Phytochemical determination and in vitro assessment of antibacterial activities of *Delonix* regia (Bojer ex Hook) against some bacteria associated with urinary tract infection

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Being a M.Sc Thesis Submitted to the Department of Biological Science,

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In Partial Fulfilment of the Requirements for the award of Master Degree (M.Sc) in Medical Microbiology

2022

Certification

This is to certify that Adenike D. Adeleke with matriculation number LCU/PG/001307 carried out this research work titled "Phytochemical determination and in vitro assessment of antibacterial activities of *Delonix* Regia (Bojer ex Hook) against some bacteria associated with urinary tract infection '' in the department of biological science, Faculty of Natural Sciences, Lead city university, Ibadan, Oyo State, for the award of Master Degree (M.Sc) in Medical Microbiology and that this has not been previously submitted.

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Dedication

This research work is dedicated to God Almighty.

Acknowledgement

All glory be to God, for all His favour, blessing, goodness, guidance and mercies.

I appreciate the staff of Lead City University Medical Microbiology Laboratory where I carried out my practical work for their support during the bench work. My gratitude goes to the Head of Department and all the Lecturers in the Department of Biological sciences, Lead City University Ibadan for their contributions during the period of my study. May God reward you all. I acknowledge the effort of my supervisor Dr B. A. Bamkefa, for teaching me the rudimentary of microbiological techniques of this research work. She devoted her time in ensuring that this research work was completed. Thank you to my parents, Chief and Mrs. S. A. Adeleke, for your endless support. You have always stood behind me, and this was no exception. Mom, thank you for fielding a ridiculous number of phone calls, for calming me down. Dad, thank you for all of your love and for always reminding me of the end goal.

Thank you to my sisters, Mrs. A.A. Atoyebi and Mayowa Adeleke, for always being there for me and for telling me that I am awesome even when I didn't feel that way.

Thank you to my partner, Dr. D. N. Ajobiewe, and my daughter Opeyemi Claire for constantly listening to me rant and talk things out, for proofreading over and over (even after long days at work and during difficult times), for cracking jokes when things became too serious, and for the sacrifices you have made in order for me to pursue a Master's degree.

Abstract

Antibiotic resistance and the side effects of synthetic medicines have led to more people using plant materials to treat a wide range of illnesses. In this study, the antibacterial activity of leaves and bark of *Delonix regia (Bojer ex Hook)*. The content's qualitative and quantitative aspects were evaluated. The effects of leaf and bark extracts of this plant on human pathogens such *as Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, and Acinetobacter nosocomialis* were examined *in vitro* with methanol, ethanol, hexane, and distilled water. Agar well diffusion technique was employed throughout the experimentation. It was found that the ethanolic extracts of the leaves had the most inhibitory impact on the test microorganisms of all the extractants. The Minimum Inhibitory Concentration (MIC) of each extract was determined. Concentrations of 59.16 mg/ml, 39.33 mg/ml, 29.5 mg/ml, and 23.6 mg/ml, both sample ethanol extracts were effective against all tested bacterial isolates, except for *Staphylococcus aureus* and *Pseudomonas aeruginosa* at doses of 39.41mg/ml and 59.16mg/ml, respectively, neither the ethanol nor the methanol extracts of the leaf nor the bark were reactive against any of the test microorganisms. Ciprofloxacin (20mm), Pefloxacin (20mm), Rocephin (14mm), all suppressed *Proteus mirabilis* (12mm) The qualitative screening of both the leaf and the bark showed substantial presence of photochemical samples. With the quantitative showing tannins (0.48) having the highest concentration in n-hexane and terpenoids (0.458). Bark was 86% effective against all isolates except *P.mirabilis and A.nosocomialis*, whereas leaf was 20% effective against S. aureus and P. aeruginosa, making it bacteriostatic (75%). Phytochemical screening helps uncover bioactive molecules that may be used to make successful drugs. This study will aid in the development of quality, purity, and sample standards for the *Delonix regia* plant, which was discovered to have a number of phytochemicals with antibacterial properties.

Keywords: *Delonix regia,* Antibiotic Resistance, Antimicrobial Activity, Phytochemical Screening

Word Count: 300 words

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Chapter One

Introduction

1.1 Background to the Study

Urinary tract infection is a global public health issue caused by several species, including viruses and bacteria, with bacteria being the most prevalent. Nearly all occurrences of urinary tract infections (UTIs) in women may be traced back to bacteria[1]. After respiratory infections, UTIs are common. 50% of women will suffer a urinary infection in their lives. The bacterial infection is one of the most important bioactivities; their ability to imitate evanish is a twenty-first-century threat to global health. With the rise in microbial infections and treatment resistance, the negative effects of synthetic chemical treatments, and the high cost of their production, it is critical to produce antimicrobials that are very effective against the bacteria that cause UTI[2,3]. In addition, an individual's health condition influences the occurrence of UTIs. Immunocompromised patients and those with chronic uncontrolled diabetes mellitus, for instance, have significantly elevated rates of UTIs because their weaker immune systems cannot successfully resist infections.

Environmental and lifestyle factors can contribute to the frequency of urinary tract infections. Multiple medical diseases are common in older persons, their handling and management regimes may raise the risk of UTIs[4]. Catheterization significantly increases the prevalence of UTIs, particularly those caused by Gram-negative bacterial infections. It has been estimated that healthcare-associated UTIs account for around 10 percent of UTI infections, with 75 percent of these instances occurring in female patients. In addition, chronic use of antibiotics to manage other therapeutic illnesses deteriorates the immune system, hence increasing the likelihood of developing UTIs. Increased sexual activity between the ages of 18 and 39 increases the pervasiveness and recurrence of UTIs in younger women. Any portion of the urinary system, as well as the kidneys, bladder, urethra, and ureter, may become infected[5]. When UTIs affect the minor urinary tract, the illness remains referred to as a bladder infection (cystitis). Commonly, kidney infections relate to infections of the upper urinary tract (pyelonephritis)[5,6].

The presence of more than two symptomatic episodes during a period of six months or more than three symptomatic episodes within a period of twelve months is considered to be a recurrent urinary tract infection (UTI)[7]. If doctors have a better understanding of the risk factors linked with recurrent UTI, they will be better able to customize preventative measures to successfully minimize the likelihood of future infections. The categorization of urinary tract infections (UTIs) is heavily reliant on risk factors[8]. Both premenopausal and postmenopausal women are susceptible to developing recurrent simple urinary tract infections (UTIs), although the risks associated with each group of women are different. Both patients and doctors continue to believe in misconceptions about risk and engage in risk-avoiding actions that are not supported by evidence, despite the fact that the degree of evidence supporting each particular alleged risk factor differs across the two groups[9]. Treatment of asymptomatic bacteriuria (ABU), which has been proven to increase the likelihood of future symptomatic UTI episodes in patients who have a history of experiencing recurrent urinary tract infections (UTIs) [8,9].

1.1.1 Classification of UTI

Generally, UTIs are categorised as superior or minor based on their location along the urinary system, however it may be hard or intolerable for clinicians to discern this distinction:

a) Minor: Infections of the bladder (cystitis)[8]

b) Superior: Infections of the kidneys (pyelonephritis)[9]

c) Most practitioners think urethritis and prostatitis reduce UTIs. One or both kidneys may get infected in paired organs. Children and adults may have UTIs[8,9,10].

1.1.2 Symptoms of UTIs

Infections of the urinary tract can manifest themselves in a number of different conducts, as well as an increase and insistent need to urinate, throbbing and burning sensations, an increase in the rate of recurrence of urination, decreased urine volumes during each urinary event, as well as a cloudy and offensive smelling urine[9]. Another very usual symptom of a urinary tract infection (UTI) is pain in the pelvic area, which may manifest itself in the lower abdomen, back, or both. This pain is more common in women. This pain may be caused by a variety of factors. Blood in the urine, which may be a reddish, pink, or cola hue, is a common symptom of a urinary tract infection. An infection in the kidney possibly will manifest itself with symptoms such as queasiness and vomiting, a high temperature, and discomfort in the upper back. UTIs are often misdiagnosed as other conditions, particularly in the elderly, due to the nonspecific character of many of these symptoms[9].

1.1.3 Causes of UTIs

Bacteria and viruses may enter the urinary system by one of two paths, both of which are common entry points for pathogens causing this illness. The urethra opens at the penis in males and the vulva in women, therefore this is the most frequent entry point[8,10]. The bacterium travels up the urethra, into the bladder, and sometimes the kidneys. Alternatively, it may be transported to the kidneys through the circulation[10].The organisms are listed below;

Bacteria

Bladder infections are frequent in young, sexually active women. There is a lower incidence of kidney infections in young women compared to bladder infections. Escherichia coli causes utmost UTIs[11]. Women are 50 times more likely than men between the ages of 20 and 50 to get a bacterial UTI. Since men's urethras are longer, it's harder for bacteria to get up high sufficient to cause an infection. UTIs happen more often in people over the age of 50, and there is less difference between men and women[11].

Viruses

Herpes simplex virus can infect the urethra, causing painful urination and bladder problems. Except a person's immune system is suppressed (by cancer, HIV/AIDS, or medication), Other viral UTIs, such those affecting the bladder or kidneys, are very rare[12,13].

Fungi

The urinary system is susceptible to infection from a variety of fungus and yeasts. This "yeast infection" is often used to refer to this kind of illness (Vaginal irritation may also be caused by yeasts [vaginitis])[13], a material that is moldy Candida is the microorganism most often associated with genital yeast infections. Candida infections are common in patients whose immune systems are compromised or who are undergoing treatment with bladder catheters. It's possible for both bacteria and fungi to infect the kidneys at the same time[14,15].

Parasites

The urinary system is susceptible to infection from a wide variety of parasites, some of which are worms. In women, trichomoniasis, a sexually transmitted illness caused by a tiny parasite, may result in a profuse, greenish-yellow, foamy discharge from the vagina[15]. Urethral and bladder infections occur seldom. The urethra is a common site of infection for trichomoniasis in males. In most cases, guys get no symptoms from it[15]. The kidneys, ureters, and bladder may all be affected by schistosomiasis, an illness caused by a fluke worm. Infectious nephritis is a leading cause of death in Asia, Africa, and South America[6,15]. Bladder schistosomiasis, if left untreated, may lead to bladder cancer by blocking the ureters or causing urine to leak blood.

A threadworm illness called filariasis may block lymphatic channels and lead to lymph fluid being excreted in the urine (chyluria). Scrotal enlargement (in males) may be caused by filariasis (elephantiasis)[16,17].

1.1.4 Risk Factor in the UTIs

Urinary tract infections (UTIs) have afflicted humans even before bacteria were identified as disease-causing agents and urology became a recognized medical specialty. Among the particular risk factors for UTIs are:

a) If there is a blockage in the urinary tract (e.g., by stones) [12]
b) Abnormal bladder function that forbids proper emptying, (e.g, spinal cord injury)
c) Anomalies of structure such as urethral diverticulum
d) Some people may have a reverse urinary tract infection, in which urine and bacteria travel up the ureters from the bladder[12]
e) Supplement of a urinary catheter or any instrument by a doctor
f) Sexual intercourse
g) Using spermicide with a diaphragm
h) Existence of irregular connection (fistula) flanked by the bladder and the vagina or the intestine and the bladder
i) Prostate enlargement [12,13,16]

1.1.5 Description of *Delonix regia*

Native to Madagascar, the flowering plant *Delonix regia* belongs to the *Fabaceae* family and the subfamily *Caesalpinioideae.* Also known, for having leaves that look like ferns and bright orange-red flowers in the summer[18,19]. Royal poinciana, flamboyant, flame of the forest, and flame tree are all English names for this spectacular tree (one of several species given this name). Before, this species was in the genus Poinciana, which was named for Phillippe de Longvilliers de Poincy, the governor of Saint Christopher in the 1600s (Saint Kitts). It is a legume that doesn't make pods.[19,20].

Delonix regia blooms are enormous, with four outspread petals in crimson or orange red up to 8 centimeters (3 inches) in length, and a fifth upright petal known as the standard, which is considerably larger and spotted with yellow and white. Corymbs of these flowers cluster at the ends and sides of branches. Yellow flowers of the wild flavida variety (Bengali: Radhachura) The pods are green and floppy while young, but become dark brown and woody when fully grown[20]. They may be up to 60 cm (24 in) in length and 2 cm (0.8 in) in width (0.8 in). It's estimated that the seeds weigh around 0.4 grams on average (6.2 grains). The compound (dual-pinnate) leaves are a bright, light green and have a distinctive feathery appearance. Each leaf is between 30 and 50 centimeters (12 and 20 inches) in length, and it has between 20 and 40 main leaflets, also known as pinnae, that are further subdivided into 10 and 20 subsidiary leaflets, known as pinnules. In humans, pollen grains are around 52 µm in length[20].

1.2 Statement of the Problem

The rise of antibiotic resistance to frequently given antibiotics for the treatment of urinary tract infections is a continuing global problem. Increasing antibiotic resistance in the population has been attributed to several factors, including the inappropriate use of antibiotics, the availability of counterfeit drugs on the market, nonadherence to standard treatment guidelines by clinicians, and a lack of laboratory resources for culture and sensitivity. In Nigeria, the frequency of urinary tract infections is high, and treatment results are poor owing to the prescribing of ineffective medications and incorrect diagnosis. Patients who are resistant to antibiotic treatment are usually treated based on clinical judgment rather than laboratory testing, and urine cultures are only conducted when necessary. Antimicrobial resistance lengthens the duration of disease, heightens the risk of complications, and contributes to prolonged hospital stays, consequently increasing patients' healthcare expenditures. The use of medicinal plants for the prevention and treatment of different ailments has expanded in recent years. Complementary treatment using medicinal plants is a potential study field worthy of particular consideration. The combination use of antibiotics and therapeutic plants showed mostly synergistic results. In several trials, herbal treatments were shown to significantly diminish bacterial resistance to antibiotics. Therefore, patients may benefit from this form of treatment in many instances. It was shown that herbal remedies might play a significant role in the treatment of a particular form of UTI.

Multiple antimicrobial mechanisms are ascribed to the antibacterial activity of plant antimicrobial compounds including diverse functional groups in their structures. Herbal remedies include chemical components that developed to defend the plant from harmful microbes and might thus prevent or cure illnesses in humans. This thesis builds up on previous researches to further explore natural products as a potential source of treatment of UTIs.

1.3 Justification of the Study

UTI is a common bacterial illness that affects women of all ages, although its frequency rises with age. Previous study has highlighted well-known related variables and risk factors for UTI, such as urinary incontinence, diabetes, sexual activity, a past history of UTI, urine retention, and oestrogen shortage. UTIs are connected with severe morbidity and death, as well as a reduction in patients' quality of life. Antibiotic therapy is an effective treatment that shortens the duration of symptoms. Resistance, harmful effects of antibiotics, and other connected issues led to the establishment of a research framework to discover alternate techniques to managing UTIs. Natural techniques have been widely employed in the treatment of numerous ailments to alleviate symptoms and enhance overall health. However, the emergence of antibiotic-resistant bacteria strains has prompted the use of more costly medications. Herbal remedies are less costly and more widely accessible than newer, more expensive contemporary treatments. This research demonstrated *Delonix regia's* activity and safety profile against specific urinary bacteria.

1.4 Aims and Objectives of the Study

The aim of this study is to investigate the phytochemical determination and in vitro assessment of antibacterial activities of *Delonix* Regia (Bojer ex Hook) against some bacteria associated with urinary tract infection. The study's objectives are as follows:

i. to evaluate the antibacterial properties of the different solvent extracts of the Delonix *regia* leaves and barks

ii. to determine the phytochemical contents of *Delonix regia* leaf and bark extracts in distilled water, ethanol, methanol and hexane

iii. to determine the number of phytochemical components, present in the plant extract and

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

1.5 Significance of the Study

Those in the medical community, in government, and in the general public who want to improve health care or increase pharmaceutical cooperation efforts for the greater good of humanity may find this research quite useful. The findings of this research will need certain efforts to be taken in order to raise the level of demand for enhanced applications of traditional medicine in the surrounding region. This study will also serve as a guide for other academics and researchers who want to do more research in this area in the near future.

1.6 Scope of the Study

This research covers the *in vitro* antibacterial potential, as well as the qualitative and quantitative analyses of *Delonix regia* leaf and bark extracts.

1.7 Limitation to the Study

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

1.8 Operational Definition of Terms

i. **Antimicrobial:** Refers to the process of killing or inhibiting the disease-causing microbes.

ii. **Phytochemical screening**: It refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds

iii. **Immunosuppressive:** (chiefly of drugs) partially or completely suppressing the immune response of an individual.

iv. **Antinociceptive:** The action or process of blocking the detection of a painful or injurious stimulus by sensory neurons.

v. **Carbunde:** A carbuncle is a red, swollen, and painful cluster of boils that are connected to each other under the skin.

vi. **Erysipelas:** is an infection typically with a skin rash, usually on any of the legs and toes, face, arms, and fingers.

vii. **Haemorrhoids**: Also known as piles are swellings containing enlarged blood vessels that are found inside or around the bottom (the rectum and anus).

viii. **Impetigo:** Is a common and highly contagious skin infection that mainly affects infants and children.

ix. **Oedema**: A condition characterized by an excess of watery fluid collecting in the cavities or tissues of the body.

x. **Hydroxyproline:** Is a neutral heterocyclic protein amino acid. It is found in collagen and as such it is common in many gelatin products.

xi. **Antipsychotic:** are used as a short-term treatment for bipolar disorder to control psychotic symptoms such as hallucinations, delusions, or mania symptoms.

xii. **Neurotransmitter:** are endogenous chemicals that enable neurotransmission. It is a type of chemical messenger which transmits signals across a chemical synapse, such as a neuromuscular junction, from one neuron (nerve cell) to another "target" neuron, muscle cell, or gland cell.

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Chapter Two

Literature Review

2.1 *Delonix regia*

Many infectious, metabolic, and malignant illnesses are treated with *Delonix* 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 *Delonix*, a member of the *Fabaceae* family and subfamily *Caesalpinioideae*. This genus contains trees that are native to Madagascar and East Africa [2].

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 [3]. 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 [4]. The notion that many pharmacologically active medications are sourced from natural resources, including medicinal plants, is widely recognized [5].

2.1.1 Scientific Classification of *Delonix regia*

Delonix is a genus of flowering plants in the *Fabaceae* and *Caesalpinioideae* subfamilies. This genus includes trees indigenous to Madagascar and East Africa. Royal Poinciana is by far the most well-known species *(D. regia)*. The genus' name is derived from the Greek words '*delos'* meaning 'obvious' and *'onyx*' meaning 'claw,' which relates to the petals. The *Delonix* tree is unarmed. The leaves are abruptly bipinnate, and the leaflets and stipules are tiny. The flowers are enormous and beautiful, arranged in terminal corymbs, while the bracts are modest. Very short calyx tube with five lobes that are valvate and subequal. Petals are 5, orbicular, imbricate, clawed, subequal. Margins are fimbriate. There are ten free, declinate, long-extended stamens. Underneath filaments are villous, whereas anthers are uniform. Subsessile and ovulated ovary. Style filiform and stigma truncate, ciliolate. The seed pods are elongated, flat, woody, and hollow. Seeds are transverse and oblong. Members of this species are flowering trees indigenous to East Africa that have been used in traditional Indian medicine to cure rheumatism, stomach ailments, and bronchitis and pneumonia in newborns, respectively. The taxonomic classification of *D.regia* is as follows:

Kingdom	*Plantae*
Clade	*Tracheophytes*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Caesalpinioideae*
Genus	*Delonix*
Species	D. *Regia*
Binomial Name	*boj. Ex Hook*

2.1.2 Active Compounds of *Delonix regia*

Delonix regia 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 *Delonix* 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 [9, 10], and a review described the many therapeutic roles of *Delonix*[11].

Delonix is an abundant source of numerous sorts of components, *Delonix regia* has a therapeutic role in health management. *Delonix*im 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 *Delonix* leaves and were known to have antibacterial and antifungal activities[13], and seeds contain important compounds such as gedunin and *Delonix*im.

2.1.3 Mechanism of Action of Active Compounds

Even though research has shown that *Delonix* has medicinal implications in the prevention and treatment of illnesses. However, the particular molecular mechanism that prevents disease is still unknown. *Delonix regia* is thought to have medicinal properties due to its high concentration of antioxidants and other beneficial chemicals such as *Delonix*im, nimbolinin, *Nimbin, nimbidin, nimbidol, salannin*, and *quercetin*[14].

Plant components of the *Delonix* tree have an antibacterial effect by inhibiting microbial development and the potential for cell wall collapse[12]. *Delonix*, a complex tetranortriterpenoid limonoid found in seeds, is the main component responsible for insect antifeedant and poisonous actions[15],[16].

2.2 Morphology of *D. regia*

D. regia is endemic to Madagascar but is now uncommon in its native region[17]. D. regia is planted as an ornamental or avenue tree along highways, in homesteads, parks, and gardens throughout the tropics. It's been planted in Indo-Pakistan for more than 100 years and introduced to many other nations in the Old and New Worlds. Introductions to these nations have been effective, and it's naturalized in areas without winter frost. It's possible that D. regia is already in every tropical nation in the globe, thus it can't be introduced[17]. Also, it scored 1 in a Pacific weed risk evaluation, suggesting a very minimal danger. It's found in moist tropical woods from sea level to 2000 m in Madagascar. Where introduced and invasive, it is found in arid lowlands and moist uplands in the Galápagos Islands, low elevation and dry to mesic disturbed sites in Hawaii, infrequently naturalized from near sea level to about 500 m in Fiji, almost monospecific stands around parent trees within disturbed marginal rainforest and along roadsides on Christmas Island, and has invaded coastal monsoon vine thickets damaged by cyclones in the Northeastern Pacific[17,18].

D. regia leaves and flowers are phytotoxic to Mikania micrantha. Mulching 1-2 g of leaf or flower powder on soil surface kills 75-90% of M. micrantha seedlings in 3 weeks. Spreading a 4% aqueous extract of D. regia leaves on M. micrantha seedlings resulted in significant mortality, showing a possible control measure of employing allelochemicals in D. regia leaves and flowers as a herbicide to manage this invasive climber. The highest allelopathic capacity of leguminous plant species against Parthenium hysterophorus came from D. regia (Dhawan et al., 2000, 2001). D. regia wood ash reduced mycelial development by 78%, 81%, and 89% in Helminthosporium sativum, Curvularia lunata, and Fusarium graminearum, respectively[18]. Insects (coleopteran storage pests), nematodes, etc. were affected.

2.3 Medicinal, Biochemical and other Qualities of *D. regia.*

D. regia is used for persistent fever, antimicrobial, constipation, inflammation, arthritis, hemoplagia, piles, boils, pyorrhea, scorpion sting, bronchitis, asthma, and dysmenorrhoea. Traditional usage lacks experimental proof[19]. This thesis gathers conventional and experimental information on *D. regia* 's benefits. The plant is antifungal, antibacterial, antioxidant, antiemetic, larvicidal, hepatoprotective, anti-diarrheal, anti-inflammatory, antimalarial, anthelmintic, antiarthritic, wound healing, and anticarcinogenic. Leaves, flowers, bark, and roots contain numerous phytochemicals, including saponins, alkaloids, carotene, hydrocarbons, phytotoxins, flavonoids, tannins, steroids, carotenoids, galactomannon, lupeol, -sitosterol, terpenoids, glycosides, and carbohydrates[19,20]. *D. regia* has been used in traditional medicine for various ailments, but more research is needed to explore the potential phyto-constituents of this plant for the prevention of other diseases and to unravel, characterize, patent, and commercialize the protective components from different parts of this plant for the benefit of humans. Shaiji in southwestern Bangladesh utilized *D. regia* flowers to treat persistent fever[20]. 250 g of flower was cooked in 1.5 l of water for 1/2 hour, and 2 ml was taken morning and evening for many days. Delonix regia flowers have antimicrobial action in a Nigerian investigation[21]. Darikal Gaon, Assam, employed medicinal herbs to heal wounds (North-East India). *D. regia* is one of 19 plant species from 16 families used to treat illnesses. Delonix regia leaves were administered on wounds [22]. In Koothanoallur and Marakkadai, Tamil Nadu, India, *D. regia* leaves are used to cure constipation, inflammation, arthritis, and hemiplagia[22].

In Pirojpur district, Bangladesh, the leaves and fruits were utilized for piles and helminthiasis[23]. Sylhet district, Bangladesh, used plants and fruits to treat piles and boils. Crushed herbs and fruits for piles and boils. Tribal regions of West Bengal, India, employ *D. regia* [23]. Infusions of flowers were used to treat bronchitis, asthma, and malarial fever. The seeds were used to treat pyorrhea. The leaves were used for rheumatism and purging. Antirheumatic and sparmogenic. Bark has antiperiodic and febrifuge properties; floral extracts kill round worms[23,24].

Patan district, North Gujarat (India) uses *D. regia* in traditional medicine. It's also used to treat gastric ulcers in Bangangte, Western Cameroon[24]. A study in Andhra Pradesh, India, validated Delonix regia's use in traditional medicine. Yanadi (an Indian tribe) utilized *D. regia* flowers to cure dysmenorrhea. Several African countries employed floral water extracts in nutritious drinks. It's used in traditional medicine [24,25]. *D. regia* leaf and bark is shown in figure 2.1 and 2.2 respectively

Figure 2.1 Leaf- *Delonix regia*[19]

Figure 2.2 Bark – *Delonix regia*[19]

2.4 Chemical Constituents of *Delonix regia*

The chemical components of *D. Regia* include anthocyanins, amino acids, tannins, flavonoids, phytosterols, alkanes, and flavonoids. It has a variety of medical applications, many of which, as was already said, have been supported by scientific research[26]. The leaves are caduceus-shaped, subulate, and abruptly bipinnate. Leaflets come in a variety of sizes and quantities. The crimson and yellow blossoms on this well named tree give the appearance of burning flames. Five, orbicular, imbricate-clawed, subequal or uppermost different, and fimbriate-margin petals are present. There are 10 stamens, a sub-sessile ovary, and numerous ovulated. Long, flat, woody, and dehiscent, the pod. Transverse oblong describes seeds. Bark is used as an antiperiodic and febrifuge because it contains leucocyanidin, lupeol, tannin, β-sitosterol, and free OH-proline as significant amino acids[26,27].

2.5 Clinical Usage of *Delonix regia* Leaves and Bark

Various pieces of the leave and bark have been used historically to cure a variety of conditions, including inflammation, rheumatism, bronchitis, diabetes, anemia, fever, gynecological diseases, and pneumonia. The plant is antioxidant, hepatoprotective, gastroprotective, wound-healing, antiarthritic, larvicidal, antimalarial, antiemetic, antibacterial, antifungal, anti-inflammatory, analgesic, antidiarrheal, antihemolytic, diuretic, and anthelmintic.

2.5.1 Antioxidant Activity

One of the main causes of disease is free radicals, also known as reactive oxygen species [14]. 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[26] 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[27]. Because of their high antioxidant content, plants' fruits, seeds, oil, leaves, bark, and roots play a significant role in disease prevention [27].

The antioxidant activity of *D. regia* leaf and bark extracts was investigated, and the results showed that all of the examined leaf and bark extracts/fractions of *Delonix* cultivated in the foothills have strong antioxidant characteristics[28]. Another major study looked at the antioxidant activity of leaves, fruits, flowers, and stem bark extracts from the Siamese *Delonix* tree, and the results showed that extracts from the leaves, flowers, and stem bark contain a lot of antioxidant potentials[29]. The antioxidant activity of the flowers and seed oil of the *Delonix regia* 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[30].

The antioxidant activity of leaves, fruits, flowers, and stem bark extracts from the Siamese *Delonix* 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].

2.5.2 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 *Delonix* help to prevent cancer by inhibiting the growth of cancer cells. A large number of epidemiological studies suggest that a high flavonoid consumption is linked to lower cancer risk[29].

Delonix oil contains a variety of *Delonix* 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, *Delonix*im, and nimbolide was investigated, and the results revealed that *Delonix*im 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].

Delonix regia and its active chemicals have an important role in cancer prevention and progression. The actual chemical process at work in this vision is unknown. *Delonix* and its components are thought to play a function in the modulation of numerous cell signaling pathways, according to research[33]. Several compounds of *Delonix regia* activate tumor suppressor genes and inactivate the activity of several genes implicated in cancer formation and progression, including VEGF, NF-B, and PI3K/Akt. *Delonix* 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. *Delonix regia* L. *Delonix* has anticancer properties via modulating multiple cell signaling pathways. *Delonix* and its constituents play a role in the prevention of malignancies through the modulation of molecular pathways, which are described below[33].

2.5.3 Anti-Inflammatory

Plants or isolated products of plants are used to treat or serve as anti-inflammatory agents. The anti-inflammatory effect of *D. regia* 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 *Delonix* 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 *Delonix* seed oil was tested on albino rats, and the results showed that *Delonix* 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 *Delonix* 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 *Delonix regia* fruit skin carbon tetrachloride extract (CTCE) and the isolated component azadiradione had considerable antinociceptive and anti-inflammatory effects[56].

2.5.4 Hepatoprotective Effect

Medicinal plants and their constituents serve an important function in hepatoprotection without causing any side effects. The hepatoprotective role of *Delonix*im-A in carbon tetrachloride (CCl_4) caused hepatotoxicity in rats was investigated, and histology and ultrastructure data revealed that pretreatment with *Delonix*im-A reduced hepatocellular necrosis dose-dependently[57]. Furthermore, the study's findings suggest that pretreatment with *Delonix*im-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 *Delonix* 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 *Delonix regia* (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 *D. regia* had moderate efficacy against carbon tetrachloride-treated rats[61]. The hepatoprotective activity of methanolic and aqueous extracts of *Delonix regia* 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 *Delonix* extract on ethanol-induced gastric mucosal lesions in rats was investigated, and the results revealed that pretreatment with *Delonix* extract protected against ethanol-induced gastric mucosal damage[62].

2.5.5 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 *D. regia* 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 *Delonix regia* leaf extracts boost wound healing activity by increasing inflammatory response and neovascularization[64].

2.5.6 Antidiabetic Activity

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

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

2.5.7 Antimicrobial Effect

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

2.5.7.1 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, regiating 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 *Delonix* extracts was tested against 21 strains of foodborne pathogens, and the results revealed that guava and *Delonix* extracts contain antibacterial components that could be effective in controlling foodborne pathogens and spoilage organisms[72]. Another study looked at the antibacterial activity of *Delonix regia* (*Delonix*) 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].

2.5.7.2 Antiviral Activity

At doses ranging from 50 to 100 g/mL, *Delonix* 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 *Delonix* bark had a direct anti-HSV-1 property[74]. *Delonix* (*Delonix regia* 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].

2.5.7.3 Antifungal Activity

The efficacy of several *Delonix* 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 *Delonix* leaf was most effective in inhibiting the growth of both fungal species[76]. Another discovery was that aqueous extracts of *Delonix* cake inhibited spore germination in three sporulating fungi, including *C. lunata*, *H. pennisetti*, and *C. gloeosporioides f. sp. Mangiferae*, and that methanol and ethanol extracts of *Delonix regia* inhibited growth in *Aspergillus flavus,*and *Alternaria sol*[76]. Previous research has shown that aqueous extracts of various elements of *Delonix*, such as *Delonix* oil and its main principles, have antifungal properties[77, 78]. The antifungal activity of *Delonix regia* 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].

2.6 General Characteristics of Pathogenic Bacteria Used

Infection occurs when bacteria invade a host and proliferate in its tissues. Disease may not always include infection, unlike infection (diabetes, for example, is a disease with no known causative agent)[80]. Microorganisms fill most ecological niches on the world. The capacity to cause sickness may be accidental or the product of evolutionary processes that have led to particular mechanisms enabling the pathogen to use the host's abundant supply of nutrients before transferring to a healthy host.

Pathogenicity frequently involves a sequence of phases with separate mechanisms at each. Some pathogens must adapt to the host by changing gene expression, and all must be easily transferred. Microorganisms have specific strategies for exploiting hosts and evading innate and acquired immune systems. Below are several pathogens.

2.6.1 The Bacteria

2.6.1.1 Staphylococcus *aureus*

Staphylococcus aureus are Gram-positive cocci that cluster but may also occur individually, in pairs, chains, or tetrads. Staphylococcus aureus can also cause disease. They are also capable of forming a bunch or a cube with eight cells, as is the case with sarcina, although bunches are more prevalent[81]. They are traditionally recognized from other Gram-positive cocci in that they produce catalase, an enzyme that destroys hydrogen peroxide. This is the basic method of differentiation. The development of coagulase, an enzyme that coagulates plasma, is what sets S. aureus from from other coagulase-negative Staphylococcus, which are typically less pathogenic than S. aureus[82].

Staphylococcus are found naturally on the skin and in mucous membranes. Impetigo, folliculitis, cellulites, furuncles, carbuncles, mastitis, pyomyostitis, septic bursitis, septic arthritis, osteomyelitis, epidural, pneumonia, and urinary tract infection are some of the diseases that may be caused by a Staphylococcus aureus infection. Infection of the heart muscle, which may include bacteremia and endocarditis[82.83]. It continues to be one of the five most prevalent causes of hospital-acquired infections and is often responsible for wound infections that develop after surgical procedures.

In the emergency department, urinary tract infections (UTIs) are often diagnosed. In urine cultures, Staphylococcus aureus is a rare isolate (0.5–6% of positive urine cultures), with the exception of individuals who have risk factors for urinary tract colonization[83].

2.6.1.1.1 Microscopic Morphology

Gram-positive S. aureus cells are spherical in form. When seen under a light microscope after Gram staining, the clusters resemble bunches of grapes. "Staphylococcus" is a Greek word meaning "a cluster of grapes (staphyle) and berries" (staphylococcus) (kokkos). A scanning electron microscope image shows cells with a smooth surface that seem to be approximately spherical[83,84]. The cells have a diameter of 0.5 to 1.0 M. Using transmission electron microscopy, researchers can see that cells have thick walls, unique membranes, and amorphous fluids inside them all.

2.6.1.1.2 Pathogenesis of *S.aureus*

There are five phases to the *S. aureus* infection process. These include

i. colonization,
ii. local infection,
iii. systemic spread and/or sepsis,
iv. metastatic infections, and
v. toxinosis.

The bacterium may persist in carrier mode in the anterior nares for weeks or months without producing infections[83,84]. Certain predisposing conditions, such as extended hospitalization, immunological suppression, surgery, the use of invasive medical equipment, and chronic metabolic illnesses, promote the progression of colonization to infection. When the organism is introduced into the skin from a site of carriage, a localized skin abscess form. This may result in a variety of clinical symptoms of localized infections, including carbuncle, cellulitis, impetigo bullosa, and wound infection. The bacteria may enter the bloodstream and spread to several organs, producing sepsis. This haematogenous dissemination may cause endocarditis, osteomyelitis, renal carbuncle, septic arthritis, and epidural abscess. Without a bloodstream infection, S. aureus extracellular toxins may cause particular symptoms. These conditions include toxic shock syndrome, scalded skin syndrome, and foot-borne gastroenteritis[84].

2.6.1.1.3 Laboratory Diagnosis

Identifying methicillin resistance *s. aureus* (MRSA) is the major goal of laboratory testing for a given *S. aureus* isolate Early detection of MRSA is critical for effective treatment with suitable antibiotics, which has been a serious public health concern since its discovery as a pathogen. Slide and tube coagulase tests, latex agglutination tests, and PCR-based assays are employed for species identification. Methicillin, oxacillin, and cefoxitin MICs, cefoxitin disk screens, oxacillin agar screens, and latex agglutination tests for PBP2a, as well as molecular approaches to identify mecA, are all used to detect MRSA in patients[84].

2.6.1.1.4 Biochemical Characteristics of S. *aureus*

S. aureus develops huge yellow or white colonies on nutrient-rich agar medium. The organism's carotenoids make the colonies yellow. Latin's 'aureus' means gold. The organism's four haemolysins make it haemolytic on blood agar (alpha, beta, gamma and delta)[84]. Almost all S. aureus isolates generate coagulase, a virulence component that aids in identification. The organism may grow on 7.5% sodium chloride mannitol-salt agar. Catalase-positive, oxidase-negative.

2.6.2.1 *Escherichia Coli*

Uropathogenic Escherichia coli (UPEC) is the leading cause of hospital- and community-acquired urinary tract infections (UTIs). Isolates from simple community-acquired UTIs have a number of virulence characteristics that facilitate the effective colonization of the urinary tract. In contrast, nosocomial UTIs may be caused by E. coli strains with virulence characteristics distinct from those isolated from community-acquired UTIs[85].

2.6.2.2 Microscopic Morphology of *E. Coli*

E. coli is a rod-shaped, gram-negative (-ve) bacterium. It measures 1-3 x 0.4-0.7 m and has a volume of 0.6 to 0.7 m. Individually or in pairs. Due of its peritrichous *flagella*, it is mobile. Some strains are non-motile. Certain strains may be fibrilled. Type 1 (hemagglutinating and mannose-sensitive) fimbriae are found in both motile and non-motile strains. Certain E. coli strains recovered from extraintestinal infections possess a polysaccharide capsule. They are sporeless[86]. They have a thin cell wall with just one or two peptidoglycan layers. These organisms are facultative anaerobes. Growth happens at temperatures ranging from 15 to 45 degrees Celsius.

2.6.2.3 Pathogenesis of *E.coli*

The *E. coli* genome is made up of a core of genes that stay the same and provide the genetic information needed for essential cellular processes and a flexible gene pool that contains genetic information that gives the bacterium traits (like virulence and fitness genes) that help it adapt to different environments[85]. E. coli is able to cause disease because it has a flexible gene pool that can add and lose genetic material. Virulence factors are the parts of pathogenic bacteria that allow them to cause infections. The presence of several putative virulence genes has been linked to the pathogenicity of E. coli[85,86].

2.6.2.4 Laboratory Diagnosis

The organism is a Gram-negative bacillus from the family *Enterobacteriaceae*. It grows easily on simple culture media with few nutrients; glucose or glycerol are often enough. Most of the time, E. coli is first found in a pathology laboratory as a lactose-fermenting, gram-negative rod that can grow both with and without oxygen, preferably at 37°C, and can either move or not move. It doesn't have oxidase, it makes indole, it doesn't ferment citrate, and it passes the methyl red test but fails the Voges-Proskauer test[86]. Several molecular techniques have made it easier to find and identify the different pathotypes more quickly. The somatic lipopolysaccharide (O), capsular (K), and flagellar (H) antigens can be used to group *E. coli* into different types. Classic serotyping is based on the Kauffman system, which uses the O and H antigens to type *E. coli*[87].

2.6.2.5 Biochemical Characteristics of *E. Coli*

It is a facultative anaerobe and an aerobe. The optimal temperature for growth is 37°C. On Nutrient agar, colonies appear as huge, greyish-white, thick, moist, smooth, opaque or transparent discs. The smooth (s) form is readily emulsified in saline, but the rough (R) form often self-agglutinates in saline. Some strains may form colonies known as "mucoid." As a result of lactose fermentation, colonies on MacConkey agar appear brilliant pink. On selective media (*Desoxycholate* citrate agar-DCA; salmonella shigella-SS medium) used to isolate salmonella, their growth is hindered; nevertheless, on DCA, which includes lactose and neutral red, their colonies appear pink. There is widespread turbidity and sediment in broth, which is dispersed by shaking[87,88].

By a normal strain of E. coli, glucose, lactose, mannitol, and maltose ferment with acid and gas generation, but sucrose does not. Triple sugar iron (TSI) produces acid and gas. The mnemonic IMV(1) C refers to the four biochemical tests often employed for entero-bacteriaceae classification: Indole (I), Methyl Red (MR), Voges Proskauer (VP), and Citrate (C) utilisation. E. coli is Indole and MR positive, VP and citrate negative (IMV(1) C++ —), does not produce H2 S, and does not hydrolyze urea. O Somatic, Greek Ohne Hauch; H—flagella; Greek Hauch; and K (Kapsular) antigens. K antigen is an envelope antigen that surrounds the O antigen, rendering the strain inagglutinable by O antiserum, and helps to virulence by blocking phagocytosis. It may be of the L, A, or B kind. Although L type is prevalent, the B antigen on enteropathogenic E. coli is medically significant[86,87,88].

2.6.3.1 *Pseudomonas aeruginosa*

Pseudomonas is a rod-shaped, thin (0.5 to 0.8 m by 1.5 to 3.0 m), Gram-negative bacteria that is motile by polar flagella; more than two flagella may sometimes be present. Some Pseudomonas bacteria, especially those isolated from patients with cystic fibrosis, are very mucoid and contain a pseudo capsule (glycocalyx) composed of polysaccharides. The glycocalyx shields pseudomonas from the host's immune system[89].

2.6.3.2 Pathogenesis of *P. aeruginosa*

Pseudomonas may infect any immune-compromised tissue or organ. Normal hosts aren't infected by Pseudomonas. As hospitals become more vulnerable, Pseudomonas has become a prevalent source of nosocomial infections. Pseudomonas causes human organ infections. Breakdown of host defense from illness or other reasons is the biggest risk for Pseudomonas infections. Invasive and toxin-producing Pseudomonas. Three stages cause infection:

i. Attachment and colonization;
ii. local invasion; and
iii. diffused systemic illness

Endogenous or external Pseudomonas infections may occur (exogenous). Pseudomonas may invade people outside of hospitals (0-24 percent)[89]. P aeruginosa adhesins attach bacteria to galactose, mannose, or sialic acid receptors on epithelial cells of the upper respiratory tract and others. Bacterial protease breaks down fibronectin and exposes pilus-specific receptors on epithelial cells. Viral infection and other factors promote Pseudomonas colonization (Opportunistic colonization). Pseudomonas pili, mucoid polysaccharide, and potentially surface-bound exoenzyme S help it colonizes[89].

2.6.3.3 Biochemical Characteristics of P. *aeruginosa*

Pseudomonas possesses oxidative metabolism. Since the organism is non-fermentative, acid is not generated from peptone water and carbohydrates.

Important biochemical properties of Pseudomonas include:

i. Oxidase test positivity;
ii. Catalase test positivity
iii. Nitrates are converted to nitrites;
iv. Arginine dihydrolase test is positive;
v. Glucose is oxidatively used Oxidative reaction in media
vi. The Indole, Methyl red (MR), Vogues Prauskar (VP), and H2S production tests all come back negative.

2.6.3.4 Laboratory Diagnosis of *P.aeruginosa*

P. aeruginosa may be extracted from any material in the laboratory using standard procedures (urine, pus, blood, ear swabs, tissue biopsies, body fluids, etc.). They grow well on normal broth and solid media such as blood agar, chocolate agar, and MacConkey agar, all of which are suggested for isolating Pseudomonas species from clinical specimens because of their ability to support their growth. Inhibitors that are selective for agar, such as cetrimide, may also be used for the purposes of isolating and perhaps identifying a microorganism[89].

2.6.4. *Klebsiella pneumoniae*

2.6.4.1 Morphology of *Klebsiella pneumoniae*

The bacteria Klebsiella pneumoniae is Gram-negative. Klebsiella pneumoniae is a bacterium with the form of a short, plump, straight rod (bacillus). Klebsiella pneumoniae is around 1–2 m 0.5–0.8 m in size (micrometer). K. pneumoniae exists alone, in pairs, in short chains, and sometimes in clusters. Klebsiella pneumoniae is a non-motile bacterium[88,90]. K. pneumoniae is a bacterium without flagella. The bacteria Klebsiella pneumoniae does not produce spores. Capsules are found in Klebsiella pneumoniae and may be readily illustrated using India ink preparation; they appear as a transparent halo on a dark backdrop. Klebsiella pneumoniae is a clinically significant pathogen and a common cause of hospital- and community-acquired urinary tract infections. This pathogen's growing resistance is limiting available treatment choices. K. pneumoniae uses several virulence factors, including capsule polysaccharides, adhesins, and iron acquisition determinants, for survival and immune evasion during infection. K. pneumoniae is often an opportunistic pathogen that mostly affects patients with compromised immune systems and typically causes hospital-acquired (HA) infections.

2.6.4.2 Pathogenesis of *Klebsiella pneumoniae*

During the quest for the pathogenic processes of Klebsiella infections, several bacterial factors that contribute to the pathogenesis of these bacteria were found. To examine the interaction between bacterial cells and their host, in vitro and in vivo models have been developed. Klebsiella pneumoniae recovered from blood culture of septicemic patient based on the following tests: Blood culture bottle incubated in Bactec[89]. After 23 hours of incubation, Bactec showed a favorable result. The positive bottle was then subculture on solid media. MacConkey agar, chocolate agar, and blood agar. After an overnight incubation, all three plates exhibited growth. The colonies on MacConkey agar seemed big, mucoid, and pink in color. The mucoid character of colonies results from the organisms' production of capsular material. Short, thick rods of Gram-negative bacteria. And Non-motile.

2.6.4.3 Biochemical Characteristics of Klebsiella *pneumoniae*

Biochemical characterisation helps distinguish bacterial species and subspecies. Bacterial species and subspecies use or don't use distinct chemical compounds. Klebsiella pneumoniae causes respiratory infections, difficulty breathing, pneumonia, and mortality. This bacterial pathogen infects dogs, piglets, and birds. Immunodeficient animals, particularly those with severe immune-deficiencies, show Klebsiella infection signs. K. pneumoniae They induce lung damage, inflammation, bleeding, and cell death (necrosis), producing thick, bloody, mucoid sputum (currant jelly sputum). These bacteria enter the lungs when someone aspirates oropharyngeal germs. Klebsiella infections are common in patients with compromised immune systems. Debilitating illnesses mostly afflict middle-aged and older males. Diabetes, alcoholism, cancer, liver illness, COPD, glucocorticoid medication, renal failure, and some occupational exposures may weaken respiratory host defenses (such as paper mill workers). Many of these illnesses are acquired by hospitalized patients (a nosocomial infection). Klebsiella bacteria cause pneumonia and bronchitis outside hospitals. These individuals often develop lung abscesses, cavitation, empyema, and pleural adhesions[89]. Even with antibiotic treatment, mortality is roughly 50%. Alcoholism and bacteremia may be fatal.

2.6.4.4 Laboratory Identification and Diagnosis

The colony morphology of *K. pneumonia* on blood agar is mucoid and ranges from 3 to 4 millimeters in diameter. K. pneumoniae colonies on MAC are pink (LF), mucoid (typically), and between three and four millimeters in diameter. The colonies that form on Hektoen enteric agar and XLD are yellow in color. There are a variety of commercially available small systems that can detect Klebsiella species in addition to other members of the Enterobacteriaceae family. K. pneumoniae has been shown to produce the enzymes glucose, lactose, and sucrose, as well as ONPG, methyl red, citrate, urease, malonate, and lysine decarboxylase.

2.6.5 *Proteus mirabilis*

2.6.5.1 Morphology of *Proteus mirabilis*

P. mirabilis is a bacillus-shaped, gram-negative bacteria with a bacillus morphology. Alternating between vegetative swimmers and hyper-flagellated swarmer cells, it is motile. It also produces an assortment of fimbriae. Proteus are rod-shaped, Gram-negative, Enterobacteriaceae family bacteria. They are widespread in nature and are also a natural component of human gut flora. They are one of the most prevalent causes of urinary tract infections (UTIs) and are linked to infection-induced kidney stones. Proteus species may also cause pyogenic lesions, ear infections, respiratory tract infections, and nosocomial infections. Species of Proteus possess pili (fimbriae). Pili are connected with sticky characteristics and, in some instances, pathogenicity[90].

2.6.5.2 Pathogenesis of *P. mirabilis*

Two phases make *P. mirabilis* pathogenic. The bacteria must colonize the urinary system and elude host defenses. Mannose-resistant fimbriae (MRF) and *P. mirabilis fimbriae* colonize the urinary system (PMF). Mobley lab at the University of Michigan Medical School studied the MRF's relevance. Their investigation produced a nasal MRF vaccination that worked in mice.

P. mirabilis has four evasion strategies[88,89,90]. First, a protease degrades secretory IgA. When infected, the host releases IgA. Three distinct flagellin genes recombine to generate new flagella that deceive the host's defenses. Third, MR/P fimbriae expression. Phase variation causes certain cells in the same population to express flagella but not others. Fourth, urease-mediated stone formation. Urease produces ammonia, which forms stones that protect bacteria.

Urease and hemolysin harm epithelial cells. Urease may harm epithelial cells by forming stones. Hemolysin, a cytotoxin, damages cells. *P. mirabilis* has seven pathogenicity factors. Urease, Zap-A metalloprotease, pore-forming hemolysin, capsular polysaccharide, fimbrial kinds, petrichous flagella, and swarming motility (Mobley)[90].

2.6.5.3 Biochemical characteristics of *P. mirabilis*

Organisms that swarm on 5% sheep blood agar, have a distinctive odor, and are oxidase-negative might be presumed to be Proteus spp. Positive isolates may be presumptively reported as *Proteus vulgaris* and negative isolates as *P. mirabilis* after further testing with spot indole. Pale or colourless (NLF) colonies. A high amount of swarming motility and Urease production[90].

2.6.5.4 Laboratory Diagnosis and Identification of *P.mirabilis*

The type of the ailment and the location of the infection both have a role in determining which sample is utilized for the isolation and identification of the Proteus species. When diagnosing a UTI, a sample of urine taken from the middle of the stream is used, however when diagnosing pyogenic lesions, the pus aspirate is utilized. The sample need to be collected in the sterile container while keeping the circumstances aseptic, and it ought to arrive at the laboratory within an hour of the collection. The nature of the specimen and the possible pathogens will determine which culture medium will be utilized to isolate the etiological agents. This will be done via the use of culture. Blood agar and MacConkey agar are two types of agar that are often used for analyzing pus and urine samples. The Proteus colonies spread out in waves over the blood agar plate, eventually forming a thin filmy layer composed of concentric circles (swarming)[90].

On a medium consisting of MacConkey agar, Proteus do not swarm and instead produce colonies that are smooth, pale, or colorless.

2.6.6 *Enterobacter Cloacae*

2.6.6.1 Morphology of *Enterobacter Cloacae*

Enterobacter cloacae is a gram-negative, rod-shaped member of the *Enterobacteriaceae* family. This bacterium spans in size from 0.3-0.6 x 0.8-2.0 m. *Enterobacter cloacae* resides in a mesophilic habitat with an ideal temperature of 37° C and moves using its peritrichous flagella. Gram-negative bacteria, such as *Enterobacter cloacae,* possess two cell walls. On the outer membrane, lipopolysaccharide's lipid-A (also known as endotoxins) produces sepsis. Lipid-A induces the release of cytokines, which may result in the circulation of toxins in the tissues and bloodstream[86,90]. The bacteria possess beta-lactamase, which is not detectable in vitro and is responsible for antibiotic resistance during therapy. This bacteria ferments glucose and can thrive in both aerobic and anaerobic environments. Beta-galactosidase, arginine dihydrolase, ornithine ecarboxylase, citrate utilization, nitrate reduction, and the Voges-Proskauer reaction are all positive for *Enterobacter cloacae*. This bacterium does not make lysine decarboxylase, hydrogen sulfide, urease, tryptophan deaminase, or indole, despite the fact that acid is produced from several carbon sources.

2.6.6.2 Pathogenesis of *E. Cloacae*

Enterobacter species, much like other enteric gram-negative rods, are equipped with a multitude of virulence factors including adhesions, endotoxin, and siderophones to acquire iron. These virulence factors allow the species to acquire iron. Due to its pervasive nature, *Enterobacter cloacae* may be acquired via the skin, GI tract, or urine system. This bacterium is an opportunistic pathogen, meaning it targets weakened people like the young, aged, or those with HIV[90]. This bacterium often causes nosocomial infections, which may be caught in the ICU. *Enterobacter cloacae* has been identified from hands, endoscopes, blood products, intra-arterial pressure devices, stethoscopes, albumim, and digital thermometers. Transoesophageal echocardiography probe and blood culturing system have cross-contaminated the cardiovascular ward. With Enterobacter cloacae outbreaks possible, frequent environment cleaning is crucial, particularly when new techniques, equipment, or staff are introduced. *E. cloacae* causes bacteremia, LRTIs, skin and soft tissue infections, UTIs, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ocular infections. Due to antibiotic resistance, these infections may cause morbidity and death[90].

2.6.6.3 Biochemical characteristics of *E.Cloacae*

Enterobacter species, much like other enteric gram-negative rods, are responsible for a broad range of nosocomial infections. These infections may impact the lungs, urinary system, intra-abdominal cavity, and intravascular devices. Neonatal septicemia and meningitis are both caused by *E. sakazakii*[90].

2.6.6.4 Laboratory Diagnosis and Identification of *E.Cloacae*

This bacterium is not difficult to differentiate from other members of the *Enterobacteraceae family* using biochemical testing, and it is simple to isolate it from clinical specimens[90].

2.6.7 *Acinetobacter nosocomialis*

2.6.7.1 Morphology of *A. nosocomialis*

Acinetobacter spp. are aerobic Gram-negative coccobacilli that are often found as free-living saprophytes in soil and water. Certain species are also often found living on the skin, in the throat, and in the secretions of healthy humans. Bacterium anitratum, Herellea vaginicola, Mima polymorpha, Achromobacter, Micrococcus calcoaceticus, Diplococcus, and Cytophaga were all names that have been used in the past to refer to strains that are now classified as belonging to the genus Acinetobacter. Over the course of many years, the taxonomic nomenclature of the Acinetobacter genus has undergone. *Acinetobacter nosocomialis* is a member of the Acinetobacter *calcoaceticus-Acinetobacter baumannii* (ACB) complex[90].

2.6.7.2 Pathogenesis of *A.nosocomialis*

Initially, Acinetobacter was thought to be a low-virulence bacteria, and little is known about its virulence mechanisms and host responses. Several putative virulence pathways associated with Acinetobacter species' capacity to attach to, infiltrate, and enter human epithelial cells have been found. Nevertheless, despite recent advancements, several concerns surrounding the virulence and pathophysiology of Acinetobacter species remain unsolved. Among the pathogenic determinants are pilus-mediated biofilm formation, an outer membrane protein A linked to apoptosis in human cells, an iron-acquisition system, lipopolysaccharides, and a quorum-sensing system. Biofilm production is a major virulence component of several A. baumannii isolates, including carbapenem-resistant strains[90].

2.6.7.3 Biochemical Characteristics of *A.nosocomialis*

Critically sick patients in intensive care units, especially those needing mechanical ventilation, and patients with wound or burn injuries are most affected by Acinetobacter spp. in the hospital environment (trauma patients)[89]. Included in the illnesses associated with Acinetobacter spp. are ventilator-associated pneumonia, infections of the skin and soft tissues, wound infections, urinary tract infections, peritonitis, secondary meningitis, and bloodstream infections. Infections of this kind are mostly caused by members of the *A. baumannii* complex; infections caused by other species of the genus Acinetobacter are uncommon and limited to catheter-related bloodstream infections and occasional outbreaks associated with point-source contamination. A. baumannii causes community-acquired illnesses seldom[88,89,90].

2.6.7.4 Laboratory Diagnosis and Identification

Standard process for isolating and diagnosing the pathogen include analyzing samples from any location of *Acinetobacter* infection[88]. Gram-negative, non-motile, oxidase-negative, glucose non-fermenting, strictly aerobic, catalase-positive The G+C content of Acinetobacter bacteria ranges between 39 and 47 percent. Depending on their stage of development, bacteria may be coccoid or coccobacillary. Most Acinetobacter spp. are metabolically adaptable and may be easily cultured on basic microbiological media, producing 2 mm in diameter, dome-shaped, smooth colonies. Some species are pale yellow or gray in color. The optimal growth temperature for clinically significant species is 37°C, while environmental organisms prefer lower temperatures. Culture in slightly acidic mineral medium containing acetate and nitrate as carbon and nitrogen sources, or in Leeds selective medium (88) or on comparable selective agars, may enhance the recovery of Acinetobacter spp. from complex microbial communities and enrich clinical and environmental specimens. Hemolytic activity on 5% sheep blood agar plates, hydrolysis of gelatin and urea, and acid production from glucose are varied[89,90].

2.7 Review of Standard Antibiotics

2.7.1 Rocephin (R)

Rocephin is a prescription drug used to treat the symptoms of a variety of bacterial infections, including intra-abdominal infections, bacterial ear infections, pelvic inflammatory disease, prosthetic joint infection, meningitis, pyelonephritis, and gonococcal infections. It is also used as a preventative measure prior to surgery[64]. Rocephin may be used alone or in conjunction with other drugs. Rocephin belongs to the family of medicines known as Third-Generation Cephalosporins. Rocephin is a sterile, semisynthetic, broad-spectrum cephalosporin antibiotic that is administered intravenously or intramuscularly. The chemical formula for ceftriaxone sodium is (6R,7R) -7-[2-(2Amino-4-thiazolyl)glyoxylamido] -8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-astriazin-3-yl)thio]methyl] -5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid, 72-(Z)(O-methyloxime), disodium salt, sesquaterhydrate[63,64].

Rocephin is a white to orange-yellow crystalline powder that is freely soluble in water, sparingly soluble in methanol, and hardly soluble in ethanol. A 1 percent aqueous solution has an approximate pH of 6.7. The color of Rocephin solutions varies from pale yellow to amber based on storage time, concentration, and diluent.

Antimicrobial Spectrum:

Staphylococcus aureus (methicillin susceptible), Coagulase negative Staphylococci, Streptococcus pneumoniae (penicillin susceptible), Streptococcus spp., Haemophilus influenzae, Moraxella catarrhalis, Neisseria meningitides, Neisseria gonorrhoeae, Enterobacteriaceae, E. coli[65]

2.7.2 Ciprofloxacin (CPX)

Ciprofloxacin is a fluoroquinolone-class antibiotic used to treat bacterial diseases including urinary tract infections and pneumonia. The FDA has approved ciprofloxacin for the treatment of urinary tract infections, sexually transmitted infections (gonorrhea and chancroid), skin, bone, and joint infections, prostatitis, typhoid fever, gastrointestinal infections, and lower respiratory tract infections, anthrax, plague, and salmonellosis[63]. In addition, ciprofloxacin is an acceptable therapeutic choice for people who have mixed infections or who have risk factors for Gram-negative infections. Through blocking bacterial DNA topoisomerase and DNA-gyrase, it limits DNA replication. Ciprofloxacin is the most effective fluoroquinolone against gram-negative bacilli bacteria (notably, the *Enterobacteriaceae* such as *Escherichia coli, Salmonella spp., Shigella spp., and Neisseria*). Additionally, ciprofloxacin is effective against certain gram-positive bacteria[66]. Ciprofloxacin is the most effective quinolone against Pseudomonas aeruginosa. Ciprofloxacin is both orally and intravenously accessible. Ciprofloxacin is used twice daily orally for 7 to 14 days, or for at least two days after the infection's signs and symptoms have subsided. The suggested oral dosage regimen is 250 mg twice day for mild to moderate urinary tract infections and 500 mg twice daily for severe or complex cases[63,64,66]. Infections of the respiratory tract or skin and soft tissues that are mild to moderate need 500 mg twice day. In contrast, a dose of 750 mg twice day is indicated for infections that are severe or complex. Ciprofloxacin should be used with meals to reduce gastrointestinal distress. Ciprofloxacin offers potential advantages in the therapy of bladder cancer. Studies *in vitro* on bladder transitional cell carcinoma tumor cells demonstrated a dose- and time-dependent suppression of cell proliferation. Ciprofloxacin concentrations that are readily obtainable in the urine of patients obtained these findings. Utilized appropriately, ciprofloxacin may be less expensive and more cost-effective than conventional parenteral regimens in some therapeutic contexts. Additional well-designed research would help define the most cost-effective use of this antibacterial drug. However, there has been a surge in ciprofloxacin resistance in E. coli-associated urinary tract infections, particularly in hospitals as compared to communities[66].

Antimicrobial spectrum:

Antibacterial spectrum of ciprofloxacin includes; *Streptococcus pneumoniae (penicillin susceptible isolates only), Enterococcus faecalis (vancomycinsusceptible isolates only), Staphylococcus epidermidis (methicillin-susceptible isolates only), Staphylococcus saprophyticus, Staphylococcus aureus (methicillin-susceptible isolates only), Streptococcus pyogenes, Gram-negative bacteria, Citrobacterkoseri (diversus), Proteus mi rabilis, Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Neisseria gonorrhoeae, Morgan ellamorganii, Haemophilus influenza, Haemophilus para influenzae,Klebsiella pneumonia, Moraxella catarrhalis, Campylobacter jejuni, Proteus vulgaris, Providencia rettgeri, Providencia stuartii, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonne*[64]

2.7.3 Streptomycin (S)

Streptomycin is an aminoglycoside antibiotic that was first derived from *Streptomyces* griseus bacterium[66]. It is the first aminoglycoside antibiotic to be identified. It is generally used to treat infections caused by aerobic gram-negative bacteria, such as brucellosis, tularemia, plague (Y. pestis), TB (in conjunction with isoniazid, pyrazinamide, and rifampin), and some instances of endocarditis when taken with beta-lactam antibiotics. In contrast to other aminoglycoside antibiotics, such as gentamycin and tobramycin, it lacks Pseudomonas aeruginosa-specific action. Antibiotic resistance has significantly narrowed the once extensive range of efficacy against gram-negative and gram-positive bacteria[67]. The resistance mechanism seems to include the blocking of its active transport into bacterial cells. Commonly resistant bacteria include *Enterobacteriaceae* and the majority of types of *Streptococci.* The use of streptomycin becomes especially relevant in the increasing topic of how to treat an infection caused by drug-resistant

Mycobacterium TB when the conventional combination treatment of rifampin, isoniazid, pyrazinamide, and ethambutol is ineffective. In these situations, streptomycin is a viable option to other antibiotics having action against M. tuberculosis, such as rifapentine, rifabutin, linezolid, and some fluoroquinolone medicines, including levofloxacin, gatifloxacin, and moxifloxacin. As with all aminoglycosides, streptomycin inhibits ribosomal peptide/protein synthesis and is bactericidal. It binds to a side of 16S rRNA situated on the smaller 30S component of the bacterial ribosome, reducing its functioning and preventing further protein synthesis by hindering the creation of peptide bonds. Hydrophilic aminoglycosides are incapable of penetrating the hydrophobic bacterial cell membrane. *Streptomycin* is normally delivered parenterally with a deep intramuscular injection since it is poorly absorbed by the digestive system. Patients who need numerous dosages or who have inadequate muscle mass may develop an intolerance to injections owing to the discomfort. In certain situations, intravenous injection might serve as an alternate form of delivery. Streptomycin has a serum half-life of about 2.5 hours, with serum drug levels peaking at around 50 mg/ml[68,69].

2.7.4 Erythromycin (E)

Erythromycin is a macrolide antibiotic produced by *Saccharopolyspora erythraea* (formally *Streptomyces erythraeus*)[70]. Erythromycin may be bacteriostatic or bactericidal depending on the organism and drug concentration. Erythromycin is used for the treatment of infections caused by susceptible strains of microorganisms in the following diseases: respiratory tract infections (upper and lower) of mild to moderate degree, pertussis (whooping cough), as adjunct to antitoxin in infections due to *Cortnebacterium diphtheriae*, in the treatment of infections due to *Corynebacterium minutissimum,* intestinal amoebiasis caused by *Entamoeba histolytica*, acute pelvic inflammatory disease cause by *Nesseria gonorrhoeae*, skin and soft tissue infections of mild to moderate severity caused by *Streptococcus pyogenes* and *Staphylococcus aureus*, primary syphilis caused by *Treponema pallidum*, infections caused by *Chlamydia trachomatis*, non-gonococcal urethritis caused by *Ureaplasma urealyticum*, and Legionnaires disease caused by *Legionella pneumoniae*[70].

Erythromycin acts by penetrating the bacterial cell membrane and binds to the 50S subunit of the bacterial rRNA complex, protein synthesis and subsequent structure and function processes critical for life or replication are inhibited. Erythromycin increases gut motility by binding to Motillin, thus it is a Motillin receptor agonist in addition to its antimicrobial properties[71].

2.7.5 Pefloxacin (PEP)

Pefloxacin belongs to the family of antibiotics known as *fluoroquinolones*, which are typically used to treat bacterial infections, such as pneumonia, typhoid fever, urinary tract, and gonorrhea (sexually transmitted disease)[72,73]. The bacterial infection is a disease in which bacteria multiply and cause illness inside the body. It may target any region of the body and multiply rapidly.

Pefloxacin includes the antimicrobial Pefloxacin, which aids in the treatment and prevention of several bacterial illnesses. It is bactericidal and acts by destroying pathogenic microorganisms. It prevents bacterial cell repair and kills the germs, stopping the illness from spreading further. Pefloxacin is an antibiotic having a wide range of activity against the majority of Gram-negative bacteria, many Gram-positive bacteria, and certain anaerobes (that lives without oxygen). However, flouroquinolones such as pefloxacin possess excellent activity against gram-negative aerobic bacteria such as *E.coli* and *Neisseria gonorrhoea* as well as gram-positive bacteria including *S. pneumoniae* and *Staphylococcus aureus*. They also possess effective activity against *shigella, salmonella, campylobacter, gonococcal* organisms, and multi drug resistant *pseudomonas* and *enterobacter*[72].

2.7.6 Gentamicin (CN)

Gentamycin is a complex of three different closely related aminoglycoside sulfates, Gentamycins C_1, C_2 and C_{1a} obtained from *Micromonospora purpurea* and related species. They are broad-spectrum antibiotics, but has been discovered to cause ear and kidney damage. They act to inhibit protein synthesis. Gentamycin is used for treatment of serious infections caused by susceptible strain of the following microorganisms: *Pseudomonas aeruginosa*, *Proteus mirabilis, Escherichia coli, Klebsiella-Enterobactor-Serratia* species, *Citrobacter* species and species of *Staphylococcus.*

Gentamycin irreversibly binds to specific 30S-subunit proteins and 16S rRNA. Specifically, gentamycin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to non-functional or toxic peptides and the breakup of polysomes into non-functional monosomes[73].

2.7.7 Ampiclox (APX)

Ampiclox Capsule belongs to the *'penicillin'* class of antibiotics used to treat a broad variety of bacterial infections. It is a broad-spectrum antibiotic used to treat bacterial infections of the ear, nose, throat, bones, lungs, and post-surgical wounds. Infections caused by bacteria are caused by the proliferation of dangerous microorganisms inside or on the body. These pathogenic bacteria generate compounds known as toxins, which may cause tissue damage and illness[74].

The 10mg capsule of Ampiclox includes two *penicillin* antibiotics, notably Ampicillin and Cloxacillin. Ampiclox Capsule 10 functions by inhibiting the production of bacterial cell covering, which is essential for their survival. This aids in treating and avoiding the spread of illnesses by eliminating microorganisms. Ampiclox Capsule is ineffective against viral illnesses, including the common cold and influenza[74].

Ampiclox Capsule are a broad-spectrum antibiotic used to treat bacterial infections of the ear, nose, throat, bones, lungs, and post-surgical wounds. The nature of Ampiclox Capsule is antibacterial. Both gram-positive and gram-negative bacteria are susceptible to its effectiveness. Infectious or dangerous bacteria may cause illness and proliferate rapidly inside the body[74]. These pathogenic bacteria generate compounds known as toxins, which may cause tissue damage and illness.

2.7.8 Zinnacef (Z)

Zinacef is a prescription medication used to treat a variety of bacterial infections, including *Pharyngitis/Tonsillitis*, Acute Bacterial Maxillary Sinusitis, Acute Bacterial Exacerbations of Chronic Bronchitis, Secondary Bacterial Infections of Acute Bronchitis, Pneumonia, Skin Infections, Urinary Tract Infections, Lyme Disease, and Gonorrhea. Zinacef may be used alone or in combination with other drugs. Zinacef is a member of the 2nd Generation c*ephalosporins* medication class. Zinacef is delivered in vials containing 750 mg, 1.5 g, or 7.5 g of cefuroxime as cefuroxime sodium and 750 mg or 1.5 g of *cefuroxime as cefuroxime* sodium, respectively. Depending on the quantity and diluent used, Zinacef solutions vary in hue from pale yellow to amber. Typically, newly formed solutions have a pH range of 6 to 8.5[75].

Zinacef is offered as a frozen, iso-osmotic, sterile, nonpyrogenic solution containing 1.5 g of cefuroxime as sodium cefuroxime. The pH has been changed using hydrochloric acid and, perhaps, sodium hydroxide. Premixed Zinacef solutions vary in hue from pale yellow to dark yellow[74,75].

2.7.9 Amoxicillin (AM)

Amoxicillin is a broad-spectrum semisynthetic antibiotic similar to ampicillin except that its resistance to gastric acid permits higher serum levels with oral administration. Amoxicillin is commonly prescribed with clavulanic acid (a β lactamase inhibitor) as it is susceptible to β-lacatamase degradation[76].

Amoxicillin is used for the treatment of infections of the ear, nose, and throat, the genitourinary tract, the skin and skin structure, and the lower respiratory tract due to susceptible strains of *Streptococcus* spp, *Streptococcus pneumoniae*, *Staphylococcus* spp., *Haemophilus influenza*, *Escherichia coli*, *Proteus mirabilis*, or *Enterococcus faecalis*[44]. Amoxicillin binds to penicillin-binding protein located inside the bacterial cell wall which leads to inhibition of bacterial cell wall synthesis[76].

2.7.10 Septrin (SXT)

Septrin is the commercial name for the antibiotic combination *cotrimoxazole*. Other manufacturers provide the same drug under a variety of brand names, including *Bactrim*. *Cotrimoxazole* consists of two substances: *sulamethoxazole* and t*rimethoprim*. Antibiotic with wide range activity. This implies that it is effective against a vast array of bacterial illnesses[77]. It is also effective against PCP *pneumonia*, malarial parasites, and a number of other disorders. *Co-trimoxazole* is a combination of the medicine's *sulfamethoxazole and trimethoprim. Sulfamethoxazole* is a sulonamide, a kind of antibiotic[77]. These antibiotics inhibit the production of folate by bacteria via a variety of mechanisms. Without folate, bacteria are unable to create DNA, preventing their growth, multiplication, and expansion. Therefore, *co-trimoxazole* prevents the transmission of infection. The remaining bacteria are either eradicated by the immune system or perish over time[77].

2.7.11 Chloramphenicol (CH)

Chloramphenicol is an antibiotic first isolated from cultures *Streptomyces venequelae* in 1947 but now produced synthetically[78,81]. It has a relatively simple structure and was the first broad-spectrum antibiotic to be discovered. It acts by interfering with bacterial protein synthesis and is mainly bacteriostatic[82]. Chloramphenicol is used for the treatment of bacterial conjunctivitis caused by Chloramphenicol susceptible organisms. It is also used in treatment of cholera as it destroys the vibrios and decreases the diarrhoea. It is effective against tetracycline-resistant vibrios. Chloramphenicol is lipid-soluble, allowing it to diffuse through the bacterial cell membrane. It then reversibly binds to the L16 protein of the 50S sub unit of bacterial ribosomes, where transfer of amino acids to growing peptide chains is prevented, thus inhibiting peptide bond formation and subsequent protein synthesis.

2.7.12 Sparfloxacin (SP)

Sparfloxacin is a *fluoroquinolone* antibiotic recommended for the treatment of bacterial infections. Sparfloxacin exerts its antibacterial effect by blocking the bacterial topoisomerase DNA gyrase. DNA gyrase is a crucial enzyme that regulates DNA topology and helps in DNA replication, repair, deactivation, and transcription[84]. Sparfloxacin is a synthetic fluoroquinolone antibacterial with a wide spectrum, similar to ofloxacin and norfloxacin. In vitro, sparfloxacin is active against a broad spectrum of gram-negative and gram-positive pathogens. Sparfloxacin exerts its antibacterial effect by blocking the bacterial topoisomerase DNA gyrase. DNA gyrase is a crucial enzyme that regulates DNA topology and helps in DNA replication, repair, deactivation, and transcription. (beta-lactam) antibiotics have a different chemical structure and method of action than quinolones. Therefore, quinolones may be effective against (beta)-lactam-resistant bacteria[85]. Despite the fact that cross-resistance between sparfloxacin and other fluoroquinolones has been found, certain bacteria resistant to other fluoroquinolones may be sensitive to sparfloxacin. The combination of sparfloxacin and rifampin is antagonistic against Staphylococcus aureus, according to in vitro experiments. Inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are necessary for bacterial DNA replication, transcription, repair, and recombination, is responsible for sparfloxacin's bactericidal activity[86].

2.7.13 Augmentin (AU)

Augmentin is an oral antibacterial combination consisting of amoxicillin trihydrate and the B-lactamase inhibitor, clavulanate potassium. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamas[87,88]. Augmentin is used to treat many different infections caused by bacteria, such as sinusitis, pneumonia, ear infections, bronchitis, urinary tract infections, and infections of the skin[88]. Augmentin binds to penicillin-binding proteins within the bacterial cell wall and inhibits bacterial cell wall synthesis. Clavulanic acids are a β-lactam, structurally related to penicillin that may inactivate certain β-lactamase emzymes[89].

2.7.14 Tarivid (CFX)

Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and Gram-negative bactaria such as *Escherichia coli*. It functions by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV which is an enzyme necessary to separate (mostly in prokaryotes, in bacteria in particular) replicated deoxyribonucleic acid, thereby inhibiting bacterial cell division[71,90]. Ofloxacin is used to treat certain infections including pneumonia, and infections of the skin, bladder, reproductive organs and prostate gland. Ofloxacin may also be used to treat bronchitis and urinary tract infections but should not be used for bronchitis and some types of urinary tract infections if other treatments are available. Ofloxacin is in a class of antibiotics called fluoroquinolones. It works by killing bacteria that cause infections[90].

2.8 Plant Constituents of Pharmacological Importance

The use of medicinal plants to cure different ailments in humans' dates back to ancient civilisation. This is accomplished by the secondary metabolites created by these plants during their metabolism, which are mostly employed to cure different human illnesses[83]. These secondary metabolites are plant components or natural substances with documented pharmacological and toxicological effects in humans, plants, and animals. The chemical substances found in plants that are classified as main or secondary metabolites depending on their chemical structure and biosynthetic origin. Secondary metabolites are known to have a variety of pharmacological activities that may be further categorised depending on their chemical structure and functional groups. Terpenoids, phenolics, flavonoids, alkaloids, and glycosides are among the most significant secondary metabolites, serving as a source of single bioactive components in nutraceuticals and contemporary pharmaceuticals. Secondary metabolites have excellent antioxidant properties and may be exploited as a natural antioxidant source in nutraceuticals. The majority of secondary metabolites have a wide spectrum of therapeutic efficacy[78,81,90]. The physiological importance of the majority of secondary metabolites has been recognized for many years. These chemicals were formerly believed to be inert metabolic byproducts, or metabolic wastes. Secondary metabolites prevent plants from being consumed by herbivores and from contracting microbial diseases. They serve as attractants (scent, color, taste) for pollinators and seed-dispersing animals (through odor, color, and flavor). They play a role in plant-plant competition and plant-microbe symbioses. Consequently, the ecological activities of secondary metabolites have a significant impact on the capacity of plants to compete and survive[91].

The secondary metabolites, which promote the reproductive success of plants by repelling fungus, bacteria, and herbivores, may render them unsuitable for human consumption. Consequently, several key agricultural plants have been bred to generate relatively low quantities of these chemicals[78]. Several of these secondary metabolites are described in detail below:

2.8.1 Alkaloids

Alkaloids are a class of naturally occurring chemicals that include nitrogen. Alkaloids are predominantly found in plants and are particularly prevalent in a particular family of flowering plants. Alkaloids possess a wide variety of therapeutic characteristics. Morphine is a potent narcotic used for pain treatment, but its addictive qualities restrict its use. Numerous alkaloids contain local anesthetic effects, but they are seldom utilized for this purpose in therapeutic settings. Cocaine (*Erythroxylon coca*) is an example of a very strong local anesthetic[78,91]. Alkaloids and plant extracts containing alkaloids have been employed as medicines, poisons, and psychoactive compounds throughout human history. It is believed that alkaloids play a significant role in the interaction between plants and their environment, since they often exhibit remarkable bioactivity. Alkaloids are known to protect some plant types against insect damage. Alkaloids are produced by several species, particularly higher plants. The alkaloid concentration in plant tissues is typically within a few percent and uniform. Depending on the kind of plant, the highest concentration is found in the leaves, fruits, roots, bark, or stem. Additionally, various plant tissues may contain distinct alkaloids[80,89].

2.8.2 Flavonoids

Plants use flavonoids for several purposes. Flavonoids provide yellow or red/blue pigmentation in flower petals to attract pollinators. Flavonoids filter UV light, fix nitrogen symbiotically, and color flowers. Flavonoids suppress plant-disease-causing pathogens like Fusarium *oxysporum*. Chemical messengers, physiological regulators, and cell cycle inhibitors. Many animals, including humans, eat flavonoids due to their vast distribution, diversity, and moderate toxicity compared to other active plant chemicals (such as alkaloids). Parsley, onions, blueberries, black tea, green tea, and bananas are flavonoid-rich foods[81,90].

2.8.3 Steroids

Plant steroids are a varied collection of plant-derived substances. In the plant world, phytosterols are common among plant steroids. It is noteworthy that some phytosterols have hypocholesterolemic action. Primarily found as secondary metabolites in genera of the Solanaceae family. Phytoecdysteroids are polyhydroxylated plant steroids, of which a number are known to display anabolic benefits without negative side effects. Steroid alkaloids are nitrogen-containing plant steroids with a variety of biological functions. In addition, traces of cholesterol and mammalian steroidal hormones, such as progesterone, have been found in certain plants[80,81,91].

2.8.4 Glycosides

A glycoside is a chemical in which a sugar is covalently bonded to another functional group. Glycosides serve various crucial functions in living beings. Numerous plants retain chemicals as inactive glycosides. The glycone may have a single sugar group (*monosaccharide*) or many sugar groups (*oligosaccharide*). The sugar group is therefore referred to as the glycone, whereas the non-sugar group is referred to as the *aglycone or genin* component of the glycoside. The glycone may have a single sugar group (*monosaccharide*) or many sugar groups (*oligosaccharide)*. Many writers demand that the sugar be bound to a non-sugar in order for the molecule to qualify as a glycoside, hence eliminating polysaccharides[80,81,92].

2.8.5 Saponins

Saponins are amphipathic, thus they may interact with cell membrane components like cholesterol and phospholipids, making them helpful for cosmetics and pharmaceuticals. Saponins have been utilized as vaccine adjuvants, such as *Quil A* from *Quillaja saponaria Molina* (commonly called *"quillaja*"). Saponins are advertised as dietary supplements and food components and utilized in traditional licorice medicine. There is no high-quality clinical evidence that they benefit human health. They might be used in subunit vaccinations and intracellular pathogen vaccines. Certain saponin concentrations are hazardous to cold-blooded animals and insects[81,89].

2.8.6 Tannins

Tannins bind and precipitate proteins, amino acids, and alkaloids. Tannins occur in blooming and seed-producing plants, especially dicotyledons. These chemicals are abundant enough to be used in commercial vegetable extracts. Any plant tissue may deposit tannins. Tannin levels vary per plant component. In the root hypodermis, tannin protects against pathogens. In the trunk, tannin is found in the secondary phloem, xylem, and between the bark and epidermis, where it influences tissue development. Tannin, found between the seed's tegument and aleuronic layer, helps maintain dormancy. Tannin in fruits and leaves decreases herbivores' appetites and acts as a natural defense. Tannins serve a vital role in the physiology and development of seeds, activating nodulation genes that favor nitrogen fixing in plants and attracting pollination insects[81,90].

2.8.7 Terpenes

Terpenes are organic hydrocarbons generated by plants, especially conifers. They give cannabis its scent. Cannabis resin glands release terpenes, an oily, aromatic material. The marijuana plant's pungent terpenes deter predators and attract pollination insects. Ingesting or smoking terpenes reduces stress. They help sleep and boost mental clarity. Terpenes in the cannabis plant have an aromatic flavor that appeals to connoisseurs, much like wine or a nice cigar. Terpenes aren't exclusive to cannabis. Many plants, notably conifers, generate them. Terpenes and terpenoids are occasionally used interchangeably. Unlike terpenes, terpenoids are oxygen-rich. Terpenes have anti-inflammatory, pain-relieving, and antimicrobial actions[78,80,81].

2.8.8 Phenolics

Plant phenolics defend against UV radiation, pathogens, parasites, and predators and contribute to color. They're in all plant organs and part of the human diet. Cereals, coffee beans, fruits, olives, vegetables, and tea leaves contain phenolic chemicals. Phenolics are vital for plant growth, including lignin and pigment production[79]. They provide plants structure and framework. Phenolic chemicals are formed by phenylpropanoid metabolization in plant shikimic acid and pentose phosphate. Simple phenolic molecules to highly polymerized compounds comprise benzene rings with hydroxyl substituents. Antioxidant phenol-containing plant compounds. They can block free radicals from reacting with other bodily components, reducing DNA damage and long-term health repercussions. Phenolic chemicals help plants grow, develop, and survive. Aromatic benzene ring molecules are vital for biotic and abiotic stress interactions[79,80,91].

2.8.9 Triterpenoid

Triterpenoid saponins are a broad collection of plant-derived natural chemicals known to be protective against pathogenic microorganisms and herbivores[92]. In addition to medical uses, saponins are used for a variety of purposes due to their diverse human-beneficial qualities.

Triterpenes also control the generation of reactive oxygen species (ROS) in the wound microenvironment, so speeding tissue healing[93]. Additionally, triterpenes may stimulate cell migration, cell proliferation, and collagen deposition. The most prevalent triterpene structures are pentacyclic — *oleanane, ursane, taraxerane, taraxastane, and lupane — and tetracyclic — dammarane and cucurbitane*. The majority of triterpenes are found on plant surfaces, such as fruit peel, stem bark, and leaves. They are produced in the cytosol by the cyclization of an epoxidized squalene, the progenitor of the varied group of polycyclic triterpenes. Triterpenoids are triterpenes containing heteroatoms, most often oxygen. Triterpene and triterpenoid are often used interchangeably. Numerous pentacyclic motifs are present in the chemistry and pharmacology (e.g. cholesterol) of triterpenoids[82,92,94].

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Chapter Three

Methodology

3.1 Materials

3.1.1 Collection and Processing of Plant Material

Delonix regia leaves and bark were collected and authenticated from Forestry Research Institute of Nigeria (FRIN) Jericho Hill, Ibadan, the plant's leaves and bark were carefully cleansed with distilled water. They were left to dry in the air at room temperature, then ground into a fine powder and put in airtight sample bottles for testing.

3.1.2 Collection of the Test Organisms

Pathogenic bacterial isolates were collected from the Medical Microbiology Laboratory of the University College Hospital in Ibadan, Nigeria. The bacteria were grown on Nutrient Agar (NA) slants and refrigerated for 24 hours before being sub-cultured onto newly prepared Petri plates for nutrient replenishment. The pathogenic bacteria collected were *Staphylococcus aureus, Klebsiella Pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter cloacae, Acinetobacter nasocomialis.*

3.2. Sterilization of Glassware and Non-glassware

All glassware were thoroughly washed with liquid detergent, rinsed with distilled water, drained, dried, then wrapped with aluminium foil and thereafter sterilized in a hot air oven at 160^{0}C for 2hours[1]. Before and after usage, the inoculating wire loop and cork borer (8mm) were disinfected by soaking them in alcohol (ethanol) and then flamed to red hot using a spirit lamp[1].

3.2.1 Preparation of Nutrient Media

Using a precise weighing scale, 28g of nutritional agar powder was weighed in accordance with the manufacturer's instructions (Mettler Toledo FA2104A). Agar powder was dispersed in sterile water in a 1000ml conical flask. The opening of the conical flask was sealed with a cotton-wool and aluminum foil stopper. After the solution was combined, it was homogenized in an 800°C water bath for 15 minutes. After removing it from the water bath the conical flask containing the homogenized nutrient agar was wrapped with aluminium foil and then sterilized in an autoclave for 15minutes at 121^{0}C at a pressure of 151b/sq inch. The medium was allowed to cool to about 47-50^{0}C before pouring into sterile petri dishes and left to solidify[2].

3.2.2 Validity of Test Organisms

After 48 hours of growth on Nutrient Agar slants in a 40 ° C refrigerator, the organisms were sub-cultured onto newly prepared petri dishes using the streak plate technique. Each isolate was put through a Gram staining method and additional biochemical tests to ensure the authenticity and viability of the test bacteria, including those for catalase, starch hydrolysis, oxidase, urease, indole, and citrate[3].

3.2.2.1 Starch Hydrolysis Biochemical Test

Starch hydrolysis test is used to assess whether the organism is capable of breaking down starch into maltose via the activity of the extra-cellular α-amylase enzyme. In starch hydrolysis test (also known as amylase test), we employ starch agar, which is a differential nutritive media. The test organisms are inoculated onto a starch plate and incubated at 30°C until growth is detected (i.e., up to 48 hours). The Petri plate is then filled with an iodine solution. The iodine becomes blue blackish indicating the presence of starch[3].

3.2.2.2 Catalase Biochemical Test

The presence of catalase, an enzyme responsible for catalyzing the release of oxygen from hydrogen peroxide, is shown by this test (H2O2). Staphylococci, which produce the enzyme catalase, are separated from streptococci, which do not produce catalase, using this method. Hydrogen peroxide may be decomposed into oxygen and water with the help of the enzyme catalase. An oxygen bubble-building enzyme may be seen in action when a bacterial isolate is injected into hydrogen peroxide. The inability to produce bubbles or a decrease in their size indicates a lack of catalase. Ideally, the culture wouldn't be any older than 24 hours. The morphologically identical *Enterococcusor Streptococcus* (catalase negative) and *Staphylococcus* (catalase positive) was distinguished with the catalase test[2,3,4].

3.2.2.3 Oxidase Biochemical Test

The oxidase test determines the existence of a cytochrome oxidase system that will catalyse the movement of electrons between electron donors in the bacteria and a redox dye- tetramethyl-p-phenylene-diamine. The oxidase test was performed to evaluate whether the organism contains the cytochrome oxidase enzyme. It was also used to separate *pseudomonads* from similar species[3].

3.2.2.4 Urease

The urease test finds organisms with the ability to hydrolyze urea into ammonia and carbon dioxide. Primarily, it was used to differentiate urease-positive *Proteeae* from other *Enterobacteriaceae*[2,3].

3.2.2.5 Indole

This test shows that some bacteria may degrade the amino acid tryptophane to indole, which accumulates in the medium. The indole synthesis assay is useful in identifying *Enterobacteria.* The amino acid tryptophan is broken down by most strains *of E. coli, P.*

vulgaris, P. rettgeri, M. morgani, and Providencia species, resulting in the production of indole. Indole was utilized as a biochemical validity test to distinguish *Proteus mirabilis* (indole negative) from all other *Proteus* species (indole positive). Furthermore, to distinguish *Klebsiella pneumoniae* (indole negative) from *Klebsiella oxytoca* (indole positive)[3].

3.2.2.6 Citrate

Citrate agar is used to examine an organism's capacity to use citrate as a source of energy. The medium comprises citrate as the only carbon source and inorganic ammonium salts (NH4H2PO4) as the sole source of nitrogen[3,4].

3.2.3 Antimicrobial Susceptibility Test

3.2.3.1 Preparation of the Extracts Dilution

30g of the powdered leaves and bark of Delonix regia were weighed and dissolved individually in 50ml of hexane, methanol, ethanol, and distilled water using a weighing scale (model number 12051). The solutions were left for 24hours but were stirred at regular intervals. Thereafter the solutions were then filtered using Whatman No 1 filter paper to obtain the extracts. Each of the concentrated filtrate were then diluted to various concentration of 100mg/ml to 59mg/ml, 39.3mg/ml, 29.5mg/ml and 23.6mg/ml.

Afterwards, the extracts were tested for their antimicrobial potency on the following bacterial isolates: *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus*, *Escherichia coli, Enterobacter cloacae*, *Acinetobacter nosocomialis*, and *Pseudomonas aeruginosa, as follows;*

The concentrations of the extracts for the antimicrobial analysis were determined using concentration formula below:

$C_1V_1 = C_2V_2$

Where C_1 = Initial concentration

C_2 = Final concentration

V_1 = Initial Volume

V_2 = Final Volume

a) 29.5g of undiluted extract converted to milligrams = 29.5g x 1000 = 2950mg

$$\text{Initial concentration} = \frac{2950\text{mg}}{50\text{ml}}$$

59mg/ml = Initial concentration

b) C_1 = 59mg/ml

C_2 =?

V_1 = 10ml

V_2 = 15ml

$$C_2 = \frac{C_1V_1}{V_2}$$

$C_2 = \frac{59mg/ml \times 10ml}{15ml}$

$C_2 = \frac{590mg/ml}{15}$

$C_2 = 39.33mg/ml$ = concentration of 1st dilution

c) $C_1 = 59mg/ml$

$C_2 = ?$

$V_1 = 10ml$

$V_2 = 20ml$

$C_2 = \frac{C_1V_I}{V_2}$

$C_2 = \frac{59mg/ml \times 10ml}{20ml}$

$C_2 = \frac{590mg}{20ml}$

$C_2 = 29.5mg/ml$ = concentration of 2nd dilution

d) $C_1 = 59mg/ml$

$C_2 = ?$

$V_1 = 10ml$

$V_2 = 25ml$

$$C_2 = \frac{C_1V_1}{V_2}$$

$$C_2 = \frac{59 \text{ X } 10}{25ml}$$

$$C_2 = \frac{590mg}{25ml}$$

C_2 =23.6mg/ml = concentration of 3^{rd} dilution

3.2.3.2 Agar Well Diffusion Test

The extracts' antibacterial activity was evaluated using the Agar well diffusion test. Using a sterile 8mm cork borer, five wells were aseptically created on the nutrient agar plate, ensuring that the control well was in the middle of the plate. With the exception of the center, which was filled with solvent alone, the extracts were poured into the wells using a syringe and needle. As a result, the proliferation of the implanted microorganisms was limited by the effectiveness of the extract ingredient. This permits the extract in the gel cavity to permeate into the solid medium. A discernible circular zone was present surrounding the well where the extract was located as evidence of this. A transparent 30mm meter rule was used to measure the diameter of the clean zone that was noticed[4].

3.2.3.3 Inhibitory Tests

Experiment organisms were streaked across the surface of the set nutrient agar plates using an inoculating wire loop. Using a sterile 8mm cork borer, a uniformly deep hole was drilled into the agar gel. Each well was then filled with 1 ml of the extracts made using various solvents. The petri dishes were left at room temperature for 30 minutes to allow for the dispersion of the extract. The controls were then established using just the solvents, without the extract. As a control for the aqueous sample, sterile distillate water was employed. After 24 hours of incubation at 37 degrees Celsius, the zone of inhibition was measured using a clear 30mm meter rule[4].

3.2.3.4 Determination of Minimum Inhibitory Concentration (MIC)

Using the agar streak method, the minimum inhibitory concentration was found. The minimum inhibitory concentration (MIC) is the lowest quantity of extract required to totally stop a test organism from developing up to 48 hours after incubation. Another way to look at it is that the MIC is the lowest amount that can cause the inoculum's viability reduce by more than 90%[5].

3.2.3.5 Antibiotics Susceptibility Test

The disk diffusion technique was used to determine antibiotic susceptibility using Gram-positive and Gram-negative antibiotic discs[6]. Each nutrient agar slant's pathogenic bacterial isolate was streaked onto a separate plate. Using sterile forceps, the antibiotics discs were then inserted aseptically on each Petri dish. The plates were then incubated at 37°C for 24 hours. In each of the plates, the inhibitory zones of the bacterial isolates were next evaluated. With a clear meter ruler, millimeters were used to measure each zone of inhibition around each antibiotic disc. The antibiotic susceptibility was based on the size of the area where the antibiotics stopped working. The inhibition zones that are shown on the agar plates are the reason why the antibiotic susceptibility tests are called antibiograms[5,6,7].

3.3 Qualitative Phytochemical Screening of the Extracts of the Leaf and Bark of *Delonix regia*

In order to determine the phytochemicals, present in the leaf and bark of *Delonix regia*, a phytochemical screening was carried out. This analysis was carried out using standard procedures[5]. The phytochemicals that were screened for are saponin, tannins, steroids, terpenoids, flavonoids, alkaloids, glycosides, triterpenes and phenolic[6,7]

3.3.1 Test for Saponins

After boiling the mixture in a water bath, the resulting powder was filtered after being comprised of two grams of the plant's leaf and bark powder and five milliliters of distilled water. The filtrate was then given an additional 3 ml of distilled water before being forcefully agitated for a period of 5 minutes. The persistent foaming that occurred upon being warned was indicative of the presence of saponins[7].

3.3.2 Test for Tannins

One gram of the powdered sample of the bark and leaf of plant was stirrer with 10ml of distilled water. This solution was then filtered with Whatman No 1 filter paper. Few drops of 1% of ferric chloride ($FeCl_3$) were added to the filtrate. The appearance of blue-black or green coloration indicated the presence of tannin[7.]

3.3.3 Test for Steroids

0.5 grams of each of the plant powders were mixed with 2 milliliters of acetic anhydride, and this was followed by the addition of 2 milliliters of concentrated sulphuric acid

(H2SO4). The presence of steroids might be determined by a shift in color from violet to blue or green[8].

3.3.4 Test for Terpenoids

Five milliliters of each plant extract were mixed with 2ml of chloroform and 3ml of concentration sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration at the interface indicated the presence of terpenoids[7,8].

3.3.5 Test for Alkaloids

In a water bath, one gram of each powder was mixed with five milliliters of aqueous hydrochloric acid solution containing 1% concentration. After treating 1 milliliter of the filtrate with a few drops of Mayer's reagent, a second 1 milliliter sample of the filtrate was treated in the same manner with Drangendorff's reagent. Evidence for the presence of alkaloids in the extract that was being analyzed was considered to be either turbidity or precipitation with either of these reagents[8].

3.3.6 Test for glycosides

2.0 ml of acetic acid was added and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides[8].

3.3.7 Tests for Flavonoids

After a few minutes of mixing, the pieces of magnesium ribbon and the concentrated HCL were added to the aqueous crude plant extract. The resulting mixture was pink, indicating the presence of flavonoid[9].

3.3.8 Test for Triterpene

To a test tube containing 5 mls of the aqueous extract of *D. regia*, 5 mls of acetic acid anhydrate were added and mixed gently. 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer[8,9].

3.3.9 Test for Phenolics

To 5 mL of extract solution in ethanol, 1 mL of distilled water and a few drops of a 10% aqueous solution of ferric chloride (FeCl3) were added. Blue coloration suggests the presence of phenols[10].

3.4 Quantitative Phytochemical Analysis

The leaf and bark extracts of *D. regia* were submitted to conventional quantitative analytical procedures. Alkaloids, flavonoids, phenolic, steroids, tannin, terpenoids, glycosides, triterpenoids, and saponins are analyzed in each of the leaf and bark extracts that were made using different organic solvents[10].

3.4.1 Determination of Alkaloids

A 500 mL conical flask was filled with 400 mL of 10% acetic acid in ethanol and a dried fine powdered plant leaf sample of 10g was added and covered for four hours. Filtered and concentrated on a water bath to one-quarter of the original volume, this was the final product. The extract was precipitated by adding concentrated ammonium hydroxide dropwise to the extract. The precipitate was collected, rinsed with dilute ammonium hydroxide, and then filtered from the rest of the solution. The alkaloid, which was dried and weighed, is the residual[11].

3.4.2 Determination of Saponins

In a conical flask, 10 g of dried fine leave particles and 50 mL of 20% ethanol were added. This mixture was cooked (55 C) for 4 hours with constant stirring. The filtrate was then extracted with 100 mL of 20% ethanol. This extract was decreased to 20 mL in 90 C water. The concentrated extract was placed in a 250 mL separating funnel with 10 mL of diethyl ether. Ether was dumped and water was collected. It was repeated. Add 30 ml of n-butanol. The n-butanol extracts were washed twice with 5% aqueous sodium chloride[11].

3.4.3 Determination of Tannin

A 50 ml plastic container was filled to the brim with 500 mg of the sample. A mechanical shaker was used to add 50 ml of distilled water and shake the mixture for an hour. A 50-ml volumetric flask was used to collect the filtrate, which was then concentrated to the required concentration. It was then pipetted into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferro-cyanide in the presence of 2 ml of 0.1 N HCL. Within ten minutes, an absorbance of 120 nm had been obtained[11,12].

3.4.4 Determination of Flavonoid

In a 250 cm^3 beaker, 2.50 g of sample was mixed with 50 cm^3 of 80% methanol in water. The beaker was then covered and left at room temperature for 24 hours. After throwing away the supernatant, the residue was taken out three more times with the same amount of ethanol. The whole solution of each wood sample was filtered through Whatman filter paper number 42 (125 mm). Later, the filtrate from each sample of wood was put into a crucible and dried over a water bath. The substance in the crucible was put in a desiccator to cool it down and then weighed until the weight stayed the same[13].

3.4.5 Determination of Phenolics

In 100 ml of distilled water, 100 mg of sample extract was dissolved. 1.5 ml of this solution was transferred to a test tube, then 1 ml 2N Folin-Ciocalteu reagent and 2 ml 20% Na2CO3 solution were added, and the volume was filled up to 8 ml with distilled water. After vigorous shaking and standing for 2 hours, the absorbance was measured at 765 nm. These data were utilized to determine total phenolic content using a gallic acid calibration curve[14].

3.4.6 Determination of Steroid

1ml of test extract of steroid solution was put into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5 percent w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml). The mixture was heated in a water-bath kept at 70±2°C for 30 minutes with periodic shaking and diluted to the mark using distilled water. The absorbance was measured at 780 nm against the reagent blank[14].

3.4.7 Determination of Terperoid

Dried plant extract 100mg (wi) was ingested and steeped in 9mL of ethanol for24 hour. The extract after filtering, was extracted with 10mL of ethanolic using separating funnel. The alcohol extract was separated in pre-weighed glass vials and waited for its full drying (wf)[15].

3.4.8 Determination of Glycosides

Each sample was weighed, then placed in a 250 cm^3 round bottom flask with about 200 cm3 of distilled water. After resting for 2 hours, autolysis was allowed to take place. After adding an antifoaming agent, the sample was distilled in a conical flask with a 250 cm^3 volume using 20 cm^3 of 2.5% NaOH (sodium hydroxide) (tannic acid). On a black backdrop, 100 cm^3 of cyanogenic glycoside, 8 cm^3 of 6 M NH4OH, and 2 cm^3 of 5% KI were added to the distillate(s), stirred, and titrated with 0.02 M AgNO3 (silver nitrate) using a microburette. The discontinuity of the turbidity implies the conclusion[15.]

Endnotes

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Chapter Four

Results and Discussion of Findings

4.0 Inhibitory Test for Bacterial Isolates

In this thesis, the antimicrobial and phytochemical properties of the extracts of the leaf and bark of *Delonix regia* plant was tested against some human pathogenic bacteria which are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* *pneumoniae, Proteus mirabilis, Enterobacter cloacae, and Acinetobacter nosocomialis*. The phytochemical constituents of these extract were evaluated using Methanol, Hexane, Ethanol and Distilled water as solvents of extraction. The solvents extract filtrates which was used against the human pathogenic bacteria and reactions was studied for potency. The bacteria isolates used for the research were isolated and obtained from University College Hospital (UCH), Ibadan after confirmatory and certification test. The experiment was repeated three times, and the mean of the triple data is shown in Table 4.1. The obtained values were analyzed to get the Mean values, Standard Deviation (SD) of the values, and Standard Errors (SE). In addition, Table 4.1 depicts the minimum inhibitory zone of D. regia leaf extract against human pathogenic microorganisms. It demonstrates that neither hexane nor distilled extracts were reactive with any of the bacterium isolates. This is contrary to the result research reported that, there were high antimicrobial activity of the hexane and distilled water extracts from the leaves of *Euphoriba royleane and Allanblackia floribunda* against *E. coli, Staphylococcus aureus, Proteus mirabilis*[1].

4.1 Minimum Inhibitory Concentration (MIC)

For each extract, four test tubes were set up, about 10 ml of nutritional broth was added, the opening was covered by means of cotton wool, and the tubes were pasteurized at 121 °C for 15 minutes. Subsequently the test extract had cooled, 1 ml was added to the first tube, which was labeled 10-1. It was then diluted step by step until it reached 10-4.

This process was done again for every other extract. Then, 0.1 ml of a test organism broth culture that had been growing for 24 hours was added to each tube with a different amount of test extract, the tubes were shaken well, and they were put in an incubator at 37 degrees Celsius for 24 hours. Optimistic controls were made up of less than 10 ml of nutrient broth and the organism on its own. Negative controls were made up of less than 10 ml of nutrient broth and the test extract on its own. The Minimal Inhibitory Concentration was found in the test tube where there was no visible growth (turbidity is a sign of growth) (MIC). MIC is the lowest concentration of extract that stops about 75% of viable inoculums from growing, as measured in vitro by a 10mm diameter. The results of the D. regia leaf extract's minimum zone of inhibition against human pathogenic bacteria are known.

Table 4:1: Minimum inhibition by Leaf extract of *Delonix Regia* against the bacterial isolates

Bacterial Isolates Used	Solvent for	A 30mg	B 10ml (A)	C 10ml (A)	D 10ml (A)	Control

	extraction	sample in 50ml Solvent 59.16mg/ml	+ 5ml Solvent 39.33mg/ml	+ 10ml Solvent 29.5mg/ml	+ 15 ml Solvent 23.6mg/ml	
Staphylococcus aureus	Methanol	27.7±0.33	17.3±0.33	15.0±0.71	10±0.0	8.00±0.1
	Hexane	-	-	-	-	-
	Ethanol	16.3±0.88	11.0±0.58	11.67±1.35	10.0±0.0	8.0±0.0
	Distilled Water	-	-	-	-	-
Pseudomonas aeruginosa	Methanol	25.0±0.58	19.0±0.58	17.3±0.19	13.0±0.58	8±0.00
	Hexane	-	-	-	-	-
	Ethanol	23.0±0.58	22.67±0.19	18.3±0.19	14.0±0.0	10.0±0.0
	Distilled Water	-	-	-	-	-
Escherichia coli	Methanol	-	-	-	-	-
	Hexane	-	-	-	-	-
	Ethanol	-	-	-	-	-
	Distilled Water	-	-	-	-	-
Klebsiella	Methanol	-	-	-	-	-

Pneumonia	Hexane	-	-	-	-	-
	Ethanol	-	-	-	-	-
	Distilled Water	-	-	-	-	-
Proteus	Methanol	-	-	-	-	-
Mirabilis	Hexane	-	-	-	-	-
	Distilled Water	-	-	-	-	-
	Ethanol	-	-	-	-	-
Enterobacter	Methanol	14.67±0.77	12.33±0.19	-	-	-
Cloacae	Hexane	-	-	-	-	-
	Ethanol	-	-	-	-	-
	Distilled water	-	-	-	-	-
Acinetobacter	Methanol	13.0±0.57	12.33±0.19	9.67±0.19	-	-
nosoconialis	Hexane	-	-	-	-	-
	Ethanol	-	-	-	-	-
	Distilled Water	-	-	-	-	-

Source: Lab work, 2022

Key: n=3; mean ± (standard error). - = negative; stock = 30mg of extract +50ml of solvent (59.16mg per ml), 39.33mg/ml = 10ml of extract +5ml of solvent, 29.5mg/ml = 10ml of extract +10ml of solvent, 23.6mg/ml = 10ml of extract +15ml of solvent and control.

Methanol extract reacted against *Staphylococcus aureus* at 27.7±0.33, *Pseodomonas aeruginosa* at 25±0.58, *Enterobacter cloacae* at 14.67±0.77 and *Acinetobacter nosocomialis* at 13±0.58. This confirms previous research showing that moringa leaf extracts are effective against *Escherichia coli* and *Pseudomonas aeruginosa*[2]. Antimicrobial activity against *Pseudomonas aeruginosa* was found to be particularly high in the ethanol extract of the leaves at 23. ±0.58 and *S. aureus* at 27.7±0.33. These result findings is in agreement with existing research findings which reported that leaves of *bryophyllumpinnnatum and aspilia Africana*[3] have been studied and found active against *P.aeruginosa and S.aureus.*

Ethanolic extract revealed potency against *S.aureus and P. aeruginosa* with the following inhibitions 59.16mg/ml, 39.41mg/ml, 28.58mg/ml, 23.66mg/ml respectively which shows the antimicrobial effectiveness against microorganism and it also demonstrates that ethanol is an effective solvent for extracting the majority of plants with medicinal use.

Table 4.2 presents the findings of the minimal zone of inhibition of the bark extract of D. regia against human pathogenic bacteria. In the same way, it was found that extracts of hexane and distilled water did not react with any of the bacteria isolates that had already been described. Methanol reacted against *Staphylococcus aureus* (13.67±0.35), *Proteus mirabilis* (15±0.58), *Klebsiella pneumoniae* (12.67±0.77), and *Pseudomonas aeruginosa* (32.67±0.19) but non-reactive against *Escherichia coli*. In the past, a bacterial illness was treated by giving a decoction of the plant parts in water. However, our data shows that making an extract with an organic solvent has a stronger effect against bacteria, which is in line with what other research has found. Methanol extract of bark of delonix regia were potent against *s. aureus* at 13.6±0.35, *P.aeruginosa* at 32.6±0.19, *K.pneumoniea* at 12.67±0.77, *P.mirabilis* at 15±0.58, and *E.cloacae* at 18±0.58 which tallies to a previous study by Azuonwu *et al*[3], that says methanol extract of bark of *moringa oleifera* exhibited activity against microorganism and in another study by bobota et al[4], states that methanolic extract of *helichrysumarenarium* and *antennariadiacia* demonstrated antimicrobial activity against the microorganism. This conclusion is consistent with previous research, which also revealed that methanol is an active solvent for the extraction of the majority of plants that are considered to be of medicinal significance. The ethanol extracts of *delonix regia* bark were shown to be effective against all seven tested isolates as presented in table 4.2. Similar to the author's results, the extract of *anacadium occidentale* bark has antimicrobial properties against both gram-positive and gram-negative organisms, and its potency may be attributed to their gram response.

Table 4:2: Minimum inhibition by bark extract of *Delonix Regia* against the bacterial isolates

Extract concentration

Bacterial Isolates Used	Solvent for extraction	A 30mg sample in 50ml Solvent 59.16mg/ml	B 10ml (A) + 5ml Solvent 39.33mg/ml	C 10ml (A) + 10ml Solvent 29.5mg/ml	D 10ml (A) + 15 ml Solvent 23.6mg/ml	Control
Staphylococcus aureus	Methanol	13.67±0.35	-	-	-	-
	Hexane	-	-	-	-	-
	Ethanol	23.67±0.19	-	-	-	8.0±0.0
	Distilled Water	-	-	-	-	-
Pseudomonas Aeruginosa	Methanol	32.67±0.19	22.67±0.77	12.3±1.35	11.67±1.35	10±0.00
	Hexane	-	-	-	-	-
	Ethanol	26.67±1.35	25.0±0.58	23.0±0.58	18.67±0.77	12.0±0.0
	Distilled Water	-	-	-	-	-
Klebsiella pneumoniae	Methanol	12.67±0.77	-	-	-	-
	Hexane	-	-	-	-	-
	Ethanol	17.0±0.58	11.3±0.19	-	-	-

	Distilled Water	-	-	-	-	-
Proteus Mirabilis	Methanol	15.0±0.58	-	-	-	-
	Hexane	-	-	-	-	-
	Ethanol	15.3±0.77	13.0±0.58	12.3±0.19	11.30±0.77	8.0±0.0
	Distilled Water	-	-	-	-	-
Enterobacter cloacae	Methanol	18.0±0.58	-	-	-	8.0±0.0
	Hexane	-	-	-	-	-
	Ethanol	19.3±0.19	12.67±0.77	-	-	-
	Distilled Water	-	-	-	-	-
Acinetobacter Nosoconialis	Methanol	-	-	-	-	-
	Hexane	-	-	-	-	-
	Ethanol	18.67±0.77	16.3±1.35	16.7±0.77	16.3±0.19	10.0±0.0
	Distilled Water	-	-	-	-	-

Key: n=3; mean ± (standard error). - = negative; stock = 30mg of extract +50ml of solvent (59.16mg per ml), 39.33mg/ml = 10ml of extract +5ml of solvent, 29.5mg/ml = 10ml of extract +10ml of solvent, 23.6mg/ml = 10ml of extract +15ml of solvent and control.

4.2 Minimum Bactericidal Concentration (MBC)

The MBC was determined by plating 0.1 ml from each MIC tube on nutrient agar and spreading it out with a clean glass rod. This was done so that the MBC could be calculated. After incubating the plates for 24 hours at 37 degrees Celsius, the plates that showed no signs of growth were used to determine the minimal concentration that was bactericidal (MBC). The effectiveness (potency) level of the solvent extraction for *delonix regia* is summarized in the table. It was discovered that the bacterial isolates (both gram positive and gram negative) employed were resistant to both methanolic and ethanolic as extract solvents. The bark of *Delonix regia* was 86% effective against all isolates studied, with the exception of *P.mirabilis and A. nosocomialis,* while the leaf of *Delonix regia* was found to be 20% effective against *S. aureus and P. aeruginosa,* suggesting that it may have bacteriostatic (<75%) capabilities. a quality similar to that of traditional mercury, dettol, and other antiseptic soaps.

4.3 Sensitivity of the Isolates to Standard Antibiotics

Recently, there has been an increase in the number of reports of clinically significant microorganisms exhibiting antibiotic resistance. The antibiotics susceptible test results are presented in Table 4.3. The test organisms were resistant to Ampiclox, Zinnacef, Tarivid, and Chloramphemicol out of 14 that was used. *Pseudomonas aeruginosa* resisted all the antibiotics used, but other antibiotics reacted against and inhibited the remaining bacterial isolates used, though with various degree of sensitivity which was measured in millimeter (mm) zone of clearance. *Staphylococcus aureus* was sensitive to Ciproflaxacin (16mm), Erythromycin (12mm), sparfloxacin (14mm), Amoxacillin (12mm), Augmentin (12mm), and Pefloxacin (18mm). *Proteus mirabilis* was sensitive to Ciproflaxacin (20mm), Pefloxacin (16mm), and Gentamicin (12mm)

Table 4:3: Diameter of zone of inhibition (mm) of gram positive and gram-negative bacterial isolates by the Standard Antibiotics Discs (SAD)

S/N	Antibiotics & Code	Concentration (mg)	*Staphylococcus aures*	*Acinetobacter nosoconialis*	*Esoherichia coli*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*P. mirabilis*	*k. pneumoniae*
1	Rocephan (R)	25	-	-	-		-	14	-
2	Ciprofloxacin (CPX)	30	16	14	20	-	20	20	20
3	Streptomycin (S)	30	-	18	12	-	12	12	12
4	Erythromycin (E)	10	12	-	-	-	-	16	14
5	Pefloxacin (PEP)	30	18	-	18	-	20	16	-
6	Gentamicin (CN)	30	12	-	-	-	-	12	14

7	Ampiclox (APX)	30	-	-	-	-	-	-	-
8	Zinnacef (Z)	20	-	-	-	-	-	-	-
9	Amoxicillin (AM)	30	12	-	-	-	-	-	-
10	Septrin (SXT)	30	-	12	14	-	-	-	16
11	Chloramphenic ololol (CH)	30	-	-	-	-	-	-	-
12	Sparfloxacin (SP)	10	14	-	16	-	14	-	14
13	Augmentin (AU)	10	12	-	-	-	-	12	-
14	Tarivid (CFX)	10	-	-	-	-	-	-	-

Source: Lab work, 2022 Key: - = negative

Table 4:4 Qualitative Phytochemical Screening of Leaf extract of *Delonix Regia*

Phytochemical Constituents	Leaf N-hexane	Methanol	Ethanol	Distilled Water
Alkaloids	-	+	+	-
Saponin	+	+	+	+
Steroids	-	+	+	+
Terpenoids	+	-	-	-
Flavonoids	+	-	-	-
Glycosides	-	+	+	+
Triterpenes	-	+	+	+
Phenolic	-	+	+	+
Tannin	+	-	-	-

Source: Lab, 2022

KEY:

+ = *Present*

- = *Absent*

D. regia N-hexane extracts showed positive findings for four phytochemical tests in qualitative analysis, methanol and ethanol showed positive results for six tests, and distilled water showed positive results for five tests, as shown in Table 4.4. The extract was tested for phytochemical elements such alkaloids, saponin, tannin, phenolics, flavonoids, glycosides, steroids, terpenoids, and triterpenes. However, among these substances, alkaloids, phenolic compounds, flavonoids, saponins, and tannins are significant secondary metabolites and the driving forces behind each plant's therapeutic properties. The extract was further put through additional analytical procedures for the measurement of phytochemical components.

Table 4:5 Qualitative Phytochemical Screening of Bark of *Delonix Regia*

Phytochemical Constituents	Bark N-hexane	Methanol	Ethanol	Distilled Water
Alkaloids	-	+	+	-
Saponin	+	+	+	+
Steroids	-	+	+	-
Terpenoids	+	-	-	-
Flavonoids	+	+	+	-
Glycosides	-	+	+	+
Triterpenes	-	+	+	-
Phenolic	-	+	+	-
Tannin	+	+	+	-

Source: Lab, 2022

KEY:

\+ = *Present*

\- = *Absent*

4.4 Result of Phytochemical Analysis of Extracts of the Leaf and Bark of Delonix Regia

Phytochemical components of *D. regia* may function as a mediator for these pharmacological actions. On *D. regia*, the presence of phytoconstituents such alkaloids, terpenes, glycosides, and flavonoids as well as their antidiarrheal and antioxidant properties have been documented. In this study, tannins, saponins, steroids, terpenoids, alkaloids, and phenolic compounds were looked for in ethanol extract of the leaf and bark of Delonix regia. The results are shown in Tables 4.5. It is impossible to overstate the importance of these phytochemicals in this plant. This is consistent with research that showed phytochemicals in medicinal plants are the key component that gives them their medicinal qualities.

Hence, the significant number of tannins in the leaves and bark of *D. regia* as found in this thesis might be responsible for the plant's use for the treatment abdominal disorders and other gastroenteritis. The results are consistent with those from a study evaluating the phytochemical and antimicrobial properties of moringa leaf extracts[3]. Some evidence for this may be gleaned from the results of a phytoconstituents screen performed on extracts of the roots and leaves of Alstonia congensis[2.]

Table 4:6 Quantitative Phytochemical Screening of Leaf of *Delonix Regia*

Phytochemical Constituents	Leaf Methanol	N-hexane	Ethanol	Distilled Water
Alkaloids	0.361	0.000	0.316	0.000
Saponin	0.089	0.118	0.073	0.164
Steroids	0.328	0.000	0.415	0.000
Terpenoids	0.000	0.458	0.000	0.000
Flavonoids	0.000	0.079	0.0042	0.000
Glycosides	0.067	0.000	0.078	0.041

Triterpenes	**0.041**	**0.000**	**0.024**	**0.010**
Phenolic	**0.211**	**0.000**	**0.129**	**0.084**
Tannin	**0.000**	**0.480**	**0.044**	**0.000**

Standard procedures were used to measure the number of phytochemicals found in the sample methanol (leaf) extract. Table 4.6 shows that of the nine compounds found, alkaloid content was the highest (0.361), followed by steroid content (0.328), and then terpenoid content, which did not react. The highest amount of Tannis (0.48 g) was found in the n-hexane leaf sample, and terpenoid has (0.458). The ethanol sample has the most steroid (0.415) and the most alkaloid (0.316). saponin has the greatest concentration in the leaf extract sample of distilled water (0.164).

Table 4:7 Quantitative Phytochemical Screening of Bark of *Delonix Regia*

Phytochemical Constituents	Bark Methanol	N-hexane	Ethanol	Distilled Water
Alkaloids	0.382	0.000	0.254	0.000
Saponin	0.052	0.093	0.094	0.123
Steroids	0.197	0.000	0.214	0.000
Terpenoids	0.000	0.385	0.000	0.000
Flavonoids	0.025	0.040	0.038	0.000
Glycosides	0.034	0.079	0.041	0.056

Triterpenes	0.024	0.000	0.014	0.014
Phenolic	0.530	0.000	0.099	0.000
Tannin	0.240	0.064	0.038	0.000

Table 4:8 Potency Evaluation of Leaf and Bark *Delonix Regia* Extract Against the Bacteria Isolates Used

S/N	ISOLATES USED	METHANOL		ETHANOL		HEXANE		DISTILLED WATER	
		LEAF	BARK	LEAF	BARK	LEAF	BARK	LEAF	BARK
1	*Staphylococcus aureus*	+	+	+	+	-	-	-	-
2	*Escherichia coli*	-	+	-	+	-	-	-	-
3	*Proteus mirabilis*	-	+	-	-	-	-	-	-
4	*Pseudomonas aeruginosa*	+	+	+	+	-	-	-	-
5	*Klebsiella pneumoniae*	-	+	-	+	-	-	-	-
6	*Acinetobater nosocomialis*	-	-	-	+	-	-	-	-
7	*Enterobacteria cloacae*	-	+	-	+	-	-	-	-
	Rate	2/7	6/7	2/7	6/7	0/7	0/7	0/7	0/7
	Percentage (%) of Potency	28.5%	86%	28.5%	86%	-	-	-	-

KEY:

\+ = *Present*

\- = *Absent*

Table 4.8 presents a summary of the efficacy (potent) level of the solvents of extraction from both the leaf and bark samples. From the table it was observed that both methanolic and ethanolic as solvents of extract was observed to be against the bacterial isolates gram positive and gram negative used. Hence could be term to be 'broad' spectrum in activities, bark sample was 86% potent on all isolates used except *P.mirabilis and A.nosocomialis* whereas leaf sample was observed to be 20% potent (average) against *S. aureus and P.aeruginosa* , hence could be reported to be bacteriostatic (<75%) in properties.

Endnote

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Chapter five

Conclusion

5.1 Summary of Findings

In this thesis, the antimicrobial and phytochemical activities of the extracts of the Delonix regia plant was tested against some human pathogenic bacteria which are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, and Acinetobacter nosocomialis.* The bacteria isolates used for the research were isolated and obtained from University College Hospital (UCH), Ibadan after confirmatory and certification test. The experiment was repeated three times, and the mean of the triple data is shown in Table 4.1. The obtained data was analyzed to get the Mean values, Standard Deviation (SD) of the values, and Standard Errors (SE). It demonstrates that neither hexane nor distilled extracts were reactive with any of the bacterium isolates. The following steps were taken for each extract: four test tubes were set up, 10 ml of nourishing broth was added, the mouth was plugged with cotton wool, and the tubes were sterilized at 121 °C for 15 minutes. The test extract was diluted serially from 10-1 (1 ml) to 10-4 (9 ml) in a series of tubes after chilling. For each subsequent extract, the same steps were taken. For 24 hours, the test organism was kept in an incubator at 37 degrees Celsius. Leaf extract has been shown to inhibit human pathogens with a minimal zone of inhibition. Methanol extract reacted against *Staphylococcus aureus* at 27.7±0.33, *Pseudomonas aeruginosa* at 25±0.58, *Enterobacter cloacae* at 14.67±0.77 and *Acinetobacter nosocomialis* at 13±0.58. Methanol extract of bark of *delonix regia* was potent against *S. aureus* and *P. Aeruginosa*. Methanol reacted against *S. aureus* (13.67±0.35), *P. mirabilis, K. pneumoniae* and *P. aeruginosa.* This is consistent with previous research, which also revealed that methanol is an effective solvent for the extraction of the majority of plants that are considered to be of medicinal significance. *D. regia* was found to be 86% effective against all isolates studied, with the exception of *P. mirabilis* and *A. nosocomialis*. 0.1 ml of each of the MIC tubes was poured onto nutrient agar plates and spread out uniformly with a sterile glass rod. After incubating the plates for 24 hours at 37 degrees Celsius, the plates that showed no signs of growth were used to determine the minimal concentration that was bactericidal (MBC). Test organisms were resistant to Ampiclox, Zinnacef, Tarivid, and Chloramphenicol out of 14 that was used. *P. aeruginosa* resisted all the antibiotics used, but other antibiotics reacted against and inhibited the remaining bacterial isolates. *S. aureus* was sensitive to Ciprofloxacin (16mm), Erythromycin (12mm) and sparfloxacillin (14mm). *P. mirabilis* was sensitive to Ciprofloxacin (20mm), Pefloxacin (16mm), and Gentamicin (12mm).

5.2 Conclusion

In *vitro* extracts of *Delonix Regia (Bojer ex Hook)* leaves and bark with ethanol, hexane, methanol and distilled water were tested on the pathogenic bacteria which are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, and Acinetobacter nosocomialis.* All of the bacterial isolates were killed by both the methanolic and ethanolic extracts of the leaf and bark. The ethanolic extract was shown to have the largest zone of inhibition against all of the test microorganisms. *D. regia (Bojer ex Hook)* leaf and bark extracts have inhibitory effects that are similar to those of the standard for antibiotics. This confirms the antibacterial properties of the plant extracts. The antibacterial action justifies the traditional use of *D. Regia (Bojer ex Hook)* in the treatment of various bacterial infections. The results also showed that ethanol is the best solvent of extraction for the parts of the plant used.

Phytochemical analysis of the leaf and bark of *D. Regia (Bojer ex Hook)* revealed the presence of Alkaloids, Saponins, Tannins, Steroids, Flavonoids, Glycosides, Triterpenes, Phenolic and Terpenoids. It can also be concluded that these phytochemicals appear to be responsible for the antimicrobial effects of this plant's extracts. The plant shows different pharmacological activities such as hepatoprotective, gastroprotective, wound-healing, antiarthritic, larvicidal, antimalarial, antiemetic, antibacterial, antifungal, anti-inflammatory, analgesic, antidiarrheal, antihemolytic, diuretic, and anthelmintic activity. The review of this plant also shows, the plants have different medicinal property.

The leaves and bark of *D. Regia (Bojer ex Hook)* possess antimicrobial properties and as such can be harnessed for the production of antimicrobial drugs. Patients should be encouraged to see a physician at a hospital or clinic, perform particular tests, and receive specific drugs and dosages depending on the results of those tests, rather than relying on indiscriminate drug usage. Antibiotic medication resistance is on the rise, and public awareness campaigns are needed to raise awareness of the problem, its causes, and possible solutions. Improved methods of extracting active antimicrobial compounds from plants, and therefore their introduction into the pharmaceutical sector, should be promoted so that they might be exploited in the creation of newer, safer, and perhaps cheaper medications. Therefore, it is firmly considered that the benefits from the aforementioned approach will surely support potential health outcomes and also jointly lessen the global trend of multidrug resistant issues.

5.3 Recommendation

The following recommendations are made from this study;

1. The use of even the most basic technology to the extraction, purification, and development of herbal products into active phytochemical constituents of leaf of delonix regia will go a long way toward improving public health.
2. It would also support government initiatives to contribute to the global herbal market in international trade.
3. Since cosmetics mostly include botanical extracts. Because it's antioxidant, anti-inflammatory, antiseptic, and antibacterial. Rural and urban communities use plants for traditional cosmetics.
4. This study finally suggests drying and storing medicinal herbs properly. Moisture fosters the growth of molds and other bacteria, destroying the plant drug's active ingredients.

5.4 Contribution to Knowledge

The major contributions of this thesis are as follows

1. assessment of antibacterial activities of *D. regia* leaves and barks.
2. determination of *D. regia* leaf and bark phytochemical content in distilled water, ethanol, methanol, and hexane.
3. identified the quantity of phytochemical constituents in the plant extract and
4. compared leaf and bark antibacterial activity.

5.5 Suggested Areas for Further Research

The medicinal capabilities of *Delonix* should be further researched, especially the antibacterial activity of the plant's stem and trunk extract and phytochemical ingredients. In the fields of pharmacy and medicine, more research is required to identify, characterize, and purify the plant's bioactive compounds in order to understand the scope of the bacteria that they can inhibit. This may deliver new antimicrobial compounds to treat antibiotic-resistant bacteria, a global concern.

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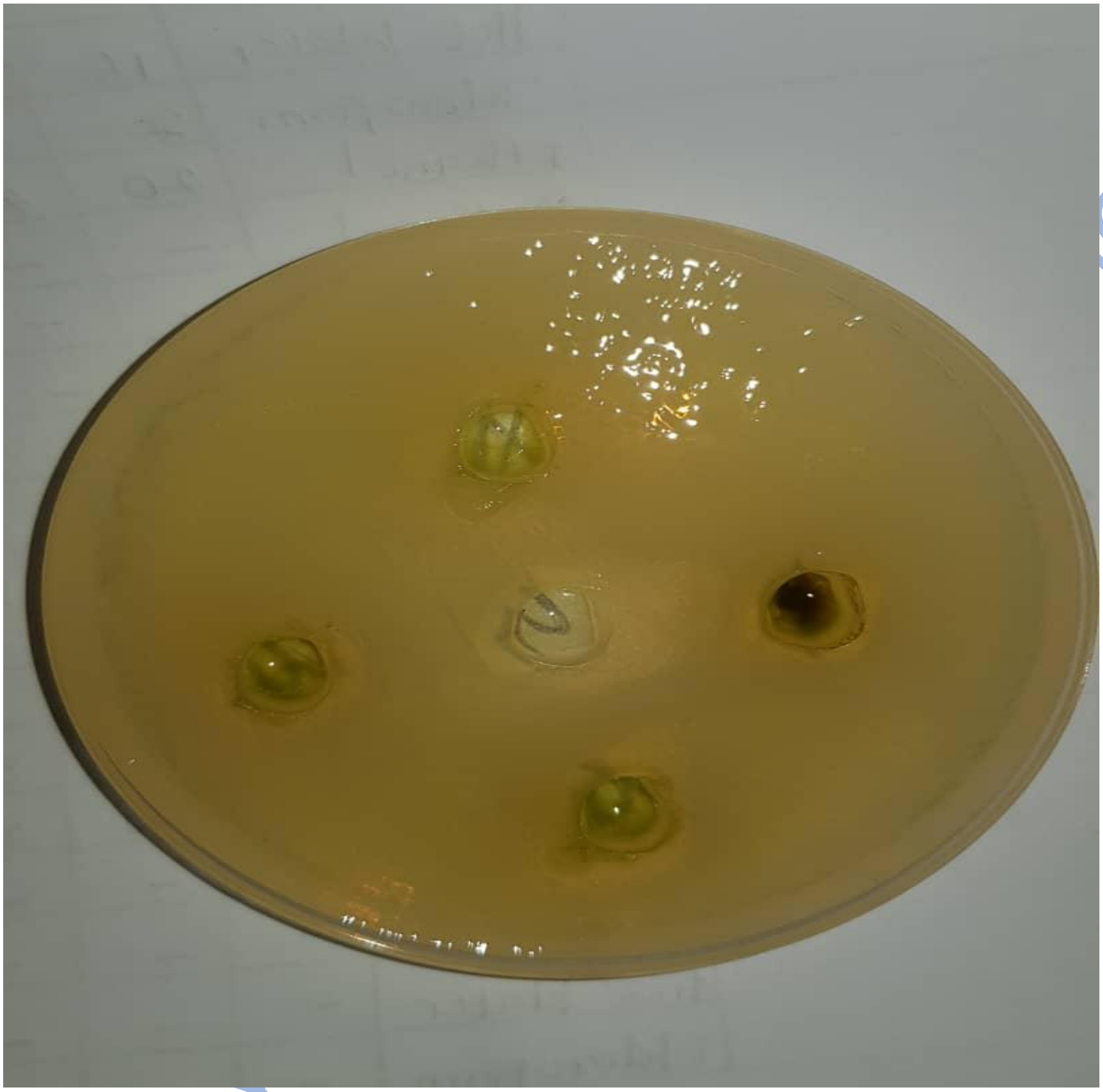
Appendices

Appendix 1: *In vitro* antimicrobial activity of hexane extract of the leaf of *Delonix regia* on *Staphylococcucs aureus* showing the zones of inhibition

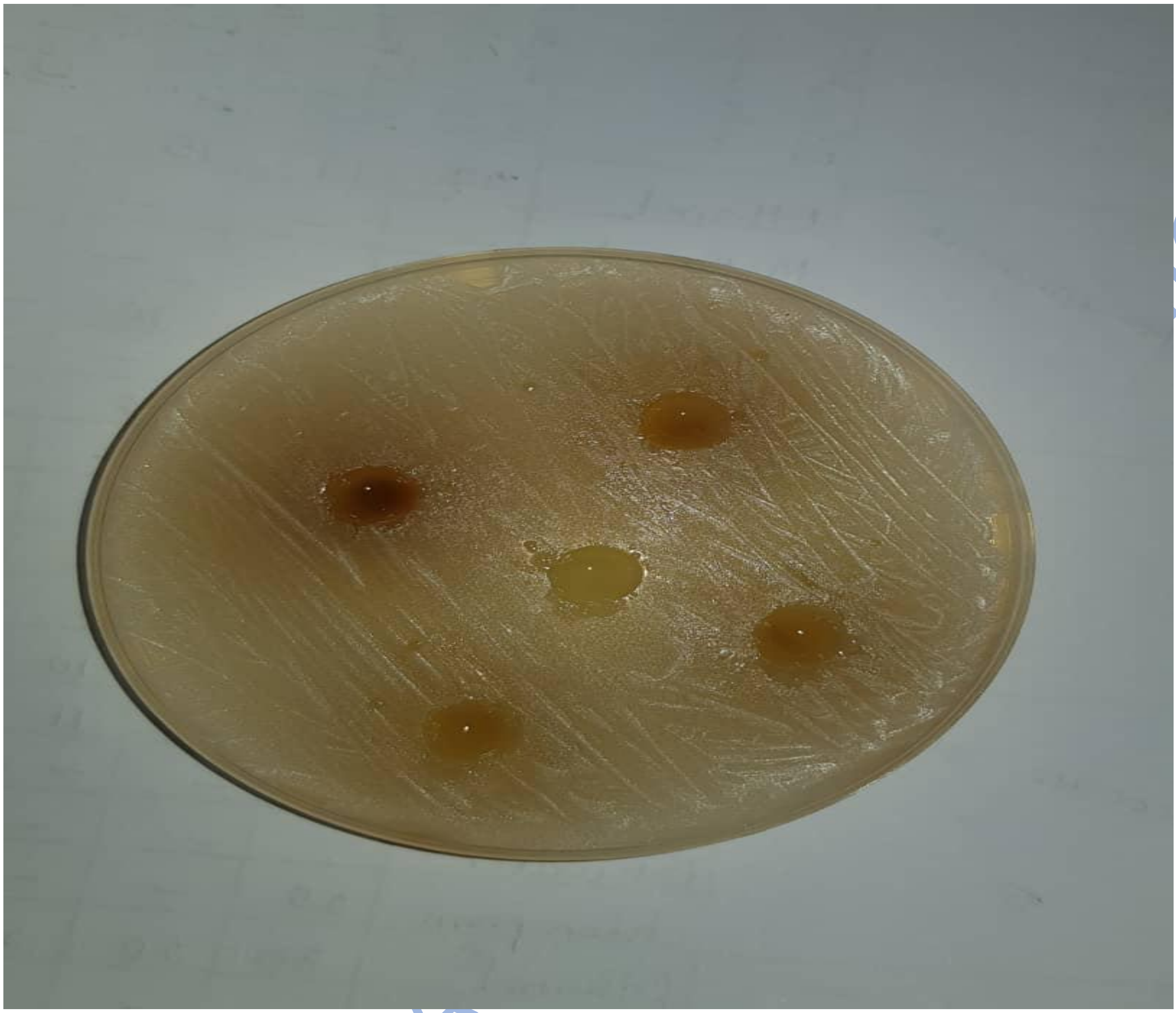
Appendix 2: *In vitro* antimicrobial activity of ethanolic extract of the leaf of *Delonix regia* on *pseudomonas aeruginosa* showing the zones of inhibition

Appendix 3: *In vitro* antimicrobial activity of methanolic ether extract of the leaf of *Delonix regia* on *Proteus mirabilis* showing the zones of inhibition

Appendix 4: *In vitro* antimicrobial activity of distilled water extract of the leaf of *Delonix regia* on *klebsiella pneumoniae* showing the zones of inhibition



Appendix 5: *In vitro* antimicrobial activities of hexane extract on the leaf of *Delonix Regia* on *Escherichia coli* showing the zones of inhibition



Appendix 6: *In vitro* antimicrobial activity of hexane extract on the bark of *Delonix regia* on *Pseudomonas aeruginosa* showing the zones of inhibition

Biodata

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B. Educational Background

Educational Institutions Attended with Dates and Qualification

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2. Book/Monographs Nil

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5. Majoy Conferences/Workshops attended Nil

6. Date ……………. & Signature …………….

University Compliance Certification

This is to certify that the Thesis of Adeleke Adenike Damilola with Matriculation Number LCU/PG/001307, in the Department of Biological Science, Faculty of Natural Science, Lead City University, Ibadan, Nigeria is in full compliance with the approved University format and style.

Date	Signature