acceptable than other primers.

This study therefore improved on the existing empirical studies by delving indepth into both the antibacterial and antibacterial analyses of Antibacterial activities using *Neem.* In the antibacterial analyses, Single nucleotide polymorphism (SNP), its frequence distance of occurrence and the functional parts of DNA genome and phylogeny of the smples were determined.

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Chapter Three

Methodology

3.1 Collection of Plant Materials

Fresh leaves of *Azadirachta indica and Morinda lucida* leaves were purchased from Bode market, Ibadan.

3.2 Preparation of Extracts

Azadirachta indica and Morinda lucida leaves were properly washed under running tap water, drained, air-dried at room temperature, and then processed into powder form using an industrial blender (Marlex) before being kept in an airtight container for future usage[1].

3.3 Collection of Test Organisms

The Medical Microbiology Laboratory, University College Hospital, Ibadan, Oyo State, provided clinical cultures of bacterial isolates for the *in vitro* antimicrobial assay. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhiii, Enterobacter cloacae, Proteus mirabilis and Staphylococcus aureus* the bacteria collected.

3.3.1 Sterilization of glassware

All laboratory glasswares, including conical flasks, glass stirring rods, beakers, measuring cylinders, and pipettes, were thoroughly cleaned with detergent solution, rinsed with distilled water, drained, dried, and sterilized in a hot air oven at 160°C for 2 hours after being wrapped in aluminum foil. Materials like McCartney bottles and bijou bottles were similarly wrapped in aluminum foil paper and sterilized in an autoclave at 121°C for 15 minutes and 15 pounds per square inch (psi) pressure[2]. In addition, throughout use, the inoculating wire loop and cock borer (8mm) were sterilized by heating to red hot in a spirit lamp flame at regular intervals.

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3.3.2 Preparation of Nutrient agar

Twenty eight grams of nutrient agar powder was weighed using an electronic weighing scale and poured into 1000ml of distilled water in a 1000ml conical flask, as directed by the manufacturer. The mouth of the conical flask was plugged with a stopper made of cotton wool covered in aluminum foil. It was swirled to combine, then heated in a water bath at 95°C for 15 minutes to homogenize it. The conical flask containing the homogenized nutrient

agar was wrapped in aluminum foil and sterilized in an autoclave at 121°C for 15 minutes at 15psi. The medium was autoclaved and then allowed to cool to 47°C before being gently and aseptically dispensed into sterile petri dishes, where it hardened.

3.4 Test Organisms

Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhiii, Enterobacter cloacae, Proteus mirabilis and Staphylococcus aureus. were utilized. These bacteria were grown on nutrient agar slants and stored at 4°C in the refrigerator. Using the streaking method, these organisms were sub-cultured into nutrient agar slants and nutrient agar plates. To prove the authenticity and viability of the test bacterium, Gram staining and additional confirmatory biochemical tests (catalase, oxidase, indole, and citrate) were performed on each of the bacteria isolates[2].

3.5 Plant extracts preparation

Ten grams of milled extract powder from *Azadirachta indica and Morinda lucida* leaves were weighed and dissolved in a 500 mL beaker, then steeped in 100 mL of each solvent separately. The beakers were covered with aluminum foil and stirred intermittently for 24 hours. Each solvent extract was filtered through filter paper into a 100ml beaker using a separation Buchner funnel after 24 hours[2]. This was then used right away, with the rest of the extract being kept in the refrigerator for further research.

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3.8 Concentration of Diluted Extracts

Concentration of each of the diluted extracts used was determined using the formula $c1v1=c2v2$ where M= concentration and V= volume (for initial and final).

Extracts of Azadirachta indica and Morinda lucida was milled into powder, and each investigates for antibacterial activity against some selected pathogenic bacteria using different concentrations of ethyl acetate, ethanol, and aqueous as solvent of extraction.

Isolated bacteria used was obtained from the laboratory unit of University College Hospital and were further subjected to standard antibiotic susceptibility test being sold commercially. Results obtained (Table 4.5) was compared with the results of the study.

25g of each of the extracts powder was dissolved into 150ml (166.7mg/ml) solvent. Further dilution was made by adding 5ml into the 10ml above to give a concentration of 111.1mg/ml. More dilution finally made serially up to the fourth fold to give a final concentration of 83.4mg/ml and 66.7mg/ml by adding 20ml and 25ml of solvents respectively.

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Concentrations

25g of each of the extract powder was dissolved into 150ml solvent.

a = 25 * 1000 = 25,000mg

25,000/150 = 166.67 mg/ml

b = 10ml extract + 5ml solvent = 15ml

$v2 = 15$

$c2 =?$

$c1v1= c2v2$

$c2 = c1v1/v2$

10 * 166.7/ 15 =

c2 = 111.1mg/ml

c = 10ml extract + 10ml solvent = 20ml

v2 = 20ml

$c2 =?$

$c1v1= c2v2$

$c2 = c1v1/v2$

10 * 166.7/ 20 =

c2 = 83.4mg/ml

d = 10ml extract + 15ml solvent = 25ml

$v2 = 25$

$c2 = ?$

$c1v1 = c2v2$

$c2 = c1v1/v2$

$10 * 166.7 / 25 =$

$C2 = 66.7 mg/ml$

3.9 Antimicrobial Screening

The antibacterial activity of each solvent extract was determined using the agar well diffusion method as per previous researches[1]. The diffusion of extracts from the cavity through the solid medium in the petri dish inhibits the growth of the cultured organism for a zone, forming a circular region around the extract, according to this procedure. This is demonstrated by the formation of a clearance zone, the diameter of which is directly proportionate to the extracts' efficacy.

3.10 Antibiotic Susceptibility Test

The disk diffusion method was used to assess antibiotic susceptibility using antibiotic discs (gram-positive and gram negative)[4]. The pathogenic bacterial isolates were streaked on each of the nutrient agar plates before the antibiotic discs were aseptically placed on each of the agar plates using sterile forceps. The agar plates were then incubated for 24 hours at 37 degrees Celsius. After that, the plates were inspected for inhibitory zones. Each antibiotic disc's inhibition zones were measured in millimeters.

3.11 Inhibitory Tests for Bacteria

Using a sterile inoculating loop, each test organism was smeared on solid nutrient agar until it covered the surface area of the petri dish. On the agar gel, a sterile cork borer with an 8mm diameter was used to cut deep and make five uniform wells. The extract was then poured into

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each well at various concentrations. To ensure appropriate diffusion, the plates were left at room temperature for 45 minutes. In the same way, each solvent of extraction was kept in one of the bored holes and served as a negative control. The plates were then incubated at 37°C for 24 hours before the zone of inhibition was determined. By comparing the differences in concentrations of the extracts with the control, the minimum inhibitory concentration (MIC) was established[6].

3.12 Phytochemical Screening

3.12.1 Qualitative Analysis

The active components of *Azadirachta indica* and *Morinda lucida* plant extracts were identified and quantified using chemical assays. The results of conventional investigations indicated the presence of phytochemicals such tannins, saponin, terpenoids like flavonoids, and alkaloids.

a. **Alkaloid:** Neem leaf powder (0.1g) was mixed with 2 mL of hexane, thoroughly stirred, and then filtered. The filtrate received 3 ml of 2% HCL. The mixture was filtered after being heated. The mixture was filtered after being heated. Alkaloid was detected by a yellow precipitate after adding a drop of picric acid to the filter[7].
b. **Terpenoids and steroids (Salkowski test):** The plant extract was combined with 1 mL chloroform and 1 mL conc. H2SO4 in a final volume of 3 mL to see the vivid red-brown coloring that indicates the presence of terpenoids[7].
c. **Flavonoids:**3 mL dilute ammonia was added to each plant extract's 2 mL aqueous filtrate. Following that, 1 mL concentrated sulphuric acid was added (H_2SO_4). The presence of flavonoids was indicated by the yellow coloring of each extract[8].
d. **Tannins:**In a test tube, 0.1 g of dried powder plant sample was cooked in 4 mL of water and then filtered. The presence of tannins was determined by adding a few drops of 0.1 percent ferric chloride to see brownish green or blue-black colouring[7].

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e. **Saponins:**One gram of the plant sample was mixed with 5 mL of 20% ethanol and kept at 55°C for 4 hours in a water bath. The residue was filtered twice and washed in 20% ethanol. In the oven, the extract was reduced to 5 mL. In a separating funnel, five milliliters of petroleum ether were added to the concentrated extract. The petroleum ether layer was removed, and the residue was treated with 3 mL of butanol before being washed with 5 mL of 5 percent sodium chloride. The butanol layer was put into a weighted Petri plate, evaporated to dryness, and the residue's weight was calculated[7].

3.12.2 Quantitative Analysis

a. Tannin

A 50ml beaker was filled with 0.20g of the sample. 20 ml of 50% methanol was added, and covered with parafilm. The sample was then placed in hot water bath for an hour at 77–80°C. It was vigorously shaken to achieve even mixing. Double layered Whatman No. 41 filter paper was used to quantitatively filter the extract I nto a 100 ml volumetric flask. Thereafter, 20 ml of water, 2.5 ml of the folin-Denis reagent, and 10 ml of 17% Na_2CO_3 were added and thoroughly mixed. Water was added to make the mixture up to mark . After 20 minutes, a bluish-green tint began to appear[7]. After color development, a Spectronic 21D spectrophotometer was used to measure the absorbance of samples and standard solutions of tannic acid at a wavelength of 760 nm. The formula below was used to calculate the % Tannin[7].

%TANNIN = absorbance of sample X average gradient factor X Dilution factor

- Wt. of Sample X 10,000

b. Alkaloid

This is a titrimetric and distillation process. 20ml of 80 percent pure alcohol was added to 2g of finely ground sample in a 100ml beaker to create a smooth paste. The liquid was then transferred to a 250 ml flask, and 1 g of magnesium oxide was added

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along with 100 ml additional alcohol. The mixture was digested for 1.5 hours in a boiling water bath with periodic shaking under a reflux air condenser. While still hot, the liquid was filtered through a tiny buchner funnel. The residue was put back into the flask and digested once more for 30 minutes with 50ml alcohol. After the alcohol had evaporated, boiling water was added to make up for the lost alcohol. Three drops of 10% HCL were applied after the entire amount of alcohol had been eliminated. The entire solution was then placed into a 250 ml volumetric flask, to which 5 ml of potassium ferrocyanide solution and 5 ml of zinc acetate solution were added and thoroughly mixed to create a homogeneous solution. The alkaloids were extracted thoroughly by shaking with five successive amounts of chloroform after the flask was allowed to stand for a few minutes, filtered through dry filter paper, and 10ml of the filterate was put into a separatory funnel. The resulting residue was diluted in 10 ml of hot distilled water, placed into a kjeldahl tube, and digested with 0.20 g of sucrose, 10 ml of concentrated sulfuric acid, and 0.02 g of selenium to produce a colorless solution for the Kjeldahl distillation method of determining the %N. By multiplying the percent nitrogen obtained by a factor of 3.26, it can be converted to % total alkaloid[8]. i.e % Total alkaloid = %N * 3.26

c. Flavonoid

A glass rod was used to swirl 80ml of 95 percent ethanol into 0.50g of finely ground sample in a 100ml beaker to avoid lumping. The mixture was put into a 100ml volumetric flask after being filtered using a Whatman No. 1 filter and adjusted with ethanol. 1ml of the leaf extract was pipetted into a volumetric flask (50ml), four drops of concentrated hydrochloric acid were added using a dropping pipette, and then 0.5 g of magnesium turnings were added to create a magenta red tint. From a 100ppm stock solution, standard flavonoid solution ranging from 0 to 5 ppm were

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made, and they were similarly processed with HCL and magnesium turnings as samples. A digital Jenway V6300 Spectrophotometer was used to measure the absorbance of the magenta red coloring of the sample and standard solutions at a wavelength of 520 nm. Using the formula below, the percentage of flavonoid is determined[8].

Absorbance of sample X average gradient factor X dilution factor

- Wt. sample X 10,000

d. Saponin

100ml of isobutyl alcohol was added to a 250ml beaker after 1g of the finely powdered sample was weighed in. To ensure even mixing, the mixture was shaken on a UDY shaker for five hours. After that, 20ml of a 40 percent saturated solution of magnesium carbonate was added after the liquid had been filtered through Whatman No. 1 filter paper into a 100ml beaker. To get a clear, colorless solution, the mixture made with saturated $MgCO_3$ was once more filtered through a Whatman No1 filter paper. A 50 ml volumetric flask was filled with 1 ml of the colorless solution, 2 ml of the 5% $FeCL_3$ solution, and the required amount of distilled water. For the blood red color to fully emerge, it was left to stand for 30 minutes. From saponin stock solution, standard Saponin solutions ranging from 0 to 10 ppm were prepared. Similar to what was done for the 1ml sample above, the standard solutions were treated with 2ml of a 5 percent $FeCL_3$ solution. After color development, a Jenway V6300 Spectrophotometer was used to measure the sample's absorbance as well as that of reference saponin solutions at a wavelength of 380 nm[8].

%Saponin = Absorbance of sample X gradient factor X dilution factor

- Wt. Of sample X10000

e. Glycoside

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A 250 ml conical flask was filled with 10 ml of extract via pipette. 50ml of chloroform was added to the extract and shaken on a vortex mixer for an hour. The mixture was filtered into a 100 ml conical flask, 10 ml of pyridine, 2 ml of sodium nitroprusside (2%) were added, and everything was firmly stirred for 10 minutes. Later, 3ml of 20% NaOH was added to create a brownish yellow color. Glycoside standards with concentrations ranging from 0 to 5 mg/ml were created from a stock 100 mg/ml standard. The standards from 0 to 5 mg/ml underwent the same processing as the sample above. At a wavelength of 510 nm, the absorbances of the sample and the standards were measured using a Spectronic 21D Digital Spectrophotometer. The formula below was used to compute percent Glucoside[7].

- Absorbance of sample X gradient factor X dilution factor
    - Wt. of sample X10000

f. **Phenol**

0.20 g of sample was weighed and poured into a 50 ml beaker after which 20 ml of acetone was added, and the mixture was adequately homogenized for 1 hour to avoid lumping. After being rinsed with acetone and thoroughly mixed, the mixture was thoroughly filtered through Whatman No. 1 filter paper into a 100ml Volumetric Flask. One milliliter of the sample extract was pipetted into a 50ml volumetric flask along with 20ml of water, three milliliters of phosphomolybdic acid, 5ml of 23% sodium carbonate, and sufficient amounts of distilled water to make the flask up to the mark. The mixture was then left to stand for 10 minutes to develop a bluish-green color.

Standard Phenol was created from a 100 mg/l stock solution from Sigma-Aldrich Chemicals, USA, with a concentration range of 0–10 mg/ml. The absorbances of the

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sample and standard phenol concentrations were measured using a digital spectrophotometer at 510 nm wavelength[8]. The formula below used to determine the proportion of phenol:

Absorbance of sample X gradient factor X dilution factor

a. Wt. of sample X 10,000

g. Terpene

A 50ml conical flask was filled with 0.50g of sample, 20ml of a 2:1 solution of chloroform and methanol, and it was shaken vigorously before being left to stand for 15 minutes. Later, the mixture was centrifuged for an additional 15 minutes after which t he resulting supernatant was discarded. The precipitate was again washed with a 20ml solution of chloroform and methanol before centrifugation. The precipitate that resulted from this was dissolved in 40ml of a 10% sodium deodocyl sulphate solution.The above was mixed well, 1ml of a 0.01M ferric chloride solution was added at 30-second intervals, and the mixture was let to stand for 30 minutes.

A 100 mg/l stock terpenes solution from Sigma-Aldrich chemicals in the United States was used to create standard terpenes with a concentration range of 0 to 5 mg/ml. On a digital spectrophotometer with a 510nm wavelength, the absorbances of the sample and standard Terpene concentrations were measured[7]. The formula below wass used to determine the percentage of terpene:

1. Absorbance of sample X gradient factor X dilution factor

a. Wt. of sample X 10,000

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Endnotes

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Chapter Four

Results and Discussion of Findings

On Table 4.1, (MIC for morinda lucida) it was observed that aqueous extracts from the sample was non-reactive against S. typhii, P. mirabilis and K. pneumoneae but inhibited E. cloava at 66.7mg/ml/ It also inhibited E. coli and S. aureus at 111.1mg/ml and P. aeruginosa at 166.7mg/ml. Similarly, ethanolic extract from the sample was observed to be non-reactive against E. coli but inhibited S. aureus at 66.7mg/ml and E. cloava, S. typhi, and P aeruginosa at 83.4mg/ml, while K. pnuemoneae was inhibited at 111.1mg/ml concentration. Solvent extract from the sample using ethyl acetate was observed to inhibit S. aureus, E cloava, and S. typhi at 66.7mg/ml, P. aeruginosa at 83.4mg/ml, E. Coli at 111.1mg/l and K. pneumoneae at 166.7mg/ml.

4.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that causes a 3-logarithmetric reduction in the size of a standard inoculum by <99.9%. It is measured and reported as ≤ 10mm diameter on agar plate. Minimum Bactericidal Concentration (MBC) is a measure of ≥ 10mm diameter on agar plate with >99.9% inoculum reduction[1].

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Table 4.1: Minimum Inhibitory Concentration for Aqeous, ethanol, and ethyl acetate extracts of *Morinda lucida* leaves against the isolates used

***Morinda lucida* Minimum Inhibitory Concentration (175.12 mg/ml)**					
Isolates	Solvent	175.12mg/ml	116.75mg/ml	87.56mg/ml	70.05mg/ml
Escherichia coli	Ethyl Acetate	10±0.58	6±0	-	-
	Ethanol	-	-	-	-
	Water	14.3±2.33	14.0±2.31	-	-
Staphylococcus aureus	Ethyl Acetate	10.3±0.88	9.3±0.33	9±0	7.7±0.33
	Ethanol	8±0.58	8±0	8.3±0.33	4±0
	Water	14±1.15	14±0.58	-	-
Enterobacter cloacae	Ethyl Acetate	15±1.53	10.7±0.33	10±0.58	5±0
	Ethanol	9.3±0.33	6.7±0.33	3±0	-
	Water	22±2.31	21.3±0.88	18±1.53	16.7±1.76
Salmonella typhiiii	Ethyl Acetate	20.7±4.23	18±0	11.7±0	11.0±0.67
	Ethanol	12±1.15	4±0	2±0	-
	Water	-	-	-	-
Klebsiella Pneumoniae	Ethyl Acetate	10±0	-	-	-
	Ethanol	8.3±0.89	6.7±0.33	-	-
	Water	-	-	-	-
Pseudomonas aeruginosa	Ethyl Acetate	16±1.15	15.3±2.03	-	8±0
	Ethanol	9.7±0.33	8±1.15	3±0	-
	Water	10±1.15	-	-	-
Proteus mirabilis	Ethyl Acetate	18.3±1.76	13±1.53	10.7±0.67	10.3±2.33
	Ethanol	4±0	-	-	-
	Water	-	-	-	-

Source: Lab work, 2021

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Table 4.2 explains the MIC for Azadirachta indica. On the table, it was observed that aqueous extracts from the sample was non-reactive against S. aureus and S. typhi but inhibited E. coli at 66.7mg/ml, and E. cloava and P aeruginosa at 111.1mg/ml. Ethanol extracts of the sample was equally observed to be non-reactive against E. cloava. It was observed to inhibit E. coli, S. typhi, and P. aeruginosa at 66.7mg/ml, S. aureus at 83.4mg/ml and K. *pneumoniae* at 111.1mg/ml. Ethyl acetate extracts from the sample also inhibited S. aureus, E. cloava, S. typhi and P. aeruginosa at 66.7mg/ml, and E. coli and K. *pneumoniae*, both being coliforms at 83.4mg/ml.

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Table 4.2: Minimum Inhibitory Concentration for Aqeous, ethanol, and ethyl acetate extracts of *Azadirachta indica* leaves against the isolates used

Azadirachta indica **Minimum Inhibitory Concentration (175.12 mg/ml)**					
Isolates	Solvent	175.12mg/ml	116.75mg/ml	87.56mg/ml	70.05mg/ml
Escherichia coli	Ethyl Acetate	15.3±0.33	15± 1.15	10±0.58	-
	Ethanol	14.3±2.65	8±0	7.7±0.3	6.7±0.33
	Water	15±2.30	10.7±1.15	10±	10±0.88
Staphylococcus aureus	Ethyl Acetate	16.3±2.03	15±0	14±1.15	13.3±2.02
	Ethanol	12.3±1.45	7±0	6.3±0.33	-
	Water	-	-	-	-
Enterobacter cloacae	Ethyl Acetate	6±0	5.7±0.33	4±0	2±0
	Ethanol	-	-	-	-
	Water	17±1.73	10±0.58	8±1	-
Salmonella typhiiii	Ethyl Acetate	13±2.31	12.7±1.45	11.3±0.33	9.3±0.33
	Ethanol	12±1.15	10±0	3±0	1±0
	Water	-	-	-	-
Klebsiella Pneumoniae	Ethyl Acetate	22.3±2.60	16.3±1.73	16±1.45	-
	Ethanol	16±0	14±2.31	-	-
	Water	12.3±1.45	8±0	-	-
Pseudomonas aeruginosa	Ethyl Acetate	19.7±2.91	18.7±0.88	16±1.73	11±0
	Ethanol	6±0	4±0	3±0	2±0
	Water	15±2.89	14.7±2.60	10±1.15	-
Proteus mirabilis	Ethyl Acetate	22±1.15	21.3±1.45	20±2.89	19±1.15
	Ethanol	27±2.02	12±1.15	10.3±0.88	-
	Water	16±0.58	12.3±0.33	-	-

Source: Lab work, 2021

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Table 4.3: Calculation of Potency for *Morinda lucida* extracts

S/N	Calculation of Potency *Morinda lucida*			
		Ethyl Acetate	Ethanol	Water
1	*Escherichia coli*	X	X	✔
2	*Staphylococcus aureus*	✔	X	✔
3	*Enterobacter cloacae*	✔	X	✔
4	*Salmonella typhiii*i	✔	✔	X
5	*Klebsiella pneumonia*	X	X	X
6	*Pseudomonas aeruginosa*	✔	X	X
7	*Proteus mirabilis*	✔	X	X
		5/7= 71%	1/7= 14%	3/7= 43%

Source: Lab work, 2021

Key

✔ − Positive

X - Negative

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4.3 Minimum Bactericidal Concentration of *Morinda lucida* and *Azadirachta indica*

Table 4.3 and 4.4 respectively expressed the efficacy and potency of morinda lucida and Azadirachta indica against the bacteria isolates used. On Table 4.3, it was observed that extract from morinda lucida using ethyl acetate as solvent extraction had 71% efficacy against the bacterial isolates used. The bacteria cut across both gram positive and gram-negative bacteria, making it broad spectrum in property. This was followed by aqueous extract (43%) where the antibacterial property of the extracts was active three bactieria (E. coli, S. aureus, and E. cloaca). Ethanolic extracts of the sample was only potent against S. typhi (14%).

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Table 4.4 expressing the potency property of Azadirachta indica also showed that ethyl acetate, if used as solvent of extraction was more potent 86% against the bacteria isolates used (except Enterobacter cloaca) while the extract using ethanol and sterile water were of similar efficacy (71% each).

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Table 4.4: Calculation of Potency for *Azadirachta indica* extracts

S/N	Calculation of Potency *Azadirachta indica*			
		Ethyl Acetate	Ethanol	Water
1	*Escherichia coli*	✔	✔	✔
2	*Staphylococcus aureus*	✔	✔	X
3	*Enterobacter cloacae*	X	X	✔
4	*Salmonella typhiii*i	✔	✔	X
5	Klebsiella *Pneumoniae*	✔	✔	✔
6	*Pseudomonas aeruginosa*	✔	X	✔
7	*Proteus mirabilis*	✔	✔	✔
		6/7= 86%	5/7= 71%	5/7= 71%

Source: Lab work, 2021

✔ − Positive

X - Negative

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4.4 Antibiotics Sensitivity Test

Table 4.5 shows the result of commercially sold antibiotics sensitivity disc carried out against the bacteria isolates used. On the table, Perfloxacin, Cirpoflaxin, and Sparfloxacin, were observed to inhibit the growth of all of the bacteria isolates used in the test. This makes them broad spectrum antibiotics. Even though ethyl acetate and ethanol extracts also displayed characteristics of broad-spectrum antibiotics, none of them mimic the efficiency of the above-mentioned antibiotics as ethyl acetate extacts did not inhibit the growth of E. cloacae, while ethanol extracts inhibited the growths of all but E. cloacae and P. aeruginosa. Roceptin was also observed to possess broad spectrum antibiotic characteristics as it inhibited the growth of both gram positive and gram-negative bacteria (except P. mirabilis). Tarivid was effective against all of the bacteria isolates except K. pneumoniae and P. mirabilis. Erythromycin displayed narrow-spectrum properties in this test as it was observed to be ineffective against S. aureus. It was also observed to be ineffective against P. mirabilis. Gentamycin was ineffective against E. coli, K. pneumoniae, and P. mirabilis while proving effective against the other four bacteria isolates. Septrin and Streptomycin were ineffective against S. aureus and *P.aeruginosa*. Furthermore, Septrin was ineffective against P. mirabilis while Streptomycin was ineffective against K. Pneumoniae. Amoxacilin and Zinnacef were ineffective against four bacteria isolates. Amoxacilin was ineffective against E. coli, K. Pneumoniae, P. aeruginosa, and P. mirabilis, while Zinnacef was observed to be ineffective against E. coli, *S. aureus, K. Pneumoniae, and P. mirabilis*. Augmentin, Chloranphenicol, and Ampiclox were observed to be ineffective against five of the bacteria isolates. The three antibiotics were observed to only be effective against E. cloacae and S. typhi. Seven of the antibiotics displayed broad-spectrum antibiotics properties as they were observed to be effective against both narrow and broad-spectrum antibiotics. They are Perfloxacin, Gentamycin, Amoxacilin, Roceptin, Cirpoflaxin, Sparfloxacin, and Tarivid.

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On the table, it was observed that, *E. cloacae* and *S. typhi* was inhibited by almost all the antibiotics used, though at various diameter of zones of clearance. Ciproflavin had the best efficacy property against the bacteria as it property was observed to be potent against both gram positive and gram negative bacteria used. The result is comparable with ethyl acetate extracts of Morinda lucida. Meanwhile Gentamycin, Ampiclox, Zinnacef, Amoxacilin. Streptomycin, Septrin, Chloramphenicol, and Augmentin were all studied to be effective only on two or three of the bacteria isolates used.

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Table 4.5: Antibiotics Sensitivity Test Using Standard Antibiotics Sensitivity Disc Against the Isolates Used

				ISOLATES USED						
S/N	Antibiotics	Code	Conc. (MG)	*E. coli*	*S. aureus*	*E. cloacae*	*S. typhii*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*
1	Perfloxacin	PEF	10	1.1	1.8	1.5	1.6	1.7	1.6	1.7
2	Gentamycin	CN	10	0	1.3	1.6	1.6	0	1.3	0
3	Ampiclox	APX	30	0	0	1.6	1.3	0	0	0
4	Zinnacef	Z	20	0	0	1.2	1.6	0	1.4	0
5	Amoxacilin	AM	30	0	1.3	2.2	2.0	0	0	0
6	Roceptin	R	25	1.0	0.6	2.0	1.8	1.8	1.7	0
7	Cirpoflaxin	CPX	10	1.0	1.6	1.6	1.8	1.6	1.8	1.4
8	Streptomycin	S	30	1.5	0	1.6	1.5	0	0	1.5
9	Septrin	SXT	30	0.7	0	1.6	1.7	1.7	0	0
10	Erythromycin	E	10	1.4	0	1.5	1.5	1.6	1.6	0
11	Chloranphenicol	CH	10	0	0	1.7	1.6	0	0	0
12	Sparfloxacin	SP	10	1.4	1.5	1.4	1.6	1.8	1.7	1.0
13	Augmentin	AU	30	0	0	1.5	1.6	0	0	0
14	Tarivid	OFX	10	1.3	1.3	1.6	1.6	0	1.5	0

Source: Lab work, 2021

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4.5 Qualitative and Quantitative analysis of *Morinda lucida* and *Azadirachta indica*

4.5.1 Qualitative and Quantitative analysis of *Morinda lucida*

Table 4.6 shows the list of phytochemical constituents present in *M. lucida* when evaluated. It was observed that the extract lacks steroid, protein, and anthrocyanin, but very rich in tannins and alkaloid. Flavonoid, saponin, glycoside, phenol, and terpenoid were also present. Similarly, table 4.7 explains the quantitative results of the phytochemical constituents where alkaloid was observed to be of highest value in the three extracts with 0.3920 in ethyl acetate, 0.4150 in ethanol, and 0.2110 in water extracts. Phenol was the second most abundant in all of the samples with a concentration of 0.0970 in water, 0.1170 in ethyl acetate, and 0.1280 ethanol. Saponin was the third-most abundant phytochemical component with the aqueous extracts having 0.0930, ethyl acetate having 0.0940, and ethanol extracts having 0.1180. Glycoside comes next with 0.0460 for aqueous extract, 0.0620 for ethyl acetate and 0.0780 for ethanol extract. Terpenoid's concentration in water extract was 0.0052, it was 0.0078 in ethyl acetate, and 0.0091 in ethanol. Tannin presence was 0.0023 in aqueous extract, 0.0047 ethyl acetate, and 0.0064 in ethanol. Flavonoid was the least present phytochemical in the samples with 0.2110 in aqueous sample, 0.3920 in ethyl acetate, and 0.4150 in ethanol.

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Table 4.6 Qualitative analysis of *Morinda lucida* extracts

S/N Parameter	ML Water	ML Ethyl Acetate	ML Ethanol
Alkaloid	+	+	+
Tannins	+	+	+
Flavonoid	+	+	+
Glycoside	+	+	+
Saponin	+	+	+
Terpenoid	+	+	+
Steroid	-	-	-
Anthocyanin	-	-	-
Protein	-	-	-
Phenol	+	-	-

KEY:

+ Present

- Absent

ML- *Morinda lucida*

Source: Lab work, 2021

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Table 4.7: Quantitative Analysis of *Morinda lucida* extracts (Unit= %)

S/N Parameter	ML Water	ML Ethyl Acetate	ML Ethanol
Alkaloid	0.2110	0.3920	0.4150
Tannins	0.0023	0.0047	0.0064
Flavonoid	0.0015	0.0036	0.0049
Glycoside	0.0460	0.0620	0.0780
Saponin	0.0930	0.0940	0.1180
Terpenoid	0.0052	0.0078	0.0091
Phenol	0.0970	0.1170	0.1280

Source: Lab work, 2021

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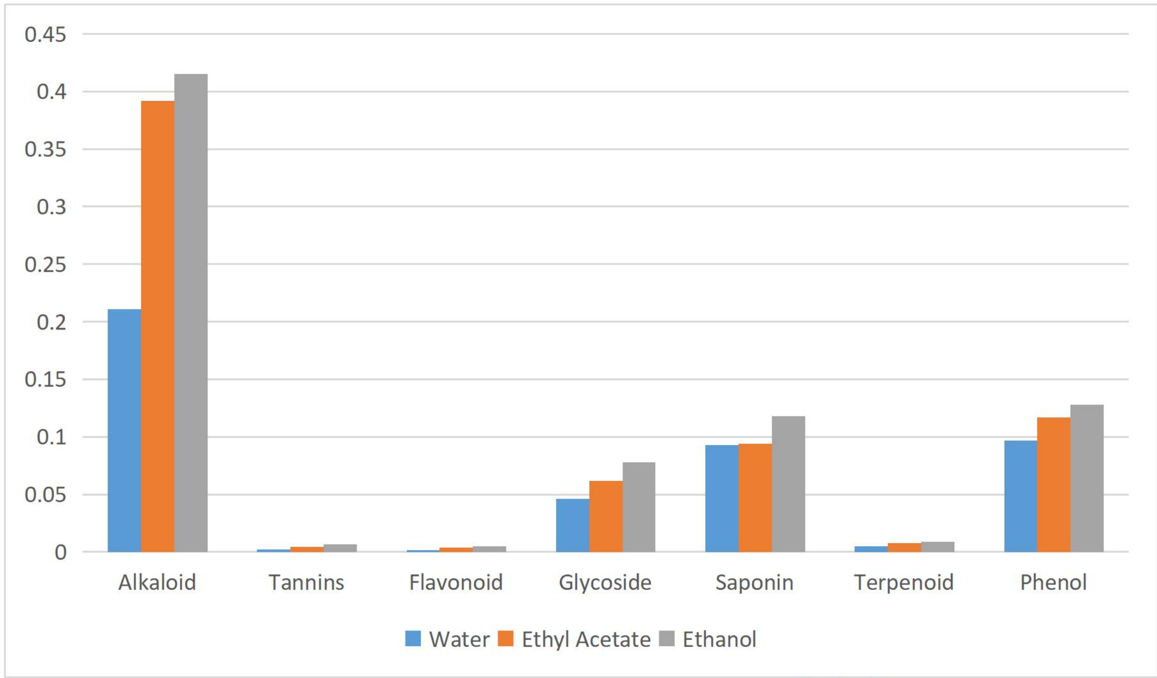

Figure 2: Concentration of Extracts Found in Morinda Lucida

Source: Project analysis, 2022

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4.5.2 Qualitative and Quantitative analysis of Azadirachta indica

Table 4.9 shows the list of phytochemical constituents present in *A. indica* when evaluated. It was observed that the extract lacks steroid, protein, and anthrocyanin, but very rich in tannins and alkaloid. Flavonoid, saponin, glycoside, phenol, and terpenoid were also present. Similarly, table 4.10 explains the quantitative results of the phytochemical constituents where alkaloid was observed to be of highest value in the three extracts with 0.3060 in ethyl acetate, 0.3280 in ethanol, and 0.1970 in water extracts. Phenol was the second most abundant in all of the samples with a concentration of 0.0630 in water, 0.1050 in ethyl acetate, and 0.1120 ethanol. Saponin was the third-most abundant phytochemical component with the aqueous extracts having 0.0520, ethyl acetate having 0.0730, and ethanol extracts having 0.0890. Glycoside comes next with 0.0340 for aqueous extract, 0.0018 for ethyl acetate and 0.0670 for ethanol extract. Terpenoid's concentration in water extract was 0.0041, it was 0.0059 in ethyl acetate, and 0.0073 in ethanol. Tannin presence was 0.0018 in aqueous extract, 0.0034 ethyl acetate, and 0.0042 in ethanol. Flavonoid was the least present phytochemical in the samples with 0.0009 in aqueous sample, 0.0018 in ethyl acetate, and 0.0025 in ethanol.

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Table 4.9 Qualitative analysis of *Azadirachta indica* extracts

S/N Parameter	NL Ethanol	NL Water	NL Ethyl Acetate
Alkaloid	+	+	+
Tannins	+	+	+
Flavonoid	+	+	+
Glycoside	+	+	+
Saponin	+	-	+
Terpenoid	+	+	+
Steroid	-	-	-
Anthocyanin	-	-	-
Protein	-	-	-
Phenol	+	+	-

KEY:

\+ Present

\- Absent

NL- Neem Leaf (*Azadirachta Indica)*

Source: Lab work, 2021

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Table 4.10: Quantitative Analysis of *Azadirachta indica* extracts (Unit= %)

S/N Parameter	NL Ethanol	NL Water	NL Ethyl Acetate
Alkaloid	0.3280	0.1970	0.3060
Tannins	0.0042	0.0018	0.0034
Flavonoid	0.0025	0.0009	0.0018
lycoside	0.0670	0.0340	0.0510
Saponin	0.0890	0.0520	0.0730
Terpenoid	0.0073	0.0041	0.0059
Phenol	0.1120	0.0630	0.1050

Source: Lab work, 2021

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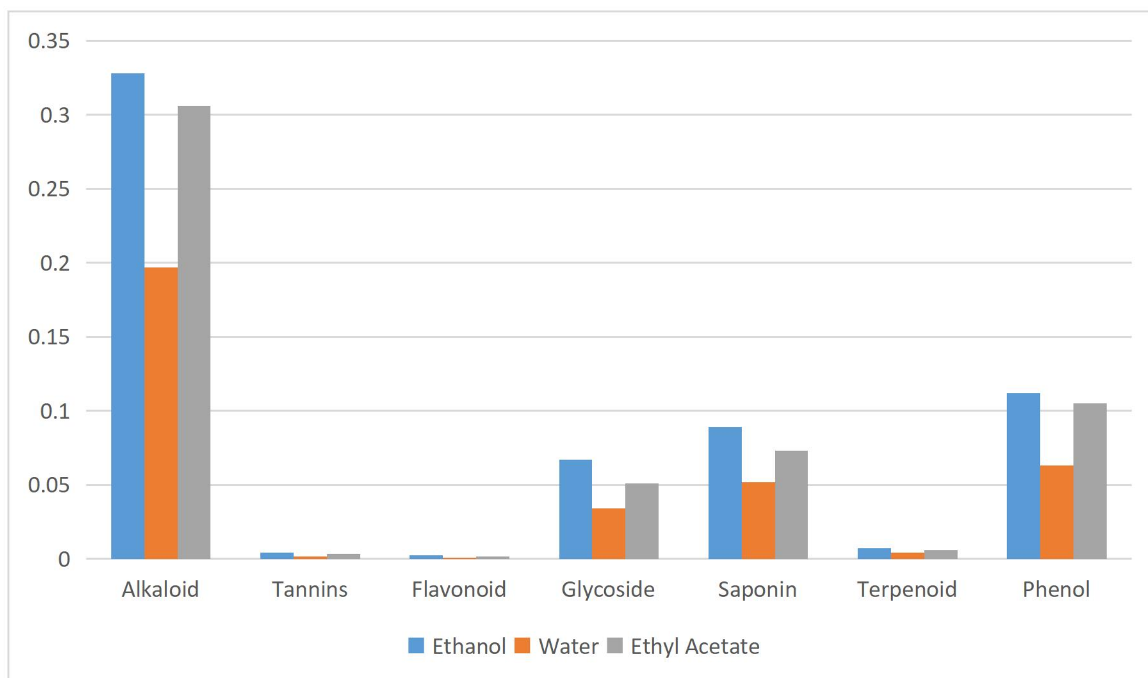

Figure 3 :Concentration of Extracts found in *Azadiracta Indica*

Sourc : Lab Work analysis 2021

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Endnotes

1. Creative Biolabs. *Minimum Bactericidal Concentration (MBC) Test.* **Creative Biolabs.** https://www.creative-biolabs.com/drug-discovery/therapeutics/minimum-actericidal-concentration-mbc-test.htm

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Chapter Five

Conclusion

5.1 Summary of Findings

Pathogenic microorganisms were studied to be the leading cause of diseases in humans, and acteria are one of the main pathogens. Synthetic drugs being used to fight off such infections and diseases have unprecedented high side effects beside the facts of being increasingly resistant to these drugs. Also, the high costs of buying these drugs made it unreachable for over 80% major populace among the third world countries.

Various researchers had carried out the efficacy of phytochemical constituents of parts of many plants against some pathogenic microorganisms using different solvents as extractant and have reported various degree of potency of such phytochemical against the associated diseases such pathogens cause. For instance, while working on *Azadirachta indica* using different solvents as extractant reported that chloroform extract of the crude sample possesses antioxidant property that makes it capable of inhibiting bacteria residing in the respiratory tract.

Consequently, three different solvents (ethanol, ethyl acetate, and aqueous) were used as extractant for this work where extracts of ethyl acetate were observed to be more potent against the bacteria isolates used. Observation shows that *morinda lucida* has 71% and *azadirachta indica* has 86% potency against the bacteria isolates. This result conformed with imilar work done by other researcher of the southwest of Nigeria.

Results of this work also showed that though ethanolic extracts and aqueous extract (Table 4.3) from *morinda lucida* were effective (43%) against bacteria isolates used, the efficacy was not as potent as ethyl acetate. This gave an improve report over a similar work done by [4] where he submitted aqueous extract of leaf and stem back of *azadirachta indica* has an effective antibacterial property against digestive and respiratory tract bacteria[3]. This was

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eported from, while working on the antimicrobial effect of aqueous chloroform and ethanolic extracts of *A. indica* on some selected human pathogenic bacteria, this report totally agreed with their summation that aqueous and ethanolic extracts are of lesser potency against the pathogen that extract from ethyl acetate

Quantitative and Qualitative Evaluation of Phytochemical composition of *m. lucida* and *A. indica* was further determined. From the result, the presence of some phytoconstituents like alkaloids, tannins, saponins, flavonoids, and phenol in the sample, perhaps confers on the plant the antimicrobial properties. In agreement with this report, they further submitted an independent report that antimicrobial potency of extract from these plants were traceable to the phytochemistry of their chemical constituents.

5.2 Recommendation

Evaluation of antimicrobial properties of *Morinda lucida* and *Azadirachta indica* using different solvents as extractant in this work showed that the use of ethyl acetate as solvent of extraction give a better phyto-constituents against bacteria (gram positive & gram negative). ence commercial production, purification and crystallization of the extract by government will help a long way to solve most of the problems associated with the listed pathogen. Aqueous extract of the *Azadirachta indica* sample was 71% effective against the bacterial isolates. The use of sterile water should be encouraged anything the extract is being used as alternative by local populace that form over 70% population of the developing countries.

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Endnotes

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Appendix I

Bio-data

A. Personal Data:

1. Full Name:	Musodeeq, Oluwatosin BELLO Plot 100, Equitable Estate, Ikorodu, Lagos State bellomusodeeq@gmail.com 09053599577
2. Date and Place of Birth:	28th January, 1997; Ketu, Lagos State
3. Nationality:	Nigerian
4. Name and Address of Next of Kin:	Bello Sulaiman Oladimeji Plot 100, Equitable Estate, Ikorodu, Lagos State

B. Educational Background:

School Attended	Date	Qualifications
❖ As-siddiq Nur &Pry School.	2000-2006	First Leaving Sch. Cert.
❖ Mahmud Ahmadiyya College.	2006-2012	S.S.C.E
❖ Olabisi. Onabanjo University	2013-2017	B.Sc Microbiology
❖ Lead City University, Ibadan.	2019-2022	M.Sc in view

C. Working Experience with Dates

Tutor 2020 -Till Date

D. Awards and Fellowship Nill

E. Membrship of Academi Professional Bodies Nill

F. Publication: 3rd FASCON Conference 2022 Poster Presentation

________________ Date

________________ Signature

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University Compliance Certification

This is to certify that the Thesis by Bello Musodeeq Oluwatosin with Matric No LCU/PG/001311 in the department of **Biological Sciences**, Faculty of Natural and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

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Signature	**Date**