Chapter One

Introduction

1.1 Background to the Study

The utilization and pursuit of medicines and dietary supplements derived from plants have seen a notable acceleration in recent years. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are actively scouring the globe for phytochemical "clues" that hold promise for the treatment of infectious diseases[1]. Globally, the prevalence of diseases that can be fatal due to pathogenic microorganisms has increased, and this has become a major source of morbidity and mortality, particularly in developing nations with worse health indices. Furthermore, the risk of incurable infections has increased due to the growing frequency of bacterial strains that are resistant to drugs, necessitating a quick search for novel therapeutic approaches[2]. Despite their effectiveness, antibiotics may have side effects that include immunological suppression, hypersensitivity, loss of important gut and mucosa bacteria, and allergic reactions[3]. This has highlighted even more how important it is to look for new medicine sources, such as plants. Nowadays, there is a greater need for and acceptance of medicinal plants, which are vital to ecosystems because they provide necessary functions. Their importance extends throughout human history, as almost all societies have used different plant parts for medicinal purposes because of their dependability, accessibility, and safety[4]. Humans have always worked closely with plants and their derivatives to treat health problems, especially in the Arabian Peninsula, China, India, and Africa, where herbal therapy has long been a key tradition[4]. Since ancient times, medicinal plants have been essential to humankind and have developed into one of the oldest sciences, particularly in

societies like China, Greece, Egypt, and India[5]. Plants have historically been the main source of medicinal remedies. Almost 50,000 different species are employed in pharmaceutical and cosmetic products, offering valuable components for the creation of new drugs[6]. Many plant species with therapeutic properties are considered medicinal plants. The plant contains active chemicals that can have direct or indirect therapeutic benefits in several portions of the plant, including the seeds, roots, leaves, fruits, and flowers. These substances, which are kept in plant tissues, have physiological effects on living things, demonstrating the use of plant materials in medicine and health care[7]. The date palm (*Phoenix dactylifera L.*), one of the most significant economic crops in the Arabian Peninsula, stands out among these plants[8]. A mainstay of Arabian cuisine, date fruits are categorized into three main cultivar groups according to their sugar content: semi-dry (like 'Dayri'), dry (like 'Thoory'), and soft (like 'Barhee')[9]. For generations, people and animals have acknowledged the nutritional and physiological advantages of date palm fruits[10]. The scientific name for dates, *Phoenix dactylifera L.*, is derived from Greek words meaning "date" and "bearing." Dates are palms of the Arecaceae family. With approximately 200 types, date palms have been farmed for over six millennia and are mostly found in Saudi Arabia, the Middle East, and Egypt[11].

A major global health and socioeconomic issue, antimicrobial resistance is thought to have contributed to 1.27 million drug-resistant bacterial infections-related fatalities in 2019[12]. The environment, food production, human and animal health, and the accomplishment of several sustainable development goals are all seriously threatened by this resistance[13], [14]. Reducing the abuse and overuse of antibiotics as well as creating novel antimicrobial drugs are necessary to combat antimicrobial resistance[15]. More than 150 million people worldwide suffer from urinary tract infections (UTIs), which are the most prevalent bacterial illnesses

globally[16]. Antibiotic resistance in UTIs has increased alarmingly in recent years due to a variety of mechanisms[17]. More than 50% of women will at some point in their lives get a UTI, making them especially vulnerable[18]. UTIs have significant social and financial repercussions, particularly in wealthy nations. There is growing interest in investigating alternative antimicrobial agents, such as plant extracts and phytochemicals, as a result of the growing problem of antimicrobial resistance[19]. Date fruits and seeds, with their complex phytochemical composition and long history of medical use, have become prospective sources of novel antibacterial agents[20]. Pain and discomfort are common side effects of UTIs, and if left untreated, they can worsen. Up to 80% of community-acquired UTIs are caused by *Escherichia coli*; however other organisms that may also be to blame include *Klebsiella pneumoniae, Proteus mirabilis,* and *Enterococcus species*[21]. Medicines are part of the standard treatment for urinary tract infections (UTIs), especially beta-lactam medicines like cephalosporins and penicillin, which are effective against gram-negative bacteria[22]. Antibiotic-resistant bacterial strains have emerged as a result of antibiotic abuse and overuse, which presents serious management issues for UTIs[23]. Bacterial beta-lactamase enzyme synthesis, which renders beta-lactam antibiotics inactive, is one of the most alarming resistances mechanisms[24]. It's crucial to look at alternate treatment options because antibiotic resistance in UTIs is becoming an increasing problem[25]. The potential antibacterial qualities of plants, particularly the availability of bioactive molecules with medicinal potential, are drawing interest in plant-based remedies[26]. Traditional medicine has traditionally used date fruits and seeds (*Phoenix dactylifera*) because they contain a variety of bioactive components, including tannins, Flavonoids, Phenolics, and phytochemicals, which have demonstrated promise in treating bacterial infections[27].

1.2 Statement of the Problem

The rise of antibiotic-resistant bacteria has led to increased morbidity, mortality, and healthcare costs. The limited availability of effective antibacterial agents against beta-lactamase-producing strains necessitates the exploration of novel antimicrobial agents from natural sources.

1.3 Justification of the Study

The emergence of antibiotic resistance in UTI-causing bacteria, especially those producing beta-lactamase enzymes, poses a significant challenge to effective treatment. Conventional antibiotics are becoming less effective, leading to treatment failures and prolonged hospital stays. The presence of beta-lactamase enzymes drastically reduces the efficacy of beta-lactam antibiotics, rendering many treatment options obsolete. This study is motivated by the need to explore untapped natural sources of antimicrobial compounds that could provide alternative treatment modalities against these resilient bacterial strains.

1.4 Aim and Objectives of the Study

The study aimed to assess the in-vitro phytochemical and antibacterial evaluation of date fruits and seed extract on beta-lactamase-producing bacteria isolated from UTI cases.

The Specific Objective of the Study is:

i. Evaluate phytochemical composition of fruit and seeds extract of *P.dactylifera* and fractionation of the crude extract.
ii. Screening of the bacterial strain for B-lactamase producing ability.
iii. To determine antibacterial potential of the *P.dactylifera* against the B-lactamase producing strain.
iv. Evaluate the antibacterial efficacy of the combination of date fruits and seeds against beta-lactamase-produced isolates of UTI.
v. To determine MIC and MBC values of date fruit and seed extracts against the tested isolates.

1.5 Research Questions

i. What are the phytochemical constituents of *Phoenix dactylifera* fruit and seed?
ii. Thus, bacterial strain produces B-lactamase?
iii. What is the antibacterial potential of*P. dactylifera* fruit and seed extracts against beta-lactamase -producing clinical UTI isolates?
iv. Can combination date fruit and seed extracts enhance their antibacterial effects?
v. What are the MIC and MBC values of date fruit and seed extracts against the tested isolates?

1.6 Significance of the Study

Contribution to the development of novel antimicrobial agents from natural sources and provides an alternative to traditional antibiotics. Shield light on the phytochemical profile of

Phoenix dactylifera and its potential applications in medicine. Inform future in-vitro studies and clinical trials.

1.7 Scope of the Study

The research work focused In vitro evaluation of *Phoenix dactylifera* extracts against beta-lactamase-producing clinical UTI isolates. Phytochemical analysis of *Phoenix dactylifera* fruits and seeds and determination of MIC and MBC values to assess the efficacy of the extracts.

1.8 Limitation of Study

An In-vitro study was carried out on a few beta-lactamase-producing isolates obtained from the University of Ibadan Teaching Hospital. Few Phytochemical analyses provide valuable insights into antibacterial activity, not directly applicable to in-vivo conditions.

1.9 Operational Definition of Terms

i. Beta-lactamase: Enzymes produced by certain bacteria that hydrolyze beta-lactam antibiotics, rendering them ineffective.
ii. Minimum Inhibitory Concentration (MIC): The lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism.
iii. Minimum Bactericidal Concentration (MBC): The lowest concentration of an antimicrobial agent required to kill a particular bacterium.
iv. Phytochemicals: Bioactive compounds found in plants that have various health benefits, including antimicrobial properties.

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Chapter Two

Literature Review

2.1 *In-vitro* Antibacterial Evaluation

Antimicrobial susceptibility testing serves several objectives, including drug development, epidemiology, and predicting therapy outcomes[1]. Following the landmark discoveries of antibiotics like tetracycline, cephalosporins, aminoglycosides, and macrolides in the 1960s, the present period faces a revival of problems due to the rising microbial resistance, putting the efficacy of these critical compounds at risk Its revival is seen in the enormous impact of treatment failures associated with multidrug-resistant bacteria, posing a global hazard to public health[2]. Against this context, the urgent priority is the discovery of new antibiotics, and natural products continue to be a significant source of innovative therapeutic compounds. These products, derived from prokaryotic bacteria, eukaryotic microorganisms, plants, and diverse animal creatures, play a significant role in the production of antimicrobial chemicals. Recent research has increasingly concentrated on studying plant and microbial extracts, essential oils, pure secondary metabolites, and newly synthesized compounds as possible antibacterial agents[3]. However, a hurdle comes when analyzing published research on the antibacterial effects of these natural compounds. The problem lies in comparing data due to the utilization of varied and non-standardized procedures, including inoculum preparation techniques, inoculum size, growth medium, incubation settings, and endpoints determination[4].

2.2 *Phoenix dactylifera L* (Date palm)

Phoenix dactylifera, also known as the Date palm, is a flowering plant species classified under the family Aracea[5], it is specifically developed for its sweet and edible fruits, acting as a cost-effective food source and a vital element of the Arabian diet [6]. The Date fruit has long held major importance as a key crop in the dry regions of the Arabian Peninsula, North Africa, and the Middle East. The cultivation of dates grew with the advent of Islam, reaching countries such as Southern Spain and Pakistan outside the Arabian Peninsula. In many areas where they are farmed, date fruits serve a key role as a primary revenue source and staple food, influencing the economy, culture, and environment[7]. The Date palm fruit has been traditionally exploited for several medicinal purposes, including treating inflammation, fever, paralysis, neurological diseases, and memory disturbances[7]. Additionally, it has been administered as an astringent for intestinal disorders, sore throats, colds, bronchial catarrh, gonorrhea, edema liver, and abdominal issues, and counteracts alcohol intoxication. Date fruits are famous for their significant number of phenolic compounds, contributing to their antioxidant activity; interestingly, different cultivars of dates demonstrate variations in total phenolic content and antioxidant activity[8]. *Phoenix dactylifera Linn*, generally known as the date palm, has earned prominence for its therapeutic benefits specifically, in the context of urinary tract infections caused by beta-lactate-producing bacteria[9]. It becomes necessary to explore the *in-vitro* antibacterial assessment and Phytochemical composition of extracts from the fruit and seeds of *Phoenix dactylifera Linn*. This exploration is vital for a full understanding of the possible therapeutic applications of this plant. Historically tied to early Judaism and Christianity, date palms are today associated extensively with the Arab Muslim world, particularly as a practice during Ramadan fasting[10]. The fruit structure comprises the pericarp, mesocarp, endocarp, and seed, with the mesocarp being the

most considerable component. The maturity process involves numerous phases such as *Hanabauk, Kimri, Khalal* (or Besser), *Rutab,* and *Tamr*, each exploited for specific consumption purposes. Besides direct eating, date fruits play a major part in culinary preparations, while the extensive use of palm trees in landscaping greatly affects the environmental landscape of the Gulf region.

Figure. 1.1 *Phoenix dactyliferaL* (Date Palm Tree)

Source[3].

Figure 2.2:*Phoenix dactylifera* (Date fruit)

Source[3]

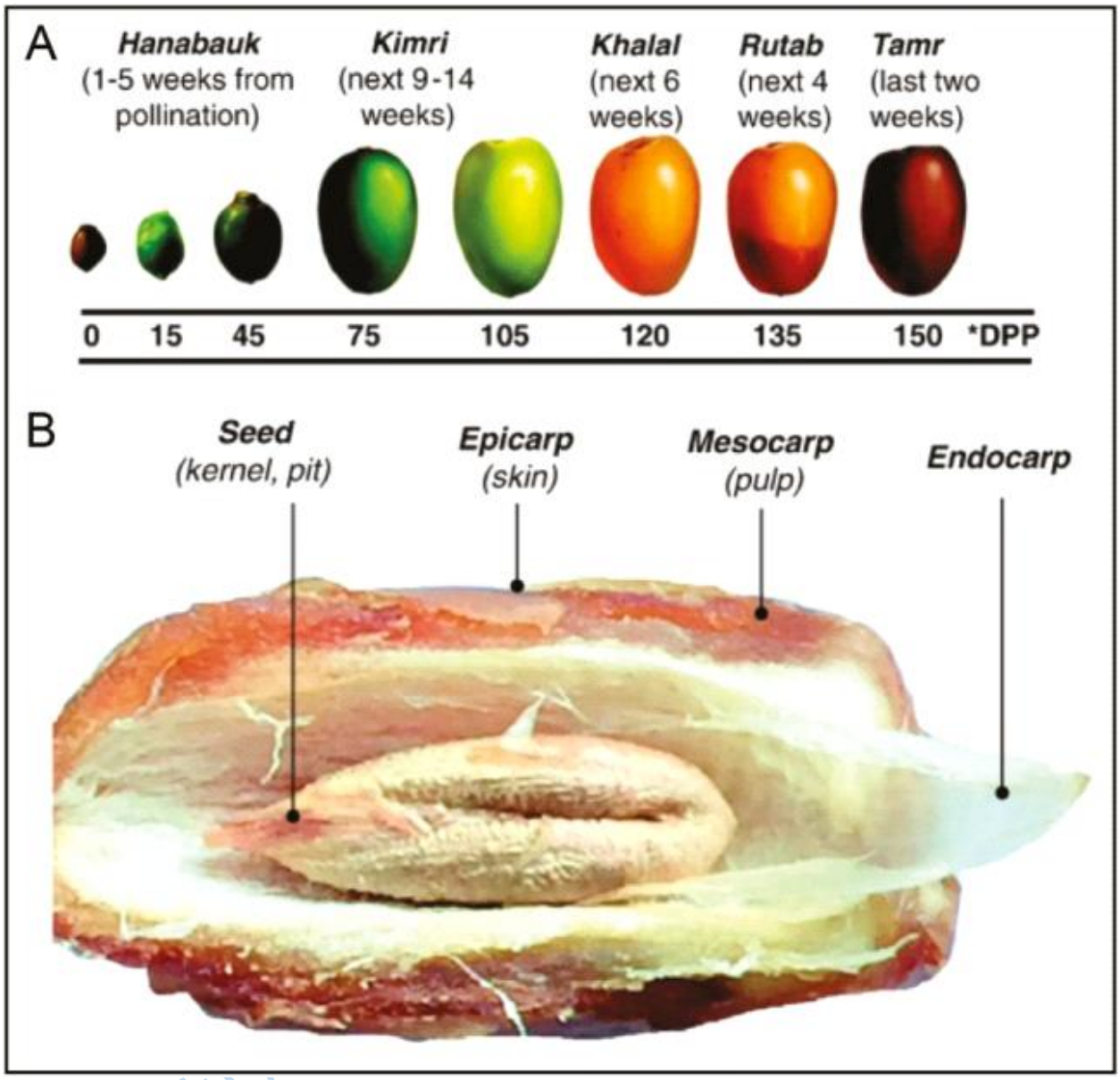

Figure 3.3: Different ripening stages of date palm fruit include the three edible stages: *Khalal, Rutab,* and *Tamr*. The anatomy of the date fruit at the *Tamr* stage displays the epicarp, mesocarp, endocarp, and seed *DPP = days post-pollination.

Source[4]

2.2.1 Date Fruit and Seed Extracts

Date fruits, scientifically known as *Phoenix dactylifera,* have a long history of human consumption due to their nutritional value and therapeutic properties. Recent scientific research has highlighted the bioactive components in date fruit and seed extracts, demonstrating their potential therapeutic applications, particularly in antimicrobial capabilities[11]. Both traditional medicine and modern scientific research recognize the potential health benefits of date fruits and seeds[12]. Various extraction methods, including aqueous extraction, ethanol extraction, and supercritical fluid extraction, are used to obtain compounds from date fruits and seeds[13]. Date fruit extracts, known for their natural sweetness, are rich in antioxidants, vitamins, and minerals, showing promise for improving cardiovascular health, aiding digestion, and serving as a natural energy source. On the other hand, date seed extracts contain a wide range of phytochemicals, such as flavonoids and phenolic compounds, which provide anti-oxidative and anti-inflammatory properties. Continued research is focused on exploring the health benefits of date fruit and seed extracts, highlighting their potential to enhance human well-being and their applications in various sectors, including food, nutraceuticals, and pharmaceuticals.

2.2.2 Composition of Date Fruit and Seed Extracts

Compounds found in extracts derived from date fruits and seeds have garnered substantial interest. These extracts boast a diverse array of bioactive elements, obtained through methods such as aqueous extraction, ethanol extraction, and supercritical fluid extraction[14]. Date fruit extracts, recognized for their inherent sweetness, are rich in antioxidants, vitamins, and minerals, making them noteworthy for potential contributions to cardiovascular health, digestion improvement, and natural energy provision[13]. In contrast, date seed extracts encompass various

phytochemicals, including flavonoid and phenolic compounds, exhibiting anti-inflammatory and antioxidant properties. Ongoing research is illuminating the potential health benefits linked to date fruit and seed extracts, underscoring their significance in promoting human well-being and exploring novel applications across sectors like the food industry, nutraceuticals, and pharmaceuticals[19]. These bioactive constituents have demonstrated the ability to inhibit the growth and proliferation of diverse microorganisms, spanning bacteria, fungi, and certain viruses[14]. Such antimicrobial attributes have positioned dates not only as sought-after ingredients in traditional remedies but also as subjects of contemporary research. Investigations are underway to explore the potential use of date extracts and compounds in food preservation, as natural antimicrobial agents in the food industry, and as potential substitutes for synthetic antimicrobial agents. The exploration of dates' antimicrobial properties underscores their importance in both culinary and medical realms, hinting at the possibility of natural remedies to combat microbial threats.

2.2.3 Mechanisms of Antimicrobial Action of Date Fruit and Seed

Due to the several modes of action that dates exhibit, research into their antibacterial properties has garnered a lot of attention. These varied actions have drawn interest from both traditional and scientific contexts[15]. The antimicrobial effects of dates are largely attributed to their bioactive constituents, such as flavonoids, tannins, and phenolic compounds. These constituents can inhibit the growth and proliferation of a variety of microorganisms, including bacteria, fungi, and certain viruses. Their interaction with microbial cell membranes, disruption of metabolic activities, and suppression of biofilm formation are the sources of their claimed antibacterial properties. Biofilms are complex colonies of bacteria that are known for their strong resistance

to antimicrobial drugs[16]. A thorough comprehension of these processes highlights the significance of dates in the field of food preservation and provides insight into the possible medicinal uses of dates. Dates are a key resource in the fight against a variety of microbial issues because they provide a natural and efficient way to treat microbial risks across a range of domains[17].

2.3 Phytochemical Analysis

Plants naturally create secondary metabolites called phytochemicals, which are essential for controlling plant cell processes in addition to giving plants their unique color, flavor, and aroma[29]. A growing body of research highlights the significant therapeutic potential of phytochemicals, which are frequently linked to negligible adverse effects. As a result, the identification and assessment of phytochemicals are vital phases in the development of new pharmaceuticals derived from plants.

Standard phytochemical tests require the identification and analysis of target phytochemical levels in addition to the extraction of active phytochemicals from plant materials[30]. Drugs, lead compounds, and components from different plant parts are showcased and isolated through the examination of Phytochemical qualities in medicinal plants. Plants' distinctive biological activity can be more easily identified thanks to their Phytochemical characteristics[31].

Fruits, stem barks, roots, and leaves are the main plant materials used to analyze Phytochemical characteristics. This thorough analysis looks at the Phytochemical components of medicinal plants using a variety of extraction techniques, including ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous extractions, to isolate distinct Phytochemical

2.3.1 Bioactive Constituent of Date Seeds

a. Phenolics Compounds: Date seeds are a good source of phenolic compounds, which have antioxidant qualities. These chemicals include Flavonoids and phenolic acids. By assisting in the neutralization of dangerous free radicals, these substances can lessen oxidative stress and the chance of developing several chronic diseases[17].

b. Fatty Acids: Fatty acids found in date seeds, such as oleic and linoleic acid, are recognized for their potential to improve heart health and reduce inflammation[18].

c. Amino Acids: The building blocks of proteins, amino acids are found in diverse forms in date seeds. These amino acids are necessary for many physiological functions, including immune system function, muscle growth and repair, and the synthesis of hormones and enzymes[19].

d. Dietary Fiber: Date seeds, like date fruit, have dietary fiber, which promotes digestive health and helps control bowel motions[20]. Potassium, magnesium, and calcium are among the minerals found in date seeds. These minerals are necessary for many body processes, including nerve transmission, bone health, and muscular function[21].

e. Saponins: Saponins are found in date seeds and are recognized to have antioxidant and anti-inflammatory qualities[22].

2.3.2 Bioactive Constituent Fruit's

a. Carotenoids: Including lutein, zeaxanthin, and β-carotene, which are potent antioxidants recognized for their function in preserving eye health and shielding cells from harm, carotenoids are found in dates[23].

b. Phenolic Compounds: Flavonoids, procyanidins, and derivatives of phenolic acid are just a few of the many phenolic compounds that are abundant in dates. Strong antioxidant characteristics allow the fruit to scavenge free radicals, lowering oxidative stress within the body[22].

c. Flavonoids: Flavonoids are a broad class of phytonutrients present in dates that are well-known for their antioxidant and anti-inflammatory qualities. They might assist in lowering the risk of a few chronic illnesses, such as cancer and heart disease[24].

d. Tannins: Studies have linked the tannins found in dates to several health advantages, including anti-inflammatory, antibacterial, and antioxidant properties[25].

e. Fiber in the Diet: Dietary fiber, both soluble and insoluble, is abundant in dates. In addition to helping with blood sugar regulation and gastrointestinal health maintenance, fiber also increases feelings of fullness, all of which can help with weight management[25].

These bioactive components support the many health advantages of date eating, such as better blood sugar regulation, decreased risk of chronic illnesses, improved digestion, and improved heart health. Dates are heavy in natural sugars and calories, so it's important to eat them in moderation[26].

2.4 Value-Added Products from Date Fruits and Seeds

Date fruits and seeds can now be used to create value-added goods, which is a growing area of interest and innovation[27]. Date fruits, which are harvested from the date palm tree, are prized for their inherent sweetness and abundance of nutrients. In addition, date seeds which are sometimes disregarded have special qualities. A variety of value-added products have been developed as a result of efforts to improve these components. Date fruit products have become popular as tempting substitutes for manufactured sweets. These products include date syrups, date pastes, and date-based sweeteners. The potential uses of date seeds as a source of bioactive chemicals, animal feed, and dietary fiber have also drawn interest. The dating industry has been proactive in exploring value-added product creation, motivated not only by significant profitability but also by the role it plays in mitigating the risk of date spoilage. Research and technology advancements continue to expand the horizons for creating innovative and nutritious products from both dates' fruits and seeds, showcasing the remarkable versatility of this ancient and revered fruit. The generation of bioethanol from abandoned date palm fruit waste via yeast (*Saccharomyces cerevisiae*) mediated fermentation has shown recent success[28]. Additionally, studies have demonstrated the possible use of date waste which uses bacteria like *S. cerevisiae* as a substrate for the manufacture of citric acid. *Aspergillus niger* and S. *Cerevisiae*[29].

2.5 Urinary Tract Infection (UTI)

A urinary tract infection (UTI) is a disorder characterized by the presence of an infection in a portion of the urinary tract[29]. It is causing cystitis, or a bladder infection when it affects the lower urinary tract, and pyelonephritis, or a kidney infection when it affects the upper urinary tract[30]. Lower urinary tract infection symptoms include painful urination, frequent urination, and a

persistent urge to urinate even when the bladder is empty[32]. Fever and flank pain are among the symptoms of a kidney infection, usually in addition to those of a lower UTI. Urine may appear bloody on rare occasions. Older people and infants may have nonspecific or ambiguous symptoms. Most infections are primarily caused by *Escherichia coli*, while other bacteria or fungi may rarely be to blame. Risk factors include sexual behavior, female anatomy, diabetes, obesity, and a history of urinary tract infections in the family[41]. Sexual activity is a risk factor, however, STIs (sexually transmitted infections) do not include urinary tract infections (UTIs). Although they can potentially come from a bloodstream infection, kidney infections typically follow bladder infections[41]. In young, healthy women, diagnosis may depend only on symptoms. It might be difficult to diagnose patients with unclear symptoms since bacteria can exist without producing an infection[38]. A urine culture can be an important diagnostic tool in complex cases or when treatment doesn't work. UTIs are one of the most frequent bacterial diseases globally, impacting millions of people each year. The development of antibiotic resistance among uropathogenic bacteria has become a serious worry, even though UTIs are typically not fatal. The ability of bacteria to resist the effects of antibiotics is known as antibiotic resistance. This resistance increases the difficulty of treating infections and lowers healthcare costs and morbidity. Geographical variations in antibiotic prescribing patterns, patient demographics, and the kinds of bacteria-producing infections all have an impact on the prevalence of antibiotic resistance in UTIs[39]. The majority of UTIs are caused by the uropathogenic bacteria *Escherichia coli* (*E. coli*), which has demonstrated increasing resistance to popular antibiotics such as beta-lactams, fluoroquinolones, and trimethoprim/sulfamethoxazole[40]. When it comes to UTIs, antibiotic resistance has several negative effects. First of all, it results in resistance to treatment, protracted sickness, and a higher chance of side effects such as bloodstream infections and pyelonephritis.

Secondly, there is a detrimental cycle created when broad-spectrum antibiotics are used as a last resort, which adds to the general increase in antibiotic resistance. There is an increasing interest in investigating complementary and alternative remedies for urinary tract infections (UTIs) due to the difficulties caused by antibiotic resistance. Plant extracts and phytochemicals are examples of natural materials that have drawn interest due to their possible antibacterial qualities. These substances offer a potentially effective means of addressing uropathogenic bacteria while avoiding problems related to antibiotic resistance[40].

2.5.1 Prevalence of UTIs

Urinary tract infections (UTIs) are a well-researched global health issue, as shown by the numerous epidemiological studies that have demonstrated UTIs' broad prevalence among a range of age groups and demographic backgrounds. Particularly women have a greater vulnerability to UTIs[41]. Two clear categories stand out among the range of UTIs. Community-acquired UTIs, which are more common in women, can be caused by several circumstances, including menopause, pregnancy, and sexual activity. This group is particularly susceptible to these infections. However, a sizable percentage of UTI cases are hospital-acquired, particularly in patients undergoing surgery and those with indwelling urinary catheters. Moreover, persistent UTIs pose difficulties for some people, leading to several episodes annually. Due to its complex prevalence, UTIs must be understood and treated as a serious public health concern, requiring all-encompassing approaches to therapy, prevention, and research[42].

2.5.2 Activity against Bacterial Resistance

Globally, the prevalence of drug resistance in microbiological infections is increasing. Traditional therapy approaches largely rely on antibiotics, which are pricey and often lead to severe consequences. On the other hand, natural products and their ingredients are becoming more and more popular as low-cost and safe substitutes for infection control[11]. The constituents of *Phoenix dactylifera* exhibit noteworthy promise in the prevention and treatment of bacterial infections[12]. Researchers used a variety of plant parts, such as leaves, seeds, fruits, and bark, to examine *Phoenix dactylifera's* antibacterial qualities. Using the disc diffusion method with kanamycin as the reference medication, they tested three extracts: aqueous, methanol, and acetone, against standard gram-positive (*S. aureus, S. pyogenes*) and gram-negative (*E. coli, P. aeruginosa*) microorganisms. Their research revealed strong antibacterial activity in all plant sections and extracts, with acetone and methanol extracts outperforming aqueous extracts in terms of efficacy. Fruit and leaf extracts outperformed seed and bark extracts in terms of antibacterial activity among the investigated components. The most active extracts against *Staphylococcus* were the methanol leaf extract and the acetone fruit extract against *P. aeruginosa* and *E. coli,* respectively[13]. The investigation additionally furnished an elaborate phytochemical profile of *Phoenix dactylifera*, accentuating the fact that leaf and fruit extracts exhibit a higher potency for antibacterial activity in comparison to seed and bark extracts. Methanol is a better solvent for antimicrobial research because it can extract a wider spectrum of chemicals than water, which is why acetone and methanol extracts have greater potency than water[14]. Except for ethyl-acetate, the fruit's richly constituent-rich portion demonstrated the strongest antibacterial action[16]. The alkaloids, flavonoids, and tannins in *Phoenix dactylifera* are probably what gives it its antibacterial qualities. Additionally, recent research indicates that *P. dactylifera* may be used

as an antimicrobial agent to lessen the side effects of medications like methylprednisolone, and its extract may be useful in the treatment of enteric diseases[15]. *Phoenix dactylifera* and its extracts, particularly those derived from methanol and acetone, exhibit significant antibacterial activity, making them promising candidates for the development of new antimicrobial agents. Additionally, preliminary evidence suggests that the pits of *Phoenix dactylifera* may also have activity against gram-negative bacteria[17].

2.5.3 Antibiotic Resistance's Impact on UTI Therapy

A recent study has brought attention to the urgent problem of antibiotic resistance in the treatment of urinary tract infections (UTIs). UTIs are common in all demographic groups, however they affect women more than men. While antibiotics traditionally give an effective UTI treatment, the rising resistance poses complications[43]. This resistance is largely caused by factors like the overuse and abuse of antibiotics in agriculture and healthcare, which has serious economic ramifications. Preliminary estimates point to likely future declines in GDP and a rise in healthcare costs. Furthermore, there are worries about the scarcity of new antibiotics, which could lead to a post-antibiotic age with few therapeutic choices. To tackle this complex issue, we need to use antibiotics responsibly, come up with new treatments, and work together globally to fight UTIs and related antibiotic resistance. Antibiotic resistance is mostly attributed to the important enzymes called beta-lactamases, which are produced by specific bacteria. These enzymes, which are members of the hydrolase family, work principally by hydrolyzing the beta-lactam ring, a structural element that is shared by important antibiotic families like cephalosporins, carbapenems, and penicillins[44]. Antibiotic resistance is greatly increased by the cleavage of this ring, which makes antibiotics ineffective.

2.5.4 Clinical Isolates of Beta-lactam Producing Bacteria (Urinary Tract)

The main defense mechanisms of Gram-negative bacteria against β-lactam antibiotics, known as Beta-lactamases, are highly evolved enzymes with a multi-million-year evolutionary history[45]. These well-studied enzymes, which at this point consist of about 2,800 different proteins, originally protected a bacterium that was generated from naturally occurring β-lactams[43]. Penicillin-binding proteins having sequence similarity to β-lactamases with an active-site serine were probably their ancestors. There are also metallo-β-lactamases, which have one or two catalytically active zinc ions. Although penicillinases in Gram-positive bacteria were discovered soon after penicillin was first used in medicine, transmissible β-lactamases that could break down freshly licensed cephalosporins, carbapenems, and monobactams eventually became important in Gram-negative pathogens. There are two main schemes on which nomenclature is based. At first, functional divisions based on substrate and inhibitor profiles were utilized. A later system divides β-lactamases into class A, B, C, and D enzymes according to amino acid sequences. Beta-lactamases are a broad group of enzymes that bacteria manufacture that function to open the beta-lactam ring and so render beta-lactam antibiotics inactive[45]. Remarkably, some beta-lactamases are encoded on chromosomes, while others are encoded on mobile genetic elements like plasmids. This enzymatic activity, especially in gram-negative bacterial pathogens, is a therapeutically relevant mechanism of resistance. A thorough knowledge of the common forms of beta-lactamases generated by different pathogens is essential for determining susceptibility, selecting appropriate treatments, and putting infection control procedures into action[46].

Pharmaceutical substances known as "beta-lactamase inhibitors" are made to prevent certain beta-lactamases from acting. As a result, they are sometimes used with antibiotics that are beta-lactam. These inhibitors include tazobactam, sulbactam, and clavulanate. Penicillinases are efficiently blocked by these medications, but not AmpC or carbapenemases[47]. While they have modest inhibitory action against extended-spectrum beta-lactamases (ESBLs) in vitro, their clinical reliability against ESBL producers is limited. Additionally, sulbactam exhibits antibacterial action against particular bacterial species, such as *A. gonorrhoeae, Bacteroides fragilis,* and *Neisseria gonorrhoeae A.baumanii*, a bacterium with a notable history of resistance to antibiotics[48]. Another beta-lactamase inhibitor is avibactam, which inhibits beta-lactamases of class A (ESBLs, most KPCs), class C (AmpC), and some class D (OXA) but not class B (MBLs). To classify beta-lactamases according to molecular homology, the Ambler classification scheme is frequently utilized. The active sites of enzymes in classes A, C, and D include serine residues, but the active sites of enzymes in class B have zinc, which indicates metallo-beta-lactamases (MBLs). Extended-spectrum beta-lactamases (ESBLs) and KPCs (*Klebsiella pneumonia* Carbapenemase) are included in class A; MBLs (NDM, IMP, and VIM) are included in class B; AmpC is included in class C; and oxacillinases (OXAs) are included in class D. The multiplicity of bacterial resistance mechanisms is highlighted by the thousands of mutations found in beta-lactamases[48].

2.5.5 Class A Ambler Classification

A class of plasmid-encoded enzymes known as extended-spectrum beta-lactamases (ESBLs) is mostly present in *Escherichia coli, Klebsiella species,* and other *Enterobacterales*[45]. Enzymes such as TEM, SHV, CTX-M, and GES are prominent instances of ESBLs. These enzymes are

capable of hydrolyzing monobactams, most cephalosporins (except cephamycins, which are not hydrolyzed by most ESBLs), and extended-spectrum penicillins, such as piperacillin. *Klebsiella pneumoniae* Carbapenemase (KPCs) are similar to ESBLs, but they also can hydrolyze carbapenems. These enzymes were first discovered in *Klebsiella pneumoniae,* but they have subsequently been found in additional Enterobacterales species. Antibiotic resistance is a serious concern that calls for a sophisticated approach to antibiotic therapy and infection control techniques due to the advent and dissemination of ESBLs and KPCs[43].

2.5.6 Class B of Amblers Classification

Metallo-beta-lactamases (MBLs), represented by VIM, IMP, and NDM, employ a zinc ion in their catalytic mechanism to hydrolyze all beta-lactams, including carbapenems[46]. Notably, monobactams continue to be resistant to MBL activity, such as aztreonam. Chromosome encoding has been reported in MBLs in some organisms, such as *Stenotrophomonas maltophilia* L1 MBL. As an alternative, they can be acquired; this is a behavior that is observed in a variety of gram-negative bacteria, including species of *Acinetobacter, Pseudomonas,* and *Klebsiella*. It is important to remember that beta-lactamase inhibitors that are currently on the market cannot block MBLs, which presents a problem for researchers trying to create effective treatment plans for diseases brought on by MBL-producing bacteria [45].

2.5.7 Class C Ambler Classification

The AmpC enzymes belong to the beta-lactamase class and are capable of hydrolyzing the majority of cephalosporins (excluding cefepime), cephamycins (like cefoxitin and cefotetan), monobactams (like aztreonam), and penicillins. AmpC beta-lactamase can be encoded on a

chromosome or plasmid. Certain antibacterials can promote the production of chromosomal AmpC, or it can be constitutively produced, especially in de-repressed mutants. Bacteria including *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* that normally do not have this beta-lactamase can also express constitutively the plasmid-encoded AmpC. Treatment options may be complicated by inducible AmpC expression since isolates, particularly in Enterobacterales, may first test responsive to third-generation cephalosporins. Among these is *K. pneumonia. Enterobacter cloacae, P. aerogenes,* and *Citrobacter freundii* are frequently linked to the presence of inducible ampicillin, which suggests using third-generation cephalosporins with caution. Although overproduction of AmpC can also occur in Proteus vulgaris, Morganella morganii, Serratia marcescens, and Providencia species, clinically relevant expression is less frequent[46].

2.5.8 Class D of Ambler Classification

OXA beta-lactamases generally target narrow-spectrum penicillin, with select forms, notably plasmid-encoded OXA, demonstrating the potential to hydrolyze carbapenems while keeping the action of numerous cephalosporins[48]. Because beta-lactamase-producing bacteria (BLPB) can produce the enzyme β-lactamase, they can have both an indirect pathogenic effect and a direct pathogenic impact in polymicrobial infections. Drugs like sulbactam and vaborbactam are designed to block beta-lactamases of classes A and C, but they are ineffective against classes D and B. Since many urinary tract clinical isolates are beta-lactamase-producing germs that become resistant to conventional antibiotics, these isolates provide a significant issue. The difficulties presented by beta-lactamase-producing bacteria in urinary tract infections have been

highlighted by research investigations, emphasizing the need to investigate alternate treatment options, such as the use of plant extracts[48].

2.5.9 Antibiotic Resistance Factors

The worldwide health issue of antibiotic resistance is intricate and widespread, with numerous underlying causes[49]. The overuse and abuse of antibiotics in a variety of contexts is a major factor. In clinical practice, healthcare practitioners may administer antibiotics unnecessarily, and patients could request them for viral diseases, where drugs are ineffective, leading to significant contributions to antibiotic resistance. The usage of antibiotics in the agriculture industry for things like promoting cattle development and preventing sickness has led to the emergence of antibiotic-resistant bacteria that can affect the food chain. The situation is made worse by the worldwide travel and trade that spreads resistant strains throughout the world, highlighting how interrelated antibiotic resistance is on a global scale[50]. Because they enable bacteria to trade and acquire resistance genes, horizontal gene transfer pathways are essential to the emergence and dissemination of resistance in bacteria. The spread of resistant bacteria is aided by insufficient infection control measures in healthcare environments, such as inadequate hand hygiene and sanitation standards. To battle this pressing public health concern, addressing the various causes of antibiotic resistance requires a holistic approach that includes increased infection control techniques, responsible antibiotic use, and international cooperation.

2.5.10 Antibiotic Resistance and Mechanisms of Action

It is essential to appreciate the mechanisms of action of β-lactamases to understand antibiotic resistance. The bacterial resistance to β-lactam antibiotics, a commonly used family of medicines

that includes cephalosporins and penicillin, is mostly dependent on these enzymes[51]. Hydrolyzing the β-lactam ring present in these antibiotics to make them ineffective is the primary function of β-lactamases. The capacity of the antibiotics to attach to their target proteins, which is necessary for the formation of bacterial cell walls, is disrupted by this hydrolysis. Thus, even when an antibiotic is present, bacteria can still proliferate and develop. Bacteria of both Gram-positive and Gram-negative species produce β-lactamases, which seriously compromise the efficacy of β-lactam antibiotics[51]. The complexity of antibiotic resistance and the need for new approaches to tackle it are highlighted by the diversity, fast evolution, and extensive distribution of β-lactamase enzymes in bacterial populations.

2.6 *Escherichia coli*

The bacterium *Escherichia coli*, also known as *E. coli,* is facultative anaerobic, rod-shaped, and gram-negative. The first description of this microbe dates back to 1885[52]. Like other natural flora, the majority of *E. coli* strains inadvertently colonize the gastrointestinal tracts of humans and animals. However certain strains of *E. coli* have developed into dangerous strains by gaining virulence factors from bacteriophages, transposons, plasmids, and/or pathogenicity islands. Based on serogroups, pathogenicity mechanisms, clinical signs, or virulence characteristics, this pathogenic *E. coli* can be divided into different groups[53]. Enterohemorrhagic *E. Coli* (EHEC) is a kind of pathogenic *Escherichia coli* that produces Shiga toxins (Stxs) and is responsible for hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which can be fatal. Human infections have been linked to several EHEC serotypes, including O26:H11, O91:H21, O111:H8, O157: NM, and O157:H7 55. The most commonly isolated serotype of EHEC from sick individuals in the UK, Japan, and the US is *E. coli* O157:H7.

The rod-shaped, facultatively anaerobic, gram-negative coliform bacteria. *Escherichia coli* belongs to the genus *Escherichia* and is frequently found in the lower intestine of warm-blooded creatures[53]. *E. coli*, is classified as Gammaproteobacterial, Enterobacteriaceae family, Genus is *Escherichia* and order is Enterobacterales and species is *coli*, Phylum: *Pseudomonadota.*

Escherichia coli typically live harmlessly in the human digestive system, but when it invades the urinary tract, it can cause serious infections. Women's anus and urethra are rather close together, which makes "wiping front to back" after using the restroom helpful in avoiding urinary tract infections. The need for prompt diagnosis and treatment is highlighted by the possibility that an untreated UTI could progress to the kidneys and cause a more serious illness.Women who are sexually active, pregnant, or old may all face an enhanced risk of having UTIs [54]. Of these pathogenic strains, *Enterohemorrhagic E. coli* (EHEC) is of special concern since it is frequently connected to outbreaks linked to contaminated water or food. Shiga toxins, which are produced by EHEC, have the potential to cause hemolytic-uremic syndrome (HUS), a disorder marked by renal failure and possibly lethal outcomes[55]. The potential for *E. coli* to develop antibiotic resistance is facilitated by its genetic plasticity and adaptability, which presents a serious risk to public health. Treatment options for infections have been limited due to the advent of multidrug-resistant *E. coli* strains, which emphasizes the significance of careful antibiotic use and surveillance.

2.6.1 Mechanisms of Resistance

The mechanisms of resistance exhibited by *Escherichia coli* (*E. coli)* are complex and multifaceted, representing a significant challenge in combating infections caused by this bacterium [56]. *E. coli,* a versatile gram-negative bacterium commonly found in the human gut, can

develop resistance to antibiotics through various mechanisms. One primary mechanism involves the production of enzymes, such as beta-lactamases, which can deactivate antibiotics like penicillin and cephalosporins by breaking down their molecular structures[57]. This enzymatic action renders these antibiotics ineffective in combating *E. coli* infections. Additionally, *E. coli* can develop efflux pumps that actively expel antibiotics from within the bacterial cell, reducing the concentration of the drug and its effectiveness. This efflux mechanism is a protective strategy employed by the bacterium to maintain its survival. Furthermore, genetic mutations within *E. coli* can alter the structure of target proteins, which antibiotics would typically bind to, making these drugs less effective in inhibiting bacterial growth. Another critical mechanism of resistance in *E. coli* is the acquisition of resistance genes through horizontal gene transfer, in this process, *E. coli* can acquire genetic material from other bacteria, which may carry resistance genes, enabling them to resist antibiotics they were previously sensitive to. This ability to share and acquire resistance genes makes *E. coli* a formidable contributor to the spread of antibiotic resistance.

2.6.2 Epidemiology of Resistance

The prevalence of antimicrobial resistance in Gram-negative bacteria, particularly *Escherichia coli,* is a growing concern in Europe[58]. *E. coli* constitutes the majority of invasive Gram-negative isolates across European countries, and the emergence of multidrug-resistant strains is complicating the treatment of severe infections. Enterobacteriaceae, including *E. coli,* are a leading cause of infections in both hospital and community settings. Multi-drug-resistant *E. coli* strains are also frequently found in animals and food products. The use of antibiotics in animals has contributed to the emergence and spread of antibiotic-resistant strains, including *E. coli* [59]. This poses a risk to humans through direct contact with animals or by consuming contaminated

food. *Escherichia coli* is known for its adaptability in various environments, and it can transfer resistance genes to potential pathogens across different habitats. Hospital effluents have been identified as a potential source of multidrug-resistant *E. coli,* contributing to the dissemination of antibiotic resistance genes in municipal sewage systems and the environment. Antimicrobial-resistant *E. coli* strains are distributed across Europe, and there are geographical variations in the percentages of resistance to specific antibiotics in different countries, these variations likely reflect differences in infection control practices and antibiotic usage. Southern regions tend to exhibit a higher prevalence of resistance.

In addition to the countries covered in the last annual European Centre for Disease Prevention and Control (ECDC) report, other European nations are also experiencing an increase in antimicrobial resistance in *E. coli* [60]. Eastern European countries have reported a significant percentage of *E. coli* isolates positive for extended-spectrum beta-lactamases (ESBLs). Turkey, in particular, has reported a high percentage of ESBL-positive strains with resistance to fluoroquinolones. Switzerland, Croatia, and Bosnia and Herzegovina have reported *E. coli* strains resistant to various antimicrobials, with fluoroquinolones resistance being prevalent. Multidrug-resistant strains have also been identified in several countries, with varying levels of surveillance data availability. Russia has reported ESBL-positive *E. coli* isolates with resistance to quinolones and aminoglycosides, particularly high resistance rates to ciprofloxacin and nalidixic acid [61].

2.6.3 Alternative Therapies

The global rise in multidrug-resistant bacteria has led to a search for innovative therapeutic strategies against infectious diseases. Several novel therapies have been developed to address

this challenge, including phage therapy, antimicrobial peptide therapy, and combination antibiotic therapies[62]. Phage therapy, which has been recognized since the 1900s, is experiencing resurgence due to the diminishing effectiveness of antibiotics against multidrug-resistant bacteria[63]. Bacteriophages (phages) are highly specific viruses that infect and kill bacteria. They can be modified to target emerging multidrug-resistant bacterial strains, Phages show promise in treating urinary tract infections (UTIs) caused by biofilm-forming uropathogenic *Escherichiacoli* (UPEC) strains[64]. Biofilm production significantly increases antibiotic resistance, but phages have been found to penetrate the extracellular matrix, degrade the biofilm, and kill bacteria. Phage therapy has also shown potential for treating sepsis and meningitis caused by multidrug-resistant neonatal meningitis-associated *E. coli* (NMEC) strains[63].

Efflux pump inhibitors represent an alternative therapeutic strategy against multidrug-resistant bacteria[66]. Efflux pumps are known antibiotic resistance mechanisms used by bacteria to actively export molecules from the cell, Efflux pump inhibitors, when used in combination with antibiotics, have demonstrated significant therapeutic potential, allowing the use of antibiotics compromised by efflux pump activity. Although not yet widely used in clinical practice, this approach holds promise for overcoming antibiotic resistance.

2.6.4 Medicinal Plants and *E. coli*

The use of medicinal plants in the context of *Escherichia coli* infections has been an area of interest in traditional and alternative medicine[67]. Medicinal plants, known for their rich content of bioactive compounds, have been explored for their potential antibacterial properties against *E. coli* and other pathogens. These plant-derived compounds can exhibit various mechanisms of action, such as disrupting bacterial cell membranes, interfering with essential enzyme systems, or

inhibiting the growth of *E. coli*. In traditional medicine, medicinal plants have been used to treat a variety of ailments, including gastrointestinal infections often caused by pathogenic *E. coli* strains. The utilization of medicinal plants in the context of *E. coli* infections reflects the ongoing exploration of natural remedies and traditional knowledge for addressing bacterial infections. This approach aligns with the broader interest in alternative healthcare systems that draw on the therapeutic properties of plants and their bioactive constituents[64].

2.6.5 Diagnosis of *E. coli*

The diagnosis of *Escherichia coli* infections typically involves a comprehensive approach that integrates clinical evaluation and various laboratory tests[68]. Healthcare providers begin with a thorough medical assessment, including a detailed medical history and a physical examination, where symptoms indicative of an *E. coli* infection, such as diarrhea or urinary tract discomfort, are carefully considered. In terms of laboratory tests, for gastrointestinal infections, a key diagnostic tool is stool cultures. This involves collecting a stool sample, which is then cultured in a laboratory to identify the presence of *E. coli* bacteria. Additionally, blood tests may be conducted to assess signs of infection and potential complications. For urinary tract infections (UTIs) caused by *E. coli,* the diagnostic process includes a urine sample analysis through urinalysis to check for signs of infection. Urine culture is another essential test, helping identify the presence of *E. coli* and determining its susceptibility to antibiotics[70].In cases where severe infections or specific *E. coli* strains like Shiga toxin-producing *E. coli* (STEC) are suspected, advanced tests may be employed. Polymerase Chain Reaction (PCR) is a molecular biology technique used to detect the presence of specific toxins or genes associated with certain *E. coli* strains, Enzyme Immunoassays are also utilized to identify specific proteins or antigens related

to *E. coli* strains. The combination of clinical evaluation and laboratory testing is instrumental in confirming *E. coli* infections, assessing their severity, and guiding appropriate treatment strategies.

2.7 *Acinetobacter baumannii*

The Dutch researcher used minimum media fortified with calcium acetate to isolate the organism from the soil for the first time in 1911[71]. About 43 years after it was first discovered as Micrococcus calco-aceticus, Brisou and Prevot proposed the moniker *Acinetobacter* (from the Greek "akinetos," meaning non-motile) to differentiate it from motile species in the *Achromobacter genus*[71]. After a thorough investigation by researchers that included Micrococcus calco-aceticus, Alcaligenes hemolysans, Mima polymorpha, Moraxella lwoffi, Herelleavaginicola, and Bacterium anitratum, the genus *Acinetobacter* was accepted in 1968[72]. Based on morphological traits, this study determined that these organisms belonged to a single genus and could not be further divided into several species. Based on a publication, the genus Acinetobacter was formally recognized by the Subcommittee on the Taxonomy of Moraxella and Allied Bacteria in 1971[73]. The Gram-negative bacterium *Acinetobacter baumannii* is usually small, nearly spherical, and rod-shaped. It bears the bacteriologist's name. As a hospital acquires infection, it is growing more significant and can be an opportunistic pathogen in humans, harming those with weakened immune systems[74]. *Acinetobacter baumannii* is bacteria frequently located in several environmental sources, including soil and numerous food items such as vegetables, meat, and fish. *A. baumannii* may sometimes colonize the skin of healthy individuals; however, this normally occurs at low levels and for brief periods. In healthy individuals, colonization in alternative bodily locations, such as the throat, nasal passages, or intestines, is

infrequent. In recent decades, *A. baumannii*has become a prominent and prevalent pathogen associated with hospital-acquired illnesses. It is predominantly linked to illnesses such as nosocomial *pneumonia* and bacteremia in patients within intensive care units (ICUs) globally. Furthermore, *A. baumannii* can induce skin and soft tissue infections, in addition to urinary tract infections. The rise of multidrug-resistant (MDR) *A. baumannii* strains has exacerbated the issue of nosocomial infections. Hospital surveillance data reveals a significant rise in *A. baumannii* infections over the last five years, predominantly isolating MDR strains from patients across multiple healthcare settings[76].

The *Acinetobacter genus* is varied, encompassing oxidase-positive and -negative, non-pigmented, Gram-negative coccobacilli. While there are over50species within the genus, most are non-harmful environmental organisms. *A. baumannii* is the most prevalent species involved with infections. Other species including *A. calco-aceticus, A. lwoffi, A. haemolyticus, A. johnsonii, A. junii, A. nosocomialis, A. pittii, A. schindleri,* and *A. ursingii* have been described as pathogens in specific situations. Some species, including *A. calco-aceticus, A. lwoffi, A. nosocomialis,* and *A. pittii,* have been identified on vegetables, meat, dairy products, and human skin, typically containing wide antibiotic resistance repertoires. *A. baumannii* strains with antibiotic resistance have been identified in commercial food, suggesting numerous pathways of transmission to human populations[77]. However, healthy persons rarely contain more pathogenic species and strains of *A. baumannii*, and *A. baumannii* is seldom recognized as a colonizer of healthy human skin.

2.7.1 Epidemiology of *Acinetobacter baumannii*

The epidemiology of *Acinetobacter baumannii* is a rising concern in hospital settings globally[77]. This multidrug-resistant bacterium is capable of causing different infections, including pneumonia, bloodstream infections, and wound infections, especially in critically ill individuals. Its epidemiology pattern is defined by its persistence in healthcare contexts, leading to nosocomial or healthcare-associated infections. *A. baumannii* is noted for its persistence on surfaces and medical equipment, making it tough to control. Historically associated with humid climes, *Acinetobacter* infections have become increasingly frequent in temperate countries since the 1970s. Its capacity to persist in many conditions and rapidly develop resistance to important types of antibiotics helps its emergence[78]. Nosocomial Acinetobacter infections show a seasonal fluctuation, with greater incidence found during the summer months. Factors such as increased humidity in the ambient air, producing favorable circumstances for *Acinetobacter* growth, may contribute to this seasonal change. Environmental variables, especially condensate from air-conditioning systems, have been highlighted as probable sources of pandemic Acinetobacter infections. *Acinetobacter* is known for its relationship with healthcare-associated illnesses, notably in critical care units (ICUs). However, it has also been related to community-acquired diseases in regions like Asia and Australia. Additionally, *Acinetobacter* infections have been documented in the context of conflicts and natural disasters, indicating its versatility and capacity to thrive in varied settings[79].

2.7.2 Diagnosis of *Acinetobacter*

The diagnosis of an *Acinetobacter* infection is typically confirmed when *Acinetobacter* is cultured from a patient's specimen (such as sputum, blood, or cerebrospinal fluid) along with other clinical findings that indicate an infection at that particular site[80]. It is crucial to note that

Acinetobacter colonization is a common occurrence, and the decision to initiate treatment should be based on distinguishing between colonization and a genuine infection. This distinction is essential due to the challenges associated with treating *Acinetobacter* infections, which can involve significant potential for toxicity [81]. Although *Acinetobacter* is relatively easy to isolate through standard cultures, identifying it can sometimes be delayed, as it does not strongly react in many of the common biochemical tests used to differentiate among gram-negative bacteria[82]. When subjected to Gram staining, *Acinetobacter species* typically appear as short, broad gram-negative rods during their rapid growth phase but adopt a more coccobacillary shape in the stationary phase. Recognizing the difference between colonization and a true infection can be challenging, especially given that many infections occur in individuals who are already colonized with the bacterium[84].

2.7.3 Transmission

Acinetobacter species are often transmitted to patients in healthcare settings through their persistence on environmental surfaces and temporary colonization of the hands of healthcare workers[75]. However, nosocomial transmission via aerosolized bacteria from infected or colonized patients has also been documented. For instance, there was a notable case where a healthcare worker developed severe pneumonia after inhaling aerosolized *A. baumannii* during endotracheal suctioning of a ventilated patient. Another study discovered that nearly 25% of air samples collected from patient rooms were contaminated with carbapenem-resistant A. baumannii (CRAB). Interestingly, these rooms had previously housed patients infected with CRAB, but the air ducts were not colonized, indicating that the patients themselves were the source of the airborne bacteria. However, another study found evidence of air contamination by

A. baumannii in only one out of twelve patient rooms evaluated, suggesting that the frequency of air contamination by *A. baumannii*varies.

2.7.4 *Acinetobacter* Virulence Factors

Acinetobacter has several virulence characteristics that have a crucial role in its capacity to generate infections in people. These virulence features encompass the capability to connect to host tissues, produce biofilms, and manufacture toxins[78]. The adhesion to host tissues permits *Acinetobacter* to establish a presence within the body, while biofilm formation bolsters its durability on surfaces and its resistance to the host's immunological defenses [79]. Furthermore, the generation of toxins can directly injure host cells and affect the functioning of the immune system[82]. A full understanding of these virulence factors is vital for the development of methods targeted at treating Acinetobacter infections. Numerous investigations have consistently demonstrated that *A. baumannii*contains a higher intrinsic capacity for virulence in humans compared to other Acinetobacter species, such as *A. calco-aceticus, A. lwoffi, A. junii, A. baylyi,* and *A. haemolyticus*. For instance, in one study, *A. baumannii*exhibited superior growth at the human body temperature of 37°C and demonstrated increased resistance to macrophage absorption when compared to these other species. The wax moth larva of Galleria was also revealed to be more vulnerable to *A. baumannii* strains, with these strains being more harmful than those of *A. baylyi* and *A. lwoffii*. Another study reported that a strain of A. junii was nonlethal in neutropenic mice, while many *A. baumannii* strains were lethal[76].

2.7.5 Antimicrobial Active Plant Compounds against *Acinetobacter baumannii*

Various plant-derived compounds have displayed antimicrobial efficacy against *Acinetobacterbaumannii*[77]. These compounds originate from diverse plant sources and exhibit the potential to impede the growth and replication of *A. baumannii*[78]. Among them are polyphenols, alkaloids, terpenoids, and essential oils. Polyphenols, for instance, those present in green tea and cranberries, have demonstrated promising antibacterial characteristics against *A. baumannii*[79]. Alkaloids, like berberine extracted from goldenseal, have also showcased antimicrobial properties[80]. Terpenoids, abundant in plants such as thyme and oregano, possess robust antimicrobial attributes, akin to essential oils from plants like tea trees and eucalyptus. These naturally occurring compounds from plants offer a promising and natural approach to combat *A. baumannii* infections.

2.8 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultatively anaerobic, rod-shaped bacteria[81]. It emerges as a mucoid lactose fermenter on MacConkey agar.

The rod-shaped, Gram-negative bacterium *Klebsiella pneumoniae* is a member of the Enterobacteriaceae family. It is a significant human pathogen known for causing a wide range of infections, particularly in individuals with compromised immune systems or underlying health conditions. *K. pneumoniae* is found in various environments, including soil, water, and the human digestive tract, where it can exist as a commensal organism without causing harm. However, under certain conditions, it can become an opportunistic pathogen[82]. This bacterial species within the *Klebsiella* genus is renowned for its pathogenic nature and its capacity to provoke various infections in humans, especially within medical environments. *Klebsiella*

pneumoniae infections can manifest as pneumonia, urinary tract infections, bloodstream infections, and surgical site infections. These infections often pose significant challenges in terms of treatment due to the development of antibiotic resistance in certain strains, including resistance to commonly used antibiotics like carbapenems, typically reserved as a last-line defense. The emergence of antibiotic resistance in *Klebsiella pneumoniae* underscores its substantial significance in healthcare facilities, emphasizing the critical importance of infection control protocols and the imperative to explore new treatment strategies. Clinical manifestations and infections associated with *Klebsiella pneumoniae* can exhibit a wide array of presentations, encompassing diverse symptoms and varying degrees of severity. This bacterium is recognized for its pathogenic nature and its capacity to induce a range of infections in humans, particularly within healthcare settings. These infections may take the form of *pneumonia*, urinary tract infections, bloodstream infections, and surgical site infections[83]. The management of *Klebsiella pneumoniae* infections can prove to be formidable due to the emergence of antibiotic resistance in certain strains, including resistance to critical antibiotics such as carbapenems. This antibiotic resistance underscores the importance of implementing stringent infection control protocols and emphasizes the pressing need for the development of innovative treatment strategies[84].

2.8.1Plant Active Compound against *Klebsiella pneumonia*

The efficacy of plant-derived active compounds against *Klebsiella pneumoniae* has been a subject of investigation by scientists[85]. These compounds are sourced from various plants and have demonstrated the potential to inhibit the growth of this bacterium[86]. Some examples of these active compounds encompass polyphenols, alkaloids, terpenoids, and essential oils. Polyphenols, such as those present in green tea and cranberries, have shown antibacterial

properties when challenged by *Klebsiella pneumoniae*. Alkaloids like berberine, extracted from sources like goldenseal, also exhibit antimicrobial effects. Terpenoids, which are commonly found in plants like thyme and oregano, possess potent antimicrobial attributes, akin to essential oils from plants such as tea trees and eucalyptus. These naturally occurring plant-derived active compounds present a promising and natural avenue for addressing infections caused by *Klebsiella pneumoniae*[87]. `

2.9 ***Pseudomonas luteola***: *Pseudomonas luteola* is an opportunistic pathogen that thrives in moist settings. The species was originally classified in the genus Chryseomonas but has now been reclassified as *Pseudomonas*[88]. Higher classification *Pseudomonas*, Scientific name *Pseudomonas luteola*, Family *Pseudomonadaceae*. *Pseudomonas luteola* is a species of bacteria that falls within the *Pseudomonas* genus. It is a Gram-negative, aerobic, non-spore-forming bacterium. *Pseudomonas* species are known for their diverse metabolic capabilities, adaptability to various environments, and opportunistic pathogenicity. Morphology and Staining: *Pseudomonas luteola* is typically rod-shaped (bacillus) and has a single, polar flagellum, which provides it with motility. As a Gram-negative bacterium, it does not retain the crystal violet stain during Gram staining, appearing pink or red under the microscope.

Metabolism: *P. luteola* is aerobic, meaning it requires oxygen for growth and energy production. It is metabolically versatile and capable of utilizing a wide range of organic compounds as carbon and energy sources.

Pigment Production: *P. luteola* is known for its characteristic yellow pigment production, which contributes to its name ("luteola" means yellow). The production of pigments by *P. luteola* can be a useful diagnostic feature when identifying the bacterium. *Pseudomonas luteola* can be found

in various environments, including soil, water, and on the surfaces of plants and animals. It is also an opportunistic pathogen, meaning it may cause infections in individuals with compromised immune systems or underlying health conditions. While *P. luteola* is generally considered to be of low pathogenicity, it has been associated with infections in humans, especially in immunocompromised individuals. Infections by *P. luteola* can include bloodstream infections, urinary tract infections, and respiratory tract infections[89]. Like many other *Pseudomonas species, P. luteola* can develop resistance to antibiotics, posing challenges in clinical settings. The bacterium's ability to form biofilms can contribute to antibiotic resistance and make eradication more difficult. *Pseudomonas luteola* has been isolated from clinical specimens, and its role in infections has been documented in various medical studies. Identification of *P. luteola* in clinical settings is typically done through microbiological techniques, including culture and molecular methods. In the laboratory, *P. luteola* can be identified based on its characteristic growth on selective media, pigment production, and biochemical tests. Molecular techniques, such as polymerase chain reaction (PCR), may also be employed for accurate identification. *Pseudomonas luteola*, like other *Pseudomonas species*, has been studied for its potential applications in bioremediation and agriculture due to its metabolic capabilities. Research continues to explore its physiology, genetics, and interactions with other microorganisms [90].

2.9.1 Plant Active Compound against *Pseudomonas luteola*

Pseudomonas luteola, a Gram-negative bacterium, has been a subject of interest in the exploration of alternative therapeutic options, particularly involving plant-derived compounds with antimicrobial properties91. Essential oils such as Tea Tree Oil and Eucalyptus Oil have

demonstrated broad-spectrum antimicrobial capabilities, including effectiveness against various bacteria, including *Pseudomonas species*. Phenolic compounds found in Cranberry Extract and Thymol from thyme have been studied for their potential to inhibit bacterial adhesion and prevent infections[91]. Alkaloids like Berberine, derived from plants like berberis and goldenseal, have shown antibacterial activity against a range of pathogens, prompting consideration for their effects on *Pseudomonas luteola*[91]. Additionally, flavonoids like Quercetin, terpenoids such as Curcumin from turmeric, and polyphenols found in Green Tea Extract, particularly catechins, have been investigated for their antimicrobial properties and their potential *against Pseudomonas luteola.* Garlic compounds, including Allicin, known for their broad-spectrum antimicrobial properties, have been studied for efficacy against various bacteria. Aloe Vera, with its Aloe Vera Gel containing compounds like anthraquinones, has also been explored for potential antimicrobial effects[92]. However, it is crucial to emphasize the importance of thorough research and consultation with professionals in microbiology and medicine before considering the use of these plant-derived compounds for treating bacterial infections. Factors such as concentration, formulation, and mode of application can significantly influence their efficacy and safety. While these natural compounds show promise, traditional antibiotics remain the primary choice in clinical settings, and any exploration of alternative therapies should be approached with caution and under the guidance of healthcare professionals[92].

2.10 *Enterobacter cloacae*

Enterobacter aerogenes was initially known as *Aerobacter aerogenes* before being added to the *Enterobacter genus* in 1960–89. This species was recommended to be called *Klebsiella mobilis* in 1971 because of its motility given by peritrichous flagella and genetic similarity to the

Klebsiella genus. It is worth noting that phenotypic differences between *E. aerogenes* and the genus *Klebsiella* include not only motility but also the presence of ornithine decarboxylase (ODC) activity and the absence of urease activity in *E. aerogenes*[94]. However, recent complete genome sequencing of a multidrug-resistant (MDR) clinical isolate (containing colistin) proposed that the species be reclassified in the genus *Klebsiella* under the name *K. aeromobilis*[95]. *E. aerogenes'* distinct phenotype can be linked to the horizontal acquisition of new genes from other *Enterobacteriaceae species*, as well as mobile components that integrated and translated as quickly as its ancestral heritage[96]. For example, the flagellar genes and assembly mechanism were acquired together from the Serratia genus. Plasmid conjugation is a hybrid of transposons and genetic elements (conjugation, integration) from multiple bacterial sources. *E. aerogenes* also has eight rRNA operons and tRNA related with the ability to translate imported genes with various codons, which improves its ability to exploit integrated foreign genes. *E. aerogenes* was engaged in a major European outbreak between 1993 and 2003 and is regarded as the archetype of opportunistic bacteria[87]. *Enterobacter cloacae* is a clinically important Gram-negative, facultative-anaerobic, rod-shaped bacterium[92].

Enterobacter cloacae can pose significant challenges in healthcare settings due to its propensity to develop antibiotic resistance[86]. Infections caused by this bacterium can manifest with a range of clinical symptoms, spanning from mild to severe, including urinary tract infections, bloodstream infections, and pneumonia. Given the diversity in the severity and antibiotic resistance profiles of *Enterobacter cloacae* infections, tailored treatment approaches are often required. Vigilance in *Enterobacter cloacae* is a bacterial species belonging to the *Enterobacter genus*[93]. It is notable for its pathogenic nature and its ability to cause a variety of infections in

humans. Monitoring antibiotic use and the exploration of alternative treatment strategies are crucial components of effectively managing infections associated with this pathogen.

2.10.1 Microbiological Characteristics

1. Shape and Arrangement: *Enterobacter cloacae* are a straight, rod-shaped bacterium with dimensions of approximately 0.5 to 1 micrometer in width and 1 to 3 micrometers in length. It typically appears as single cells or short chains[94].

2. Gram Stain: It is Gram-negative, meaning it does not retain the violet crystal stain and appears red/pink after Gram staining due to the thin peptidoglycan layer in its cell wall.

3. Motility: *Enterobacter cloacae* are often motile due to their possession of peritrichous flagella. This motility allows the bacterium to move toward favorable environments and nutrients.

4. Metabolism: It is a facultative anaerobe, capable of both aerobic and anaerobic respiration. *Enterobacter cloacae* can ferment various sugars and is metabolically versatile[95].

2.10.2 Pathogenicity and Clinical Significance

The pathogenicity and clinical relevance of *E. cloacae* are significant. This bacterium is recognized for its capacity to induce various infections in humans, and it holds clinical importance, particularly within healthcare environments[96]. Infections associated with *E. cloacae* can manifest with a wide array of clinical symptoms, ranging from mild to severe, encompassing conditions like urinary tract infections, bloodstream infections, and pneumonia. The clinical significance of this bacterium is further emphasized by its ability to develop resistance to antibiotics, which can complicate the treatment process[97]. The management of *E. cloacae*-related

infections often requires individualized treatment strategies, as the severity and antibiotic resistance profiles of these infections can vary. Vigilance in monitoring antibiotic usage and the exploration of alternative treatment approaches are essential components in effectively addressing infections linked to this pathogenic bacterium[98].

2.10.3 Plant Active Compound against *Enterobacter Cloacae*

Researchers have been investigating the effectiveness of plant-derived active substances in treating *Enterobacter cloacae* [104]. These chemicals, derived from various plants, show potential in limiting the growth of this bacterium[105]. Such active chemicals include polyphenols, alkaloids, terpenoids, and essential oils. When polyphenols contained in green tea and cranberries were tested against *Enterobacter cloacae*, they demonstrated antibacterial activity. Berberine, an alkaloid derived from goldenseal, has also been shown to have antibacterial properties. Terpenoids, which are present in plants such as thyme and oregano, have strong antibacterial properties, similar to essential oils found in tea trees and eucalyptus. These naturally occurring plant-derived active substances represent a promising and natural approach to treating *Enterobacter cloacae* infections.

2.11 *Raoultella ornithinolytical*

Raoultella ornithinolytica is a non-motile, aerobic, encapsulated Gram-negative rod that belongs to the *Enterobacteriaceae* family[100]. Initially classified as *Klebsiella ornithinolytica* in cluster II of the *Klebsiella* genus, alongside environmental organisms such as *Klebsiella terrigena*, *Klebsiella planticola, and Klebsiella trevisanii*, advances in phylogenetic testing, including 16S rRNA and rpoB sequence analysis, led to the further division of the *Klebsiella genus* into two

genera. As a result, the *Raoultella genus* was established in 2001, and species that were previously classified as cluster II in the *Klebsiella genus* were relocated and rebranded within this new genus. The *Raoultella genus* is named after Didier Raoult, a French bacteriologist at the Université de la Méditerranée in Marseille, France[101]. *R. ornithinolytica* has been found in a variety of settings, including water, soil, insects, fish, ticks, and termites. *Raoultella planticola* isa gram-negative bacterium from the *Raoultella genus*[102]. *R. planticola* has a similar look to *Klebsiella pneumoniae* and must be identified through growth habits or DNA analysis. Numerous strains have been identified[103]. *Raoultella ornithinolytica* is a bacterial species within the *Raoultella genus*. It is known for its pathogenic nature and ability to induce various infections in humans. This bacterium can pose significant challenges, particularly in healthcare environments, due to its potential to develop antibiotic resistance. Infections caused by *Raoultella ornithinolytica* can present with a wide array of clinical symptoms, ranging from mild to severe, including urinary tract infections, bloodstream infections, and respiratory tract infections[104]. Given the diversity in the severity and antibiotic resistance profiles of these infections, treatment strategies often need to be customized for individual cases. To effectively manage infections associated with this pathogenic bacterium, vigilant monitoring of antibiotic use and exploration of alternative treatment approaches are essential[105].

2.11.1 Plant Active Compound against *Raoultella ornithinolytica*

Researchers have been investigating the potential of plant-derived active compounds to combat *Raoultella ornithinolytica*[106]. These compounds, derived from various plants, have shown promise in inhibiting this bacterium's growth. Examples of such active compounds include polyphenols, alkaloids, terpenoids, and essential oils. For instance, polyphenols found in green

tea and cranberries have shown antibacterial properties when tested against Raoultella ornithinolytica. Alkaloids like berberine, extracted from sources like goldenseal, have also exhibited antimicrobial effects. Terpenoids, which are commonly found in plants like thyme and oregano, possess potent antimicrobial properties similar to essential oils from plants such as tea trees and eucalyptus. These naturally occurring plant-derived active compounds offer a potential and natural avenue for addressing infections caused by *Raoultella ornithinolytica*[107].

2.12 Standard Antibiotics

2.12.1 Pefloxacin: is an asymmetric dihydrate structure made up of one norfloxacin molecule and two distinct water molecules. It is one of the fluorinated quinolone carboxylic acid derivatives with significant bactericidal activity. Its mode of action is based on its capacity to bind to the DNA gyrase enzyme and inhibit bacterial DNA replication. Pefloxacin, with a chemical formula of C16 H19Cl FN3O3 and a molecular weight of 355.79, has bacteriologic and pharmacokinetic properties that suggest it could be effective in treating urinary tract infections. Studies have indicated a comparable success rate in curing typhoid illness within seven days[108].

2.12.2 Gentamycin is an antibiotic composed of four closely related aminoglycoside sulfates produced from *Micromonospora purpurea* and related species. Although it has broad-spectrum activity, it has been linked to side effects such as ear and kidney damage. Gentamycin inhibits protein synthesis by irreversibly binding to particular 30S-subunit proteins and 16S rRNA, disrupting the initiation complex and producing nonfunctional or toxic peptides. It is used to treat severe infections caused by susceptible strains of different bacteria[109].

2.12.3 Ampiclox: Ampiclox, a mixture of ampicillin and cloxacillin, is a bactericidal antibiotic regimen that inhibits bacterial cell wall construction. Cloxacillin is resistant to staphylococcal β-lactamases, but ampicillin covers a wider range of bacteria. Ampiclox has better susceptibility rates than ampicillin alone, especially against β-lactamase-producing staphylococci. It has high absorption, tissue penetration, consistent pharmacokinetics, and renal elimination[110].

2.12.4 Zinacef, which contains cefuroxime, is an antibiotic used to treat bacterial infections in a variety of body sites and surgical procedures.

2.12.5 Amoxicillin, a broad-spectrum semi-synthetic antibiotic, belongs to the cephalosporin class and kills or inhibits bacterial growth. It is similar to ampicillin but has higher serum levels due to increased resistance to gastric acid[111].

2.12.6 Rocephin, a broad-spectrum cephalosporin antibiotic, is administered intravenously or intramuscularly and is effective against bacterial infections of the ear, nose, throat, genitourinary tract, skin, and lower respiratory tract. It is frequently combined with clavulanic acid to inhibit β-lactamase degradation. Ceftriaxone sodium, its chemical composition, is soluble in water but only moderately so in methanol and ethanol. Rocephin's antibacterial activity is governed by its sodium content and its pH-dependent solubility, making it useful against a variety of infections[113].

2.12.7 Ciprofloxacin: Ciprofloxacin, a fluoroquinolone antibiotic, is used to treat a variety of bacterial infections, including those of the joints, skin, bones, respiratory, and intra-abdominal regions. Its outstanding safety profile and efficacy against resistant infections make it a popular

choice in clinical settings. Ciprofloxacin's chemical formula is C17H18FN3O3, and it demonstrates excellent bioavailability with limited first-pass metabolism[114].

2.12.8 Streptomycin, an aminoglycoside antibiotic generated by Streptomyces griseus, is commonly used to treat aerobic Gram-negative bacterial infections. Its method involves irreversible binding to particular 30S-subunit proteins and 16S rRNA, which disrupts protein synthesis and produces non-functional peptides. Streptomycin works particularly well against tuberculosis and other mycobacterial illnesses[115].

2.12.9 Augmentin: Augmentin, an oral antibacterial mixture of amoxicillin trihydrate and potassium clavulanate, has a wider range of action and activity against amoxicillin-resistant bacteria. It is used to treat a variety of bacterial diseases, including sinusitis, pneumonia, ear infections, bronchitis, urinary tract infections, and skin infections. Augmentin inhibits bacterial cell wall formation by attaching to penicillin-binding proteins. It also contains clavulanic acid to inhibit β-lactamase enzymes[115].

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Chapter Three

Methodology

3.1 Collection, Identification and Processing of Plant Samples

Date fruits (*P. dactylifera*) were procured from a local fruit settlement at Mokola Sabo, Ibadan. It was washed thoroughly with running tap water, rinsed in distilled water, and shade dried for one week in open air. The fruit pulp was removed, and the seeds were then shade dried and grounded using mortar and pestle, later pulverized into powder for experimental use[2]. Fresh leaf of *P. dactylifera* was obtained in February, 2023. The leave was identified and authenticated, with herbarium number UI 42345.

3.1.1 Collection of the Test Microorganism

Bacterial isolates from urinary tract infection were obtained from the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria. The isolates were *Raoultella Ornithinolytical, Klebsiella pneumoniae, Escherichia coli, Pseudomonas luteola, Enterobacter cloacaeandAcinetobacter baumannii.*

3.2 Preparation of Plant Extracts

The plant extract was prepared using cold maceration extraction method. A mechanical shaker set to 220 rpm was used to extract the *Phoenix dactylifera* fruits and seeds samples for 48 hours. The samples were subsequently weighed at 15 gramme (15 g) using an electronic weighing scale (a Mettler Toledo FA2104A). The same process was carried out using ethyl acetate, methanol, chloroform, and aqueous. The solutions were left for 24 hours and stirred at regular intervals. The solvents were filtered after concentration using a rotary evaporator at 40 °C to produce the extracts, which were then filtered using Whatman No. 1 filter paper with a pore size of 100 mm by 195 mm.

Each of the concentrated filtrates was diluted to various concentrations starting from 150 mg/ml to 100 mg/ml, 75 mg/ml, and 60 mg/ml[2]. Afterwards, the extracts were tested for their antibacterial potency on the following bacterial isolates: *Acinetobacter baumannii, Pseudomonas luteola*, *Klebsiella pneumoniae*, *Escherichia coli, Enterobacter cloacae*, and *Rauitella ornithinolytica*[6].

3.3 Phytochemical Screening of the Extracts of the Fruit and Seed of *Phoenix dactylifera L*

3.3.1 Qualitative Determinations Procedure

Phytochemical Screening of the Extracts of the Fruit and Seed of *Phoenix dactylifera L*

Phytochemical screening was carried out to identify the phytochemicals present in the fruit and seeds of *P.dactyliferaL.*This analysis was carried out using standard procedures. The phytochemicals that were screened include saponin, tannins, terpenoids, phenol, flavonoids and alkaloids.

3.3.2 Qualitative Analysis

a. Test for Tannins

One gram (1 g) of the powdered sample of Fruit and seeds of the plant was stirred with 10 ml of distilled water, separately. The solutions were filtered with Whatman No 1 filter papers. A few drops of 1% ferric chloride (FeCl3) were added to each of the filtrates. The appearance of blue-black or green coloration indicated the presence of tannins[19].

b. Test for Saponins

The general method involved in the qualitative analysis of saponins is 2 mL of aqueous extract was added to 2 mL of distilled water then shaken for 15 min in a graduated cylinder. Observation of 1 cm foam layer indicate positive response to the presence of saponins[20].

c. Test for Terpenoids

Five (5) ml of each Fruit and seed extract was mixed with 2mL of chloroform. Three (3) ml of concentrated sulphuric acid (H_2SO_4) was carefully added until a layer was formed. A reddish-brown coloration at the interface indicated the presence of Terpenoids[21].

d. Test for Alkaloids

One gram (1 g) of each of the powdered samples was stirred with 5 mL of 1% dilute hydrochloric acid in a water bath. One (1) ml of the filtrates, each was treated with a few drops of Mayer's reagent. One (1) mL portion was taken from each solution and treated with a few

drops of Drangendorff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract being evaluated[22].

e. Test for Phenolics

FeCl3 Test: 1 mL of the filtered plant extract sample was added to few drops of 10% ferric chloride solution. Green blue or violet color appears which indicates the presence of phenolic compounds[23].

f. Test for Flavonoids

Shinoda Test: Crude extract was mixed with a few fragments of magnesium ribbon and concentrated HCl was added dropwise. The pink scarlet color appeared after a few minutes which indicated the presence of Flavonoids. Sodium hydroxide Test: To 1 mL of the plant extract, add a few drops of dilute NaOH solution. An intense yellow color is observed. It becomes colorless with the addition of a few drops of dilute acid which indicates the presence of Flavonoids. Lead acetate Test: To the plant aqueous extract, add 10% lead acetate solution. A yellow precipitate is formed which indicates the presence of Flavonoids[24].

3.3.3 Quantitative Determinations Procedure

a. Tannin

A 50 mL beaker was filled with a 0.20 g sample. After adding 20 mL of 50% methanol and covering it with paraffin, the mixture was left in a water bath set between 77 and 80 °C for an hour. To guarantee a consistent mixing, it was shaken vigorously. A double-layered Whatman No. 41 filter paper was used to quantitatively filter the extract into a 100 mL volumetric flask. Additionally, 20 mL of water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 were added, and everything was thoroughly mixed. After thoroughly mixing the mixture with water

and letting it rest for 20 minutes, the mixture developed a bluish-green color. Tannin working standard solutions in the 0–10 ppm range were handled identically to 1 mL of sample. The absorbance of the Tannic acid standard solutions as well as samples was read after color development on a Spectronic 21D spectrophotometer at a wavelength of 760 nm. % Tannin was calculated using the formula[25].

$$\%\text{TANNIN} = \frac{\text{absorbance of sample X average gradient factor X Dilution factor}}{\text{Wt. of Sample X } 10{,}000}$$

b. Alkaloids Procedure

This process involves distillation and titrimetry. Two grams of finely ground material were weighed into a 100-milliliter beaker, and twenty milliliters of 80% absolute alcohol were added to create a smooth paste. The mixture was then moved to a 250-milliliter flask, where additional alcohol was added to reach 100 milliliters, and one gram of 99 magnesium oxide was added. The mixture was digested for one and a half hours under a ref lux air condenser with periodic shaking, and it was filtered while still hot through a tiny Buchner funnel. The residue was reintroduced to the flask and re-digested for 30 min with 50 mL alcohol after which the alcohol was evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10% HCl was added. The entire mixture was then put into a 250 mL volumetric flask. 5 mL of potassium ferrocyanide solution and 5 mL of zinc acetate solution was added, and they were well combined to create a homogeneous solution[26].

The flask was allowed to stand for a few minutes, filtered through a dry filter paper, and 10 mL of the filtrate was transferred into a separator y funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 mL hot distilled water and transferred into a Kjeldahl tube with the addition of

0.20g sucrose and 10 mL Conc.H_2SO_4 and 0.02 g selenium for digestion to a colorless solution to determine %N by Kjeldahl distillation method9. %Nitrogen got was converted to % total alkaloid by multiplying by a factor of 3.26 i.e. % Total alkaloid = %N X 3.26

% Alkaloids = %N X 3.26.

c. Flavonoids Determination

Finely 0.50 g ground sample was weighed into a 100 mL beaker and 80 mL of 95%, Ethanol was added and stirred with a glass rod to prevent lumping. The mixture was filtered through a Whatman No.1. filter into a 100 mL volumetric flask and made up to mark with Ethanol. 1 mL of the extract was pipetted into a 50 mL volumetric flask and four drops of Conc. HCL was added via a dropping pipette after which 0.5 g of magnesium turnings were added to develop a magenta red coloration. Standard flavonoid solution of range 0-5 ppm was prepared from 100 ppm stock solution and treated similarly with HCl and magnesium turnings like sample. The absorbance of magenta red coloration of sample and standard solutions was read on a digital 100 Jenway V6300 Spectrophotometer at a wavelength of 520 nm[27]. The percentage of flavonoids was calculated using the formula.

$$\frac{\text{The absorbance of sample x average gradient factor x dilution factor}}{\text{Wt. sample x 10,000}}$$

d. Saponin

Finely 1 g ground sample was weighed into a 250 mL beaker and 100 mL of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman No1 filter paper into a 100 mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated MgCO3 was again filtered through a Whatman No1 filter paper to obtain a clear

colorless solution. 1 mL of the colorless solution was pipetted into a 50 mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for blood red color to develop. 0-10 ppm standard Saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 mL of 5% $FeCl_3$ solution done for the 1 mL sample above. The absorbance of the sample as well as standard saponin solutions was read after color development in a Jenway V6300 Spectrophotometer at a wavelength of 380min[28].

$$\%\text{Saponin} = \frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x10000}}$$

e. Determination of Terpenoids

A sample of 0.50 g was weighed into a 50 mL Conical Flask, and 20 mL of 2:1 Chloroform-Methanol mixture was added, shaken thoroughly, and allowed to stand for 15 minutes. The mixture was later centrifuged for another 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20 mL chloroform-methanol mixture for re-centrifugation.

The resultant precipitate was dissolved in 40 of 10% Sodium Dodecyl Sulphate solution 1ml of 0.01 M Ferric Chloride solution was added to the above at 30 +s intervals shaken well, and allowed to stand for 30 minutes. Standard Terpenes of the concentration range 0-5 mg/ml were prepared from 100 mg/l stock Terpenes solution from Sigma-Aldrich chemicals, U.S.A[29]. The absorbances of the sample as well as that of standard concentrations of Terpenes were read on a Digital Spectrophotometer at a wavelength of 510 nm. The percentage of Terpenoids is calculated using the formula:

$$\frac{\text{The absorbance of Sample x Gradient Factor x Dilution Factor}}{}$$

Wt. of sample x 10,000

f. Determination of Phenol

A sample of 0.20 g was weighed into a 50 mL beaker, and 20 mL of acetone was added and homogenized properly for 1 hr. to prevent lumping. The mixture was filtered through a Whatman No.1 filter paper into a 100 mL Volumetric Flask using acetone to rinse and make up to mark with distilled water with thorough mixing. 1 mL of sample extract was pipetted into a 50 mL Volumetric flask, 20 mL water was added, 3mL of phosphomolybdic acid was added followed by the addition of 5 mL of 23% Na_2CO_3 and mix thoroughly, make up to mark with distilled water and allowed to stand for 10 min to develop bluish-green colour[30]. Standard Phenol of the concentration range 0-10 mg/mL was prepared from 100 mg/L stock Phenol solution from Sigma-Aldrich chemicals, U.S.A. The absorbances of the sample as well as that of 105. Standard concentrations of Phenol were read on a Digital Spectrophotometer at a wavelength of 510 nm. The percentage of Phenol was calculated using the formula:

$$\frac{\text{The absorbance of sample x gradient factor x dilution factor}}{\text{Wt. of sample x 10,000}}$$

3.4Antimicrobial Susceptibility of Microorganism from the Urinary tract

3.4.1 Preparation of the Extracts Dilution

Stock solution of the plant fractions were prepared by mixing 15g of each extract in 100ml of solvent (150 mg/ml). Then other concentrations were prepared from the stock solutions by using law of equivalence proportion formula:

$$C_1V_1 = C_2V_2$$

The final concentration of each of the extracts of the (MIC) was determined.

3.5 Determination of the Susceptibility Profile of the Isolates to different Antibiotics discs

Susceptibility is a term used when microorganisms such as bacteria and fungi are unable to proliferate in the presence of one or more antimicrobial medications. Susceptibility of antibacterial testing is performed on bacteria causing an individual's infection after they have been recovered in a culture of the samples. Testing is performed to establish the possible effectiveness of specific antibiotics on the bacteria and/or to determine if the bacteria have evolved resistance to certain antibiotics. The findings of this test can be used to assist identify the drug(s) that will likely be most effective in treating an illness. The antimicrobial susceptibility pattern to antibiotics was determined according to the Kirby-Bauer method with modifications by the Clinical Laboratory Standards Institute. Different kinds of antibiotic discs (Oxoid®, UK) utilized in this study include amoxicillin/clavulanic acid, cefepime, ceftazidime, ciprofloxacin, pefloxacin, ceftriaxone, nalidixic acid, gentamycin, imipenem, and nitrofurantoin[7]. Before each antibiotic disc was placed on each of the media surfaces, the pathogenic bacteria isolate from the nutrient agar slants were inoculated into normal saline, compared with the McFarland standard of 0.05, then streaked on each of the nutrient agar dishes, after which the antibiotic discs were aseptically placed on each of the Petri plates using sterile forceps. The agar plates were then incubated at 37º for 24 hours. Afterward, the plates were inspected for zones of inhibition. The zone of inhibition around each antibiotic disc was measured in millimeters using a clear ruler. Antibiotic susceptibility was determined from the reading obtained from the diameter of the zone of inhibition. The antibiotic susceptibility patterns from each of the zones of inhibition identified on the agar plates are referred to as Antibiogram[7].

3.6 Validity of Test Microorganisms

The organisms were maintained in nutrient agar slants for 48 hours in a refrigerator at about 4°C before they were further sub-cultured into freshly prepared Petri dishes using the streak plate method[4].

3.7 Biochemical Tests Identification of Tested Microorganisms

3.7.1 Gram Staining

3.7.2 Gram's Staining Procedure

Thin smear of pure bacterial culture was made on clean glass slide air dried and heat fixed. Smear was covered with crystal violet for 30 seconds, washed with distilled water and smear was flooded with Gram's iodine solution for 60 seconds, washed with 95% ethyl alcohol and then distilled water again, the smear will be covered with safranin for 30 seconds, wash with distilled water and blot dried. Air dried and observed under microscope2

3.7.3 Oxidase Test

Impregnated oxidase strip was put on a clean petri plate, a sterile inoculating loop was used to pick up a well-isolated colony of test bacteria from fresh culture and make a smear on the oxidase strip. It was observed for 60 seconds for color change from initial color to purplethat indicate oxidase positive result.

3.7.4 Determination of Identification of Bacterial UsingAPI (Analytical Profile Index)

API (Analytical Profile Index) 20E is a biochemical panel which was used for the identification of the tested bacterial,the kits that was used include strips that contain up to 20 miniature biochemical tests for the identification of *Klebsiella spp., Escherichia coli, Pseudomonas spp., Enterobacter spp., Acinetobacter spp.,* and *Raoultella spp*.

Method

The confirmatory of the culture of tested isolates was carried out a quick oxidase test for cytochrome c oxidase by picked a single isolated colony from a pure culture. A suspension of the culture was made in sterile distilled water then using a Pasteur pipette to fill up (up to the brim) the API 20E Biochemical Test Strip which contains dehydrated bacterial media/bio-chemical reagents in 20 separate compartments with the bacterial suspension and add sterile oil into the ADH, LDC, ODC, H_2S and URE compartments. Some drops of water were added into the tray and then put the API Test strip and close the tray. Then mark the tray with identification number (Organism ID), date and your initials. Incubate the tray at 37oC for 18 to 24 hours.

I. After 24 hours Api test strip was brought out from incubator and read the color change, while TDA, IND and VP was added with the following reagents, a drop of ferric chloride was added to TDA, while a drop Kovacs reagent was added to IND, lastly a drop of drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol) was added to VP.

ThenVPAPI Reading Scale (color chart) was used by marking each test as positive or negative on the lid of the tray. The wells were marked off into triplets by black triangles, for which scores are allocated.Then add up the scores for the positive wells only in each triplet.

Three test reactions are added together at a time to give a 7-digit number, which can then be looked up in the code book. The highest score possible for a triplet is 7 (the sum of 1, 2 and 4) and the lowest is 0.

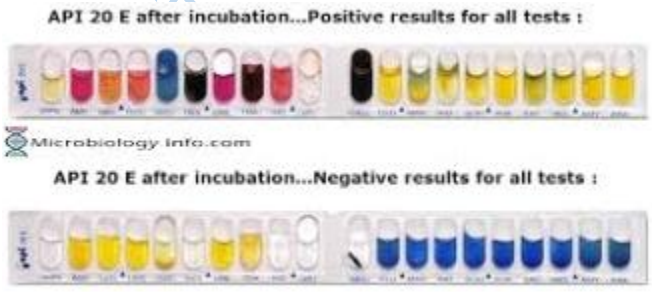

Figure 4.4: API 20 E

Source: Microbiology info.com

3.8 Beta-lactamase Production Confirmatory Testing

3.8.1 Nitrocefin Disk Test: The Nitrocefin biochemical test was carried out for the confirmatory testing for beta-lactamase producing strains of the tested UTIs isolates. Nitrocefin is chromogenic cephalosporins that changes from yellow to red when the amide bond in the beta-lactam ring is hydrolyzed by beta-lactamase. It is sensitive to hydrolysis by all known β-Lactamases produced Gram-negative bacteria[13].

Bacterial colony growth overnight on Muller- Hilton agar plate was picked with sterile wire loop then transfer a small amount of the bacterial colonies onto the moistened Nitrocefin disc and ensure good contact between the bacteria and the disc.

Allow the inoculated disc to incubate at room temperature then observe for a color change for up to 60 minutes. In some cases, incubation at 35-37°C for 15-30 minutes may be required for slow-reacting organisms.

b. Interpretation of Results:

- **Positive Result:** A rapid color change from yellow to red within a few minutes indicates the presence of beta-lactamase activity.
- **Negative Result:** No color change or a color change that occurs after an extended period (beyond 60 minutes) suggests the absence of beta-lactamase activity.

Test results was compared with the positive and negative controls to ensure the accuracy of the test.

3.8.2 Double Disc Synergy Test (DDST): The Double Disk Test (DDST) is a phenotypic test used to detect the production of extended -spectrum beta-lactamase (ESBLs) and other beta-lactamase enzymes in bacterial isolates.

i. Procedure:

Muller-Hilton agar plate was prepared according to the manufacturer manual, then plates was label with the bacterial isolates and inoculate the agar with the isolates to give evenly distribution.Beta-lactam antibiotic disks were place on the inoculated plate ,15-20mm apart and Beta-lactamase inhibitor disk was place adjacent to the beta-lactam antibiotic.

The plate was Incubated for 18-24hours at35-37^{o}C,

The Common Disk used for the identification were

- Cefotaxime (30µg) +Clavulanic acid (10 µg).
- Ceftazidime (30µg) +Clavulanic acid (10 µg).
- Aztreonam (30µg) +Clavulanic acid (10 µg).

ii. Interpretation

The zone of inhibition in diameter were measure around each disk and recorded.

Observation of the zone of inhibition between the beta-lactam antibiotic and beta-lactamase inhibitor disc indicates ESBL production.

- Positive result: enhance zone of inhibition (>5mm) between disks.
- Negative result: No zone of inhibition observes.

3.9 Preparation of Nutrient Agar Medium

According to the manufacturer's specification, 28 g of nutrient agar powder was weighed using the sensitive weighing balance. The agar powder was dissolved in a conical flask containing 1000 ml of distilled water. A stopper, made with cotton wool and wrapped with aluminum foil, was used to plug the mouth of the conical flask. The solution was mixed thoroughly and then homogenized by placing it in a water bath at 800 °C for 15 minutes. After removing it from the water bath, the conical flask containing the homogenized nutrient agar was wrapped with

aluminum foil and then sterilized in an autoclave for 15 minutes at 121^{0}C at a pressure of 151 b/sq inch. The medium was allowed to cool to about 47-50°C before pouring into sterile Petri dishes and left to solidify [2].

3.9.1 Preparation of Mueller Hinton Agar

Thirty-eight grams (38g) of Mueller Hinton agar powder (CM0337B) was weigh and poured into 1L of distilled water, mixed properly to dissolve the agar completely. Mixture was sterilized in an autoclaving at 121°C for 15 minutes. The agar were poured into Petri dishes and were allowed to solidify[3].

3.10Determination of Anti-bacterial Activities of the Plant Extract against Test Pathogen

Using inoculating wire loop, the test organisms was streaked on the surface of the solidified nutrient agar plates. A sterile 6 mm cork-borer was used to make a uniform deep well into the agar gel. Each of the well was afterwards filled with 1 ml of the extracts prepared in different solvents. The Petri plates were allowed to stand for 30minutes at room temperature to allow the proper diffusion of extract. The controls were then set up using the solvents alone, without the extracts. Sterilized distilled water was used as control for the aqueous sample. The plates were then incubated at 37°C for 24hours after which the zone of inhibition was measured with the transparent ruler.

3.11 Determination of Minimum Inhibitory Concentration of *Phoenix dactylifera L.*

Standardized suspension of each clinical bacterial isolate was injected into separate series of test tubes containing nutritional broth. Varied concentrations of the extracts were subsequently put into the inoculated test tubes. Cotton wool and aluminum foil were used to cover the test tubes. The preparations were incubated at 37 ºC for 24 hours. Reading tubes without turbidity were

recorded as MIC in which MIC is the minimum inhibitory concentration of an antimicrobial agent that can reduce the observable growth (turbidity) of test microorganisms following overnight incubation. The MICs were tested for all date fruit and seed extracts using the agar diffusion method[12].

3.11.1 Determination of Minimum Bactericidal Concentration (MBC)

The lowest bactericidal concentration of the plants extract was determined by sub culturing the test tubes from minimum inhibitory concentration tubes that showed no growth on nutrient agar and incubated for 24hours at 37ºC. Plates containing new solidified nutrient agar were seeded with inoculum from tubes with no turbidity in the MIC setup and incubated at 37ºC for 24hrs. The plate with the least concentration of the extract from MIC that showed no growth was recorded as the MBC[11].

3.12 Statistical Analysis:

All collected data were entered into a computer, and analyzed using the statistical package for the social sciences (SPSS) version 23. The data were subjected to descriptive (i.e. frequency distribution tables, percentages and proportions, mean and standard deviations) and inferential statistics (i.e. t-test and F-test) statistical treatment. Descriptive statistics was employed to describe characteristics of the study variables; t-test was used to compare the means of extract concentrations; and F-test was also used to compare the means within the extract concentrations and the various solvents for extraction. The level of statistical significance was set at $p \leq 0.05$ for all analysis in this study. The results were displayed in tables and percentages.

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Chapter Four

Results and Discussion of the Findings

4.1 Results of the Findings

4.1.1 Phytochemical Analysis of Plant Extract

The qualitative phytochemical analysis of the *P.dactylifera* fruit and seed extract using methanol, ethyl-acetate,chloroform and aqueous solvent are show in Table 4.1Phenol, Flavonoid,Saponin, alkaloid, terpenoids, and Tannin were the phytochemical detected in varied concentration in the fruit and seed methanolic and ethyl-acetate extracts in which chloroform and aqueous extract was not detected.

The phytochemical screening and quantitative analysis of *Phoenix dactylifera* fruits and seeds in table 4.1 revealed significant variations in the presence and concentration of bioactive compounds across different solvent extractions. The screening indicated that the ethyl acetate and methanol extracts contained substantial amounts of phytochemicals, while the chloroform and aqueous extracts showed no detectable compounds. Terpenoids were moderately present in both the ethyl acetate and methanol extracts of the fruits, with lower quantities in the seed extracts. Flavonoids, phenols, alkaloids, tannins, and saponins were all detected in the ethyl acetate and methanol extracts, with higher concentrations in the methanol fractions. In contrast, the chloroform and aqueous extracts lacked these phytochemicals entirely.

Quantitative analysis in table 4.2 further confirmed that the methanol extracts, particularly from the fruits, had the highest concentrations of phenols (208.8 mg/mL), Flavonoid (94.46±1.04), this could be the antibacterial behind the treatment of beta-lactamase produced urinary pathogens, alkaloids (174.2 mg/mL), tannins (142.2 mg/mL), and saponins (170 mg/mL). The seeds also contained significant amounts of these compounds in methanol extracts, though at slightly lower levels. Ethyl acetate extracts showed moderate concentrations of these phytochemicals, with phenols, flavonoid, alkaloids, and tannins being present in the range of 125.3 mg/mL to 135.9 mg/ml. Terpenoids, were detected at lower concentrations compared to the other compounds, with 0.128 mg/mL in the methanol extract of fruits and 0.071 mg/mL in seeds.

4.1.1 Phytochemical Analysis of Plant Extract

Qualitative Evaluation of Date (*Phoenix dactylifera)* Fruits and Seeds Extract

Phytochemical contents	Ethyl acetate		Methanol		Chloroform		Aqueous	
	F	S	F	S	F	S	F	S
Terpenoids	++	+	+++	+	-	-	-	-
Flavonoids	++	+	++	+	-	-	-	-
Phenols	++	++	++	+	-	-	-	-

Alkaloids	++	++	+++	++	-	-	-	-
Tannins	++	++	+++	++	-	-	-	-
Saponins	++	+	++	+ +	-	-	-	-

Key: + = **Low,** ++ = **Moderate,** +++ = **Heavy,** - = **Nil, F=Fruit**, **S= Seeds**.

Source: Author`s Field Work, 2024.

Table 4.2: Quantitative Phytochemical Test Result of *Phoenix dactylifera* Fruit and Seeds

Phytochemical	**Ethyl-Acetate**		**Methanol**		Chloroform		**Aqueous**	
	F	**S**	**F**	**S**	**F**	**S**	**F**	**S**
Terpenoid (mg/mL)	0.094±0.05 0.067±0.08		0.128±0.01	0.071±0.06	-	-	-	-
Flavonoid	34.20±0.34 32.10±0.07		94.46±1.04	54.46±1.02	-	-	-	-
Alkaloid	129.0±12.0		174.2±1.08	100.5±12.1	-	-	-	-

	127.2±0.19						
	125.3±0.04						
Tannins		142.2±1.03	134.5±0.07	-	-	-	-
	120.0±0.02						
	128.1±13.2	170±13.5					
Saponi							
	100.0±12.0	166.1±12.1		-	-	-	-
	135.9±12.1	208.8±9.95					
Phenol							
	134.6±11.1	200.7±19.6		-	-	-	-

Source: Author`s Field Work, 2024.

The results of the antibacterial evaluation of *Phoenix dactylifera L.* fruit and seed extracts presented in table 4.3, shows antibacterial effects against few tested isolates, reveal significant variations in efficacy against beta-lactamase-producing bacterial isolates. For the fruit extracts, methanol showed the highest activity against *Klebsiella pneumoniae*, methanol extract produced inhibition zones of 16.0 ± 1.41 mm at 150 mg/mL and 11.0 ± 1.41 mm at 100 mg/mL, with no activity at lower concentrations or with other solvents. For *Escherichia coli*, ethyl acetate extract demonstrated better inhibition, with zones ranging from 17.0 ± 1.41 mm at 150 mg/mL to 11.0 ± 1.41 mm at 75 mg/mL, while methanol extract showed similar but slightly lower efficacy. Other

isolates, such as *Enterobacter cloacae*, exhibited moderate sensitivity to methanol, with the highest inhibition zone of 19.5 ± 7.78 mm at 150 mg/mL. In contrast, *Acinetobacter baumannii*, *Pseudomonas luteola*, and *Rauitella ornithinolytica* showed minimal to no response to the fruit extracts.

For seed extracts presented in table 4.4, methanol and ethyl acetate were the most active solvents. against *Klebsiella pneumoniae*, methanol extract produced inhibition zones of 15.5 ± 0.70 mm at 150 mg/mL, reducing to 11.5 ± 0.70 mm at 75 mg/mL.*Escherichia coli* showed sensitivity to both ethyl acetate and methanol extracts, with ethyl acetate achieving inhibition zones from 15.0 ± 1.41 mm at 150 mg/mL to 7.5 ± 0.70 mm at 60 mg/mL. However, other isolates, such as *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Rauitella ornithinolytica*, exhibited little to no response to the seed extracts.

The synergistic combination of fruit and seed extracts presented in table 4.5 enhanced antibacterial activity against *Klebsiella pneumoniae*, methanol extract showed inhibition zones from 22.0 ± 1.41 mm at 150 mg/mL to 15.5 ± 0.70 mm at 75 mg/mL, while ethyl acetate showed zones ranging from 20.0 ± 0.33 mm to 16.0 ± 1.41 mm across the same concentrations. Similar trends were observed with *Escherichia coli*, where methanol and ethyl acetate extracts produced strong inhibition zones of up to 20.0 ± 1.41 mm at 150 mg/mL.

The minimum inhibitory concentration (MIC) was identified for each extract and isolate. For *Klebsiella pneumoniae*, methanol extract had an MIC of 100 mg/mL with fruit extract and 75 mg/mL with seed extract. *Escherichia coli* showed an MIC of 75 mg/mL with ethyl acetate extract for both fruit and seed extracts. The minimum bactericidal concentration (MBC) was not directly determined but would require further testing to establish the lowest concentration

capable of killing 99.9% of bacteria. These results suggest that methanol and ethyl acetate extracts of *Phoenix dactylifera L.* have potential as antimicrobial agents, particularly when used in combination.

Table 4.3: Antibacterial Activity of Fruit Extract of *Phoenix dactylifera L* Urinary Tract Pathogens

Isolates	Solvent Extraction	150mg/Ml	100mg/mL	75mg/mL	60mg/mL	Control
	Ethyl acetate	-	-	-	-	
Klebsiella pneumoniae	**Methanol**	16.0±1.41	11.0±1.41	-	-	-
	Chloroform	-	-	-	-	
	Aqueous	-	-	-	-	
	Ethyl acetate	17.0±1.41	14.0±1.41	11.0±1.41	-	-
Escherichia coli	**Methanol**	16.0±1.41	11.5±2.12	10.0±0	-	-
	Chlorofom	6.0±0	-	-	-	

	Aqueous	6.0±0	-	-	-	
	Ethyl acetate	11.0±1.41	-	-	-	
Enterobacter cloacae	**Methanol**	19.5±7.78	7.0±1.41	-	-	-
	Chlorofom	-	-	-	-	
	Aqueous	-	-	-	-	
	Ethyl acetate	-	-	-	-	
Acinetobacter baumannii	**Methanol**	-	-	-	-	
	Chlorofom	-	-	-	-	-
	Aqueous	-	-	-	-	
	Ethyl acetate	13.0±4.24	11.5±0.71	10.0±0	-	-
Pseudomonas Luteola	**Methanol**	14.5±0.71	13.5±2.12	11.0±1.41	10.5±3.54	-
	Chlorofom	-	-	-	-	
	Aqueous	-	-	-	-	
	Ethyl acetate	-	-	-	-	
Rauitellaorni thinolytical	**Methanol**	-	-	-	-	-
	Chlorofom	-	-	-	-	
	Aqueous	-	-	-	-	

Results were presented as mean ± standard deviation. – represent no zone of inhibition, and DMSO(Negative Control).
Source: Author`s Field Work, 2024.

Table 4.4: Antibacterial Activity of Seeds Extract of *Phoenix dactylifera L*, against Urinary Tract Pathogen

Solvent Extraction	Isolates	150mg/ml	100mg/ml	75mg/ml	60mg/ml	Control
Ethyl acetate	***K. pneumonia***	-	-	-	-	
Methanol		15.5±0.70	12.0±2.82	11.5±0.70	-	-
Chlorofom		-	-	-	-	
Aqueous		-	-	-	-	
Ethyl acetate	***E. coli***	15.0±1.41	12.5±3.07	9.0±1.41	7.5±0.70	-
Methanol		14.5±2.12	12.5±3.53	7.0±1.41	-	-
Chlorofom		-	-	-	-	

Aqueous		-	-	-	-	
Ethyl acetate	***E. cloacae***	-	-	-	-	
Methanol		-	-	-	-	-
Chlorofom		-	-	-	-	
Aqueous		-	-	-	-	
Ethyl acetate	***A.baumannii***	-	-	-	-	
Methanol		-	-	-	-	-
Chlorofom		-	-	-	-	
Aqueous		-	-	-	-	
Ethyl acetate	***P. Luteola***	16.5±3.53	14.0±2.82	13.0±4.24	6.0±0	-
Methanol		18.0±1.41	14.5±0.70	13.0±1.41	11.5±3.5	-
Chlorofom		-	-	-	-	-
Aqueous		-	-	-	-	
Ethyl acetate	***R.ornithinolytical***	-	-	-	-	
Methanol		-	-	-	-	
Chlorofom		-	-	-	-	-
Aqueous		-	-	-	-	

Results were presented as mean ± standard deviation of duplicate determination. –

Source: Author`s Field Work,2024.

Table 4.5: Antibacterial Effect of Date Fruit and Seeds Extracts Combination Urinary Tract Pathogen

Isolates	Solvent Extraction	150mg/ml	100mg/ml	75mg/ml	60mg/ml	Control
	Ethyl acetate	20.0±0.33	18.0±0.14	16.0±1.414	-	-
K. Pneumonia	**Methanol**	22.0±1.41	18.5±0.70	15.5±0.70	-	-
	Chloroform	-	-	-	-	-
	Aqueous	-	-	-	-	-
E. Coli	**Ethyl**	19.5±2.1	16.5±2.1	10.0±0.01		-

	acetate	2	2			
	Methanol	20.0±1.41	17.0±2.82	7.5±2.12	-	-
	Chlorofom	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Ethyl acetate	19.0±1.41	7.0±1.41	-	-	-
E. cloacae	**Methanol**	19.0±1.41	14.0±2.82	-	-	-
	Chlorofom	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Ethyl acetate	16.5±2.12	10.0±0.01	-	-	-
A. baumannii	**Methanol**	19.0±1.41	11.0±1.41	-	-	-
	Chlorofom	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Ethyl acetate	16.5±2.12	14.0±2.82	13.0±1.41	-	-
P. Luteola	**Methanol**	19.0±1.41	14.5±0.70	13.0±1.41	-	-
	Chlorofom	-	-	-	-	-
	Aqueous	-	-	-	-	-
	Ethyl acetate	16.5±2.12	11.0±1.41	-	-	-
R.ornithinolytical	**Methanol**	19.0±1.41	12.0±1.41	-	-	-
	Chlorofom	-	-	-	-	
	Aqueous	-	-	-	-	

Results were presented as mean ± standard deviation of duplicate determination. – represent no zone of inhibition.

Source: Author`s Field Work,2024.

The susceptibility testing of multiple antibiotic discs against six bacterial isolates (*Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, Acinetobacter baumannii, Rauitella ornithinolytica,* and *Pseudomonas luteola*) revealed varying levels of resistance and sensitivity, based on the Clinical Laboratory Standards Institute (CLSI) reference values. For Amoxicillin (Am, 30 µg), all isolates showed an inhibition zone of 6 mm, which is below the CLSI resistance threshold, indicating complete resistance across all isolates, indicating a 100%resistance rate.Nalidixic acid showed moderate inhibition zone of,10 mm for *K. pneumoniae*, 12 mm for *E.*

coli, and 6 mm for the other isolates with 16.67% of isolates being intermediate, 16.67% intermediate, and 66.67% resistant.Amikacin (AK, 30 µg) exhibited better activity, with sensitivity, intermediate, and resistance rates equally distributed at 33.33%.Amikacin presented inhibition zones of 19 mm for both *K. pneumoniae* and *E. coli,* which classify as intermediate (17-19 mm), while *Enterobacter cloacae* showed resistance at 16 mm. Other isolates, including *A. baumannii*(12 mm), *R. ornithinolytica* (11 mm), and *P. luteola* (18 mm), also fell in the resistant or intermediate categories.Nitrofurantoin displayed 50% sensitivity, 16.67% intermediate response, and 33.33% resistance, indicating moderate efficacy. Gentamycin had no sensitive isolates, with 50% intermediate and 50% resistance rates, suggesting limited effectiveness, only *Enterobacter cloacae* met the susceptibility standard with an 18 mm zone (≥17 mm), while the other isolates had inhibition zones between 10 mm and 13 mm, indicating resistance (≤14 mm).

Gentamycin (CN, 10 µg) did not achieve the susceptibility threshold (≥18 mm) for any isolate; inhibition zones ranged from 10-14 mm, reflecting resistance across all bacterial strains. Augmentin (AUG, 30 µg) was generally ineffective, with inhibition zones indicating resistance intermediate rates of 50%,for most isolates, except *P. luteola,* which reached 17 mm, classifying as intermediate. Ciprofloxacin (CIP, 10 µg) showed intermediate inhibition zones for *K. pneumoniae* (18 mm) and *P. luteola* (16 mm) i.e.,66.67% resistance and only 33.33% intermediate response, while other isolates were below the resistance thresholds.

With Ceftriaxone (CRO, 25 µg), none of the bacterial isolates met the susceptibility criteria of ≥23 mm, with all isolates falling into intermediate rates equally distributed at 50% and 33.33%, respectively, cefepime also resistance. Ceftriaxone and Cefepime both had no sensitive isolates, Ceftazidime (CAZ, 30 µg) and Cefepime (FEP, 30 µg) displayed limited efficacy, against *K.*

pneumoniae at the intermediate threshold (17 mm for CAZ), while most other values signified with 50% sensitivity, 33.33% intermediate response, and 16.67% resistance. Finally, Imipenem (IMP, 10 µg) demonstrated the highest effectiveness, especially with *Enterobacter cloacae* (21 mm) and *R. ornithinolytica* (22 mm) reaching susceptibility levels (≥20 mm), Imipenem emerged as the most effective antibiotic, with 83.33% sensitivity, 16.67% intermediate response, and no resistance observed.

Overall, the high resistance levels among these bacterial isolates indicate limited susceptibility, with only a few cases of intermediate sensitivity observed primarily for Amikacin, Imipenem, and Nitrofurantoin.

Table 4.6: Antibiotic Susceptibility Pattern against Various Bacterial Isolates Tested

Antibiotic Disc	Code	Conc.	*Klebsiella pneumoniae*	*Escherichia coli*	*Enterobacter cloacae*	*Acinetobacter baumannii*	*Rauitellaornithinolytica*	*PseudomonasLuteola*
Amoxicillin	Am	30ug	6m ®	6m (R)	6m ®	6m ®	6m ®	6m ®

Nalidixic acid	NAL	30u	10m (R)	12m (R)	6m ®	6m ®	6m ®	6m ®
Amikacin	AK	30u	19m (S)	19m (S)	16m (S)	12m ®	11m ®	18m (S)
Nitrofurant on	NIT	30u	12m ®	10m ®	18m (S)	12m ®	13m ®	10m ®
Gentamy	CN	10u ®	12m ®	11m ®	14m ®	13m ®	13m ®	10m ®
Augmentin	Aug	30u	16m (I)	16m (I)	16m (I)	13m ®	14m (I)	17m (I)
Ciproflox acin	Cip	10u	18m (S)	12m ®	10m ®	12m ®	14m (I)	16m (I)
Ceftriaxone	Cro	25u	15m (I)	16m (I)	12m (I)	10m (I)	12m (I)	11m (I)
Ceftaxidim e	CAZ	30u	17m (I)	15m (I)	16m (I)	14m (I)	17m (I)	15m (I)
Cefepime	FEP	30u	18m (S)	18m (S)	18m (S)	14m (I)	16m (I0)	8m ®
Imipenem	IMP	10u	14m (I)	18m (S)	21m (S)	20m (S)	22m (S)	16m (I)

Source: Author`s Field Work, 2024

S- indicate (sensitive), I-indicate (intermediate), R-indicate(resistance).

Table 4.7Outlines the reaction potency of different solvent extracts, which provides qualitative outcomes for each bacterial isolate tested against the extracts, where - indicates a negative reaction (no antibacterial activity) and √ indicates a positive reaction (presence of antibacterial activity). Ethyl acetate extracts showed positive reactions against Escherichia coli and Pseudomonas luteola, resulting in a total potency of 2 out of 6 isolates (33%), while methanol

extracts showed positive reactions against Klebsiella pneumoniae, Escherichia coli, and Pseudomonas luteola, achieving a total potency of 3 out of 6 isolates (50%).

Both chloroform and aqueous extracts showed no positive reactions, resulting in a total potency of 0 out of 6 isolates (0%).

Table 4.7: Percentage Reaction Potency of extract of *Phoenix dactylifera* against the Bacterial Isolates Used

Isolates	Ethyl acetate	Methanol	Chloroform	Aqueous
K. Pneumonia	-	√	-	-

E. Coli	√	√	-	-
E. cloacae	-	-	-	-
A. Baumannii	-	-	-	-
R. Ornithinolytica	-	-	-	-
P. Luteola	√	√	-	-
Total	2	3	0	0
Total potent	2/6	3/6	0/6	0/6
% Potency	33%	50%	0%	0%

√-indicate positive

--indicate negative

Source: Author`s Laboratory Work, 2024.

4.2 Discussion of Finding

The findings from this study highlight the antimicrobial potential of *Phoenix dactylifera* (date fruit and seeds) extracts against beta-lactamase-producing bacterial isolates from urinary tract

infections, an area of growing concern due to the increasing resistance among Gram-negative bacteria. Beta-lactamase enzymes are known to degrade beta-lactam antibiotics, leading to multidrug-resistant infections that are challenging to treat. Consequently, the search for alternative and complementary therapies has gained significant attention, with plant-derived compounds being explored for their bioactive properties[1]. The qualitative analysis of phytochemical screening in Table 4.1 reveals that both the fruit and seed extracts are rich in terpenoids, flavonoids, phenols, alkaloids, tannins, and saponins, particularly in methanol extracts. These compounds are widely known in the literature for their antimicrobial properties, with alkaloids and flavonoids in particular being linked to the inhibition of bacterial cell walls and metabolic pathways. Quantitative analysis in Table 4.2 was showed higher concentrations of phenols, flavonoid, Tannin and alkaloids in methanol extracts, further corroborating their antimicrobial efficacy, this are likely to be antibacterial effect on beta-lactamase produced bacteria. The observed potency of these phytochemicals aligns with other studies, which suggest that flavonoids and tannins can damage bacterial cell walls and interrupt enzyme activities[2]. Tables 4.1 and 4.2 highlight the phytochemical contents of the various solvent extracts in which both the fruits and seeds were rich in terpenoids, flavonoids, phenols, alkaloids, and tannins, particularly in methanol and ethyl acetate extracts. Quantitative analysis showed that methanol extracts contained the highest concentrations of these bioactive compounds, with phenols (208.8 ± 9.95 mg/ml in fruit and 200.7 ± 19.6 mg/ml in seeds) and alkaloids (174.2 ± 1.08 mg/ml in fruits) being the most abundant. These findings align with previous studies, which have identified phenols, flavonoids, and alkaloids as major phytochemical constituents responsible for the antimicrobial properties of date fruits[3]. The high concentration of phenolic compounds, in

particular, may contribute to the extracts' ability to scavenge free radicals and inhibit bacterial growth by disrupting cell walls and membranes [4].

The study's analysis of various solvent extracts (methanol, ethyl acetate, chloroform, and aqueous) demonstrated that methanol and ethyl acetate extracts of both the fruit and seeds exhibited noteworthy antibacterial activity. Methanol extracts, in particular, showed inhibition zones that were sometimes comparable to conventional antibiotics in terms of effectiveness, though generally lower in magnitude. In Table 4.3, methanol extracts of the fruit displayed significant inhibitory effects against *Klebsiella pneumonia* at 150 mg/mL with intermediate inhibition zone of16.0 ± 1.41mm indicate significant, as the concentration decreases the inhibition zone decreases to 0±0mm at 60mg/ml, this alignment with earlier research, which also identified methanol as a potent solvent for extracting bioactive components in date fruits[5]. This moderate level of activity, although less potent than certain antibiotics (e.g., Ciprofloxacin with a CLSI sensitivity threshold of ≥26 mm), is significant, especially considering that natural extracts often act through mechanisms different from those of conventional antibiotics, potentially reducing the likelihood of cross-resistance.

Similarly, Table 4.2 highlights the effectiveness of methanol and ethyl acetate seed extracts, with methanol showing potent inhibition against *Pseudomonas luteola* (18.0 ± 1.41 mm). This activity supports findings in current literature that suggest methanol is particularly effective for extracting bioactive compounds like alkaloids, flavonoids, and phenols from *Phoenix dactylifera*, in which flavonoid poses Quercetin and kaempferol inhibit beta-lactamase enzyme and exhibit antimicrobial activity[7]. The antibacterial activity of seed extracts is consistent with existing literature, where the seed extracts of *Phoenix dactylifera* have been reported to contain bioactive

compounds like saponins, flavonoids, and phenolics, which have shown antimicrobial potential. These compounds might disrupt bacterial cell walls or inhibit enzyme activity, thereby contributing to the observed inhibitory effects[8].

The results indicate that ethyl acetate seed extracts also provided considerable inhibition zones against *E. coli, E. cloacae* and*Pseudomonas luteola* (17.0 ± 1.41-11.0±1.41mm) at 150 mg/mL, suggesting the presence of moderately potent antimicrobial agents within these solvent fractions. This finding aligns with existing literature where ethyl acetate has been identified as a superior solvent for isolating phytochemicals, particularly phenolic and flavonoid compounds, which are known for their antimicrobial properties[9]. In this study, p-values were statistically significant ($p < 0.05$) for many of the extracts, further supporting the efficacy of these plant-based antimicrobials in inhibiting pathogenic bacterial growth. Notably, these zones were generally lower than those seen with CLSI-standard antibiotics such as Gentamycin (≥18 mm) and Amikacin (≥20 mm) but indicate a promising supplementary role in antimicrobial therapy.

Table 4.3 reveals a notable enhancement in activity when combining the fruit and seed extracts, particularly in methanol and ethyl acetate fractions. The methanolic extract of combine fruit and seed extract showed the highest zones of inhibition against all the isolates at the highest concentration of 150 mg/mL with inhibition zone of (22.0±1.41-19.0±1.41) 0.01*-0.1414* as the extract concentration reduced the inhibition zone also reduced. Ethyl-acetate also have same pattern with methanolic extract across all the tested isolates at 150mg/ml with the inhibition zone of (20.0±0.33-16.5±2.12) 0.01*-0.1257*. Chloroform and Aqueous extract was showed no inhibition zone across all the extracts with the various concentrations. This indicating that synergistic interactions may boost antimicrobial efficacy. This aligns with existing research,

where combining plant extracts is shown to increase bioactivity, making them effective against a broader range of bacterial pathogens, the combined effect of fruit and seed extracts exhibited a stronger inhibitory action than individual extracts, particularly against *Klebsiella pneumonia, Escherichia coli*, and Pseudomonas luteola[10]. Synergistic effects between plant extracts have been documented in various studies, where different phytochemicals work together to produce a more potent antibacterial action[11]. This could be due to complementary mechanisms of action, such as the disruption of bacterial membranes and inhibition of protein synthesis.

When comparing the extracts' efficacy with conventional antibiotics as seen in Table 4.7, specific antibiotics like amikacin and imipenem displayed relatively strong inhibition across multiple bacterial isolates, with zones exceeding 16 mm. However, traditional antibiotics like amoxicillin showed minimal to no inhibition, particularly against resistant strains, with zones limited to 6 mm. This suggests that, while standard antibiotics remain effective, particularly imipenem and amikacin, *Phoenix dactylifera* extracts could serve as a viable alternative or complementary options, especially against resistant strains where antibiotics like amoxicillin fail. Methanol extracts emerged as the most potent fraction, comparable to the stronger conventional antibiotics, particularly for pathogens such as *Escherichia coli* and *Pseudomonas luteola*. These findings suggest that methanol and ethyl acetate extracts of *Phoenix dactylifera* could potentially enhance or complement existing antibiotic treatments, especially as resistance continues to limit the efficacy of traditional antibiotics[12]. This synergistic effect is corroborated by findings in similar studies, which demonstrated that combinations of plant extracts could lead to enhanced efficacy, likely due to the complementary actions of various phytochemicals[8]. Compounds such as flavonoids, phenol, tannins, and alkaloids are commonly found in date fruits and are reported

to have antimicrobial and antioxidant properties, which may work together to inhibit bacterial growth more effectively than isolated compounds[13].

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Chapter Five

5.1 Summary of Findings

This study evaluated the phytochemical and antibacterial activity of the extracts from *Phoenix dactylifera L.* fruit and seeds with standard antibiotics against beta-lactamase-producing bacteria isolated from urinary tract infections. It was discovered that bio-active presents in date fruit and seeds were the most responsible for the susceptibility testing against the tested isolates. Among the standard antibiotics tested were Amikacin and Imipenem which demonstrated the highest efficacy, with inhibition zones reaching up to 22 mm against most bacterial isolates. Ciprofloxacin showed moderate activity, with inhibition zones ranging between 12–18 mm. In contrast, Amoxicillin and Nalidixic acid exhibited minimal activity, with inhibition zones as low as 6 mm, reflecting resistance among the tested isolates.

The fruit extracts of *Phoenix dactylifera L.* showed significant antibacterial activity, particularly when methanol was used as the extraction solvent. Methanol extracts exhibited the highest inhibition zones, especially against, *Klebsiella pneumoniae* (16.0±1.41 mm) *Escherichia coli* (17.0±1.41 mm) and *Enterobacter cloacae* (19.5±7.78 mm) at 150 mg/mL. Ethyl acetate extracts demonstrated moderate activity, while chloroform and aqueous extracts did not show measurable inhibition zones. Similarly, the seed extracts showed notable activity, with methanol extracts being the most effective. These inhibited *Escherichia coli* and *Pseudomonas luteola* with zones of inhibition of 15.0±1.41 mm and 18.0±1.41 mm, respectively.

Methanol proved to be the most efficient solvent, yielding higher antibacterial activity than ethyl acetate, chloroform, and aqueous solvents. Additionally, the antibacterial activity of both fruit

and seed extracts was dose-dependent, with the highest concentration (150 mg/mL) producing the most significant inhibition zones. Lower concentrations (75 mg/mL and 60 mg/mL) exhibited reduced activity.

Synergistic effects were observed when combining fruit and seed extracts, with enhanced activity against *Klebsiella pneumoniae* and *Pseudomonas luteola*. This suggests that combining different parts of the plant might improve antimicrobial efficacy.

The findings indicate that methanol-based extracts of *Phoenix dactylifera L.* possess strong antimicrobial properties against drug-resistant pathogens, particularly beta-lactamase producers. These results underscore the potential of plant-based antimicrobials as alternatives to conventional antibiotics and highlight the need for further research to optimize extraction methods and isolate active compounds.

5.2 Conclusion

While the inhibition zones of *Phoenix dactylifera* extracts generally fall short of those produced by high-potency antibiotics as per CLSI standards, the results indicate a substantial antimicrobial potential, especially with methanol and ethyl acetate extracts, either alone or in combination. This supports the potential application of *Phoenix dactylifera* extracts as complementary antimicrobial agents, particularly for resistant infections. The study underscores the value of exploring phytochemicals as alternative therapies, with implications for integrating such natural compounds into broader antimicrobial strategies, particularly against antibiotic-resistant pathogens. The current treatment of many diseases relies on synthetic drugs, which are often expensive and can interfere with genetic and metabolic pathways, leading to unwanted side effects. As a result, there is a need for safer, more effective approaches to prevent the development and progression of diseases.

5.3 Recommendation

Based on the findings of this study, the following recommendations are made: Additional studies should be conducted to explore the detailed mechanisms of action of the bioactive compounds in *Phoenix dactylifera* extracts. This includes identifying the specific compounds responsible for the antibacterial activity and understanding their interaction with bacterial cells. Clinical trials are essential to evaluate the safety and efficacy of these extracts in human subjects. This will help in determining the appropriate dosages and potential side effects. The synergistic effects observed with fruit and seed extract combinations suggest that combination therapy could be a promising approach. Future studies should investigate the optimal combinations and concentrations for maximum antibacterial efficacy. Standardizing the extraction process and formulating the extracts into suitable dosage forms (e.g., capsules, ointments) will be crucial for practical therapeutic applications. This includes ensuring that the extracts' phytochemical content and stability are consistent. While this study focused on urinary tract clinical isolates, future research should explore the efficacy of *Phoenix dactylifera* extracts against a broader range of pathogens, including those responsible for other types of infections. Investigating other solvents for extraction may reveal even more effective methods for isolating the bioactive compounds. This could enhance the antibacterial efficacy of the extracts.

5.4 Contribution to Knowledge

This study would lead to the development of some drug-like candidates in *P.dactylifera*that could be used to formulate novel and more effective antibacterial drugs. It can equally be use as an alternative to antiseptic due to high phenol present in both fruit and seed.

5.5 Suggested Areas of Research

i. Further studies should explore the individual phytochemical constituents within the solvent extracts to identify specific compounds responsible for medicinal properties.

ii. Investigate the mechanisms through which different phytochemicals exert their biological effects, particularly their antimicrobial, antioxidant, and anti-inflammatory activities.

iii. Conduct comparative studies on the phytochemical contents and antimicrobial properties of *Phoenix dactylifera* varieties from different geographical regions.

iv. Evaluate the clinical efficacy of phytochemical-rich extracts from *Phoenix dactylifera* in treating various diseases through well-designed clinical trials.

5.6 Research Limitations

i. The study was limited to four solvents (ethyl acetate, methanol, chloroform, and aqueous), and other solvents could be investigated for their extraction efficiency.

ii. The quantitative analysis was limited to a few phytochemicals, and a more comprehensive analysis including other bioactive compounds would provide a more complete profile.

iii. The antimicrobial susceptibility testing was based on selected antibiotics, and including a broader range of antibiotics would give a more extensive understanding of resistance patterns.

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This is to certify that, this Thesis written by **Wasiu Bode Oyekola with** Matric No. **LCU/PG/000533**, in the Department of Biological Science, Faculty of Natural and applied Science Lead City University Ibadan is in full compliance with the approved university format and style.

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