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ANTIMICROBIAL EFFECTS OF FLUOR BEARING SCHIFF BASE DERIVATIVES AND THEIR METAL COMPLEXES ON *Streptococcus pyogenes*, *Proteus mirabilis* AND *Klebsiella pneumoniae*

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FLOR İÇEREN SCHİFF BAZI TÜREVLERİNİN VE BUNLARIN METAL KOMPLEKSLERİNİN *Streptococcus pyogenes, Proteus mirabilis* VE *Klebsiella pneumoniae* ÜZERİNDEKİ ANTİMİKROBİYAL ETKİLERİ

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Patojenlerde mevcut ilaçlara karşı direnç gelişimi dünyanın en büyük sağlık sorunlarından biridir. Bu çalışmada, yeni antibakteriyel ilaç araştırmalarına katkı amacıyla, iki florofenil-bütilsalisisil aldimin Schiff bazı ligandı ile bunların bakır ve paladyum kompleksleri içeren *Klebsiella pneumoniae, Proteus mirabilis* ve *Streptococcus pyogenes* üzerindeki antibakteriyel aktivitesi MTT analizi ile araştırıldı. Kimyasallar ve pozitif kontrol (gentamisin) mikroplakalar üzerinde üçlü düzende 1µM, 10µM, 100µM ve 1000µM olacak şekilde ekildi ve üzerine 0.5 McFarland dozunda bakteri suşları eklendi. 18 saat inkübasyondan sonra, bileşiklerin her birinin MIC_{50} değerlerini saptamak için, bir canlılık göstergesi olarak MTT boyası uygulandıktan sonra formazan kristallerini çözmek için çözücü olarak dimetil sülfoksit kullanıldı. Her bir kuyucuk için 570 nm dalga boyunda bir mikroplaka okuyucuyla elde edilen OD değerleri, her kimyasalın MIC_{50}'sini hesaplamak için kullanıldı. Gentamisin MIC_{50} değerleri *K. pneumoniae, P. mirabilis* ve *S. pyogenes* için sırasıyla 2,09, 1,87 ve 1,68 µM olarak belirlendi, ve *K. pneumoniae* ve *P. mirabilis*'e karşı en güçlü MIC_{50} değerleri C1 ve C4 tarafından sırasıyla 6,06 ve 5,31 µM olarak belirlendi. *S. pyogenes* neredeyse tüm bileşiklere karşı güçlü etkinlik sergilerken, en etkili MIC_{50} değeri C3 ile 3,72 µM olarak belirlendi. Çalışmamızda özellikle Schiff bazı bakır kompleksinin *S. pyogenes* üzerine güçlü antibakteriyel etkisinin dikkate değer olduğunu düşünmekteyiz.

ANAHTAR KELİMELER: Antibakteriyel direnç, Schiff bazları, MTT tahlili, MIC_{50}

ABSTRACT

MSc Thesis

ANTIMICROBIAL EFFECTS OF FLUOR BEARING SCHIFF BASE DERIVATIVES AND THEIR METAL COMPLEXES ON *Streptococcus pyogenes, Proteus mirabilis* AND *Klebsiella pneumoniae*

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The development of resistance in pathogens to existing drugs is one of the world's biggest health problems. In this study, in order to contribute to new antibacterial drug research, two fluorophenyl-butylsalicysyl aldimine Schiff base ligands and their antibacterial activities on *Klebsiella pneumoniae, Proteus mirabilis and Streptococcus pyogenes* containing copper and palladium complexes were investigated by MTT analysis. Chemicals and positive control (gentamicin) were seeded on microplates in triple order as 1µM, 10µM, 100µM and 1000µM, and bacterial strains were added at a dose of 0.5 McFarland. After 18 h incubation, dimethyl sulfoxide was used as a solvent to dissolve formazan crystals after applying MTT dye as a viability indicator to determine the MIC_{50} values of each of the compounds. OD values obtained with a microplate reader at 570 nm wavelength for each well were used to calculate the MIC_{50} of each chemical. Gentamicin MIC_{50} values were determined to be 2.09, 1.87, and 1.68 µM for *K. pneumoniae, P. mirabilis, and S. pyogenes*, respectively, and the strongest MIC_{50} values against *K. pneumoniae and P. mirabilis* were by C1 and C4, respectively. It was determined as 6.06 and 5.31 µM. While *S. pyogenes* exhibited strong activity against almost all compounds, the most effective MIC_{50} value was determined as 3.72 µM with C3. In our study, we think that the strong antibacterial effect of Schiff base copper complex on *S. pyogenes* is particularly noteworthy.

KEYWORDS: Antibacterial resistance, Schiff bases, MTT assay, MIC_{50}

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LIST OF SYMBOL AND ABBRIVATIONS

ATCC	American Type Culture Collection
Co	Cobalt
Cu	Copper
DMSO	Dimethyl sulfoxide
ELIZA	Enzyme-Linked immunosorbent assay
GAS	Group A *streptococci*
IZ	Inhibition zones
KPC	*Klebsiella pneumoniae* carbapenemase
M.W	Molecular weight
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
mL	Mililitre
mm	Millimeter
mM	Millimolar
MRSA	Methicillin-Resistant *Staphylococcus aureus*
PLA	Pyogenic liver abscess
SBs	Schiff bases
SPSS	Statistical Package for the Social Science
STSS	*Streptococcal* toxic shock syndrome
UTI	Urinary Tract Infection
WHO	World Health Organization
μg	Microgram
μL	Microlitre

1. INTRODUCTION

At the current century, the pathogen predominance has had a remarkable negative influence on the treatment of microbial infections. Despite constant technological advancement and the continuous efforts of researchers, the global escalation of microbial infection is still a threat to humanity so that in the developing scientific epoch, infectious diseases come into sight as a result of technological breakthroughs, this also leads to an extensive mortality and morbidity rate worldwide (Ray et al., 2017).

Antibiotic resistance is a phenomenon in which bacteria acquire the ability to endure and stop responding to antibiotics as a result of alterations and mutations that occur in them due to antibiotic misuse. Raising the cost and toxicity of alternative medicines, extending hospital stays, making it difficult to treat disease, increasing the risk of disease outbreaks, and reducing our treatment arsenal are consequences of antibiotic resistance (Conly, 2002; Chinemerem et al., 2022).

Although antibiotics have been used as an important weapon to combat infection, they have also been used in many other areas, such as health care systems, animal husbandry, food production, and agriculture, since their discovery in 1924 (Kundar and Gokarn, 2022). As described in (Fodor et al., 2020), after the first discovery of Antibiotic resistance in the 1940s. According to the Wellcome Trust and the UK government, the death rate from resistant infections is 700,000 a year, which is expected to rise to 10 million by 2050. Therefore, by 2050, this figure will exceed the cancer death rate of 8.2 million in 2019 (Wall, 2019).

Klebsiella pneumoniae (*K. pneumoniae*) is one of the most ESCAPE- or antibiotic-resistant pathogens, according to recent World Health Organization (WHO) publications, associated with both hospital-acquired and community-acquired pathogens (Chatupheeraphat et al., 2023). Both strains of *K. pneumonia*

(conventional and hyper virulent) are capable of causing a wide range of infections, such as Friedländer's pneumonia, rhinoscleroma, and the emerging disease pyogenic liver abscess (PLA)(Brisse et al., 2009; Cardenas-Alvarez et al., 2023). *Streptococcus pneumoniae, Staphylococcus aureus, K. pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Moraxella catarrhalis*, and *Escherichia coli*, as demonstrated, are the main causes of typical pneumonia (Assefa, 2022). (Basu, 2009) revealed that, recently, the important pathogen of PLA is *K. pneumoniae*. Despite that fact, *Escherichia coli* have been declared as a major pathogen in western nations. A large number of plassmids found in *Klebsiella spp*. are responsible for resistance to most-β lactams, including extended-spectrum cephalosporins and, most currently, carbapenamcetanes. In addition to topoisomerase mutations, porin loss and energy-dependent egress are the two co-occurring mutations present in the vast majority of multidrug-resistant *Klebsiella* isolates, which have been shown to produce a fluoroquinolone-resistant phenotype in *K. pneumonia* (Schneiders et al., 2003).

Gramm-negative *Proteus mirabilis* (*P. mirabilis*) are multi host opportunistic pathogens, with their high virulence factor becoming a major public health issue. *Proteus mirabilis* is a zoonotic bacterium that has been observed in several species of animals, such as dogs, monkeys, sheep, goats, raccoons, chicks, ducks, turtles, and other mammals. Although can be seen in a wide range of settings, such as soil, contaminated water, and sewage (El-Tarabili et al., 2022; Liu et al, 2023). It has been reported that the most common conditions caused by *P. mirabilis* are diabetic foot infection, pyelonephritis, urethritis, bacteremia, myelitis, otitis media (Meng et al., 2023), respiratory tract infection (RTI), urinary tract infection (UTI), and gastrointestinal tract infection (Liu et al., 2023). According to studies carried out on 617 *Proteus* species collected in Łódź (2006–2011) from various clinical sources, showed that 80–90% of all *proteus* infections belonged to *P. mirabilis* (Palusiak, 2022).

Gram-positive and highly host-compatible *Streptococcus pyogenes* (group A streptococci; GAS) can cause both mild infections such as impetigo and pharyngitis

as well as severe diseases such as septicemia, streptococcal toxic shock syndrome (STSS), and necrotizing fasciitis (Brouwer et al., 2023). Approximately, there are 616 million cases of GAS pharyngitis worldwide, and acute GAS infections are thought to cause 517,000 deaths per year. 9,000 to 11,500 severe cases of GAS and 1,000 to 1,800 deaths are reported annually in the United States, according to the Centers for Disease Control and Prevention (Dao et al., 2023). Regarding (Pletz et al., 2006) Fluoroquinolone resistance among *S. pyogenes* is widely reported due to a number of processes. In addition, *S. pyogenes* acquired resistance against erythromycin and similar drugs through mutations in the target site and efflux mechanism (the M phenotype) (Bingen et al., 2000).

Schiff bases are a class of ligands that, due to their ease of manufacturing, coordination properties, potential biological implications, and astonishing diversity of structural properties, are frequently employed in coordination chemistry (Oboňová et al., 2023). The C=N double bond atom is the main feature of Schiff bases; these substances are also known as imines by International Union of Pure and Applied Chemistry (IUPAC), and as azomethine by many others. Schiff bases have various uses for a variety of ailments due to their susceptibility structures, such as antimicrobial, antiviral, antituberculosis, antioxidant, and anticancer (Alfonso-Herrera et al., 2022). Despite the wide range of their biological activity, they have also been used in coordination chemistry: in analytical chemistry, as optical chemo sensors, as dyes, as polymers, and in catalysis (Beč et al., 2023).

The coordination of Schiff bases with metal complexes has been widely used in medicine, according to the findings obtained by testing the ligand, DBAPB, and their complexes against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Ligands and DBAPB is inert against pathogens other than *E. coli*. However, all the complexes showed antibacterial activity, and the high antibacterial effect is shown with Co (II) and Cu (II) against *S. aureus* (El-ghamry et al., 2022). On the other hand, according to the results achieved by (Gaikwad et al., 2022) the metal complexes display lower antibacterial activity against both Gram-positive and Gram-negative bacteria tested than the ligands.

In the absence of effective antimicrobials and widespread dissemination of resistance pathogens, we aimed to develop a new and alternative antibiotic by utilizing the micro dilution method to assess the antibacterial activity of our six fluorinated Schiff bases and their Cupper and palladium complexes against three standard resistance acquisition pathogenic bacteria strains: *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. We applied a modified MTT assay to detect the antibacterial effectiveness of each chemical. Hopefully, our tested compounds will be selected as antibacterial substitute agents.

2. LITERATURE REVIEW

2.1. ANTIMICROBIAL and ANTIMICROBIAL RESISTANCE

2.1.1. Definition of Antimicrobial

An antimicrobial agent can be defined as any natural, semi-synthetic, or synthetic material with the ability to obstruct, slow down, or even completely eradicate the growth and reproduction of microbes (Zhou et al., 2015). Antimicrobials are extensively applied to infection treatment in humans, animals, and plants, such as antibiotics, antivirals, antifungals, and antiparasitics (Dutt et al., 2022).

The inventor of more than 20 antibiotics, Professor Selman Waksman, first coined the term "antibiotics" in 1941. While the "antibiose" term was much earlier used by Paul Vuillemin in 1890 to explain a substance that interferes the action of certain microbes (Dhingra et al., 2020). Therefore, antimicrobials, especially antibiotics, remained the first line of treatment for a long time in medical history (Watts et al., 2017).

2.1.2. Antimicrobial Resistance

Across several decades, with the common intention of curing and remedying mild to severe infections, numerous antimicrobials have evolved and been marketed around the world. Despite the essential role of antimicrobials in boosting human health and lengthening lives, their effectiveness has been severely reduced by the appearance of the antimicrobial resistance (AMR) phenomenon in response to antimicrobials (Murugaiyan et al., 2022).

AMR stands as a serious global peril to human health currently, and millions of people are affected by antimicrobial resistance bacteria annually around the world, with numerous dying as a consequence (Rončević et al., 2019). Also, the constantly rising spreading rate of resistant pathogens is a great danger to global health security (GHS), because getting serious illnesses and higher expenses for antibiotics are the consequences of infections caused by microorganisms resistant to antimicrobial drugs (Ribeiro et al., 2022; Wang et all., 2024).

According to (Shinu et al., 2022), antimicrobial resistance was first identified for penicillin in the 1940s, just a few years after penicillin was initially used as an antibiotic. Then, antimicrobial resistance was common to nearly all of the antimicrobials. Overall, 25,000 deaths in the European Union (EU) and 700,000 around the world were caused by infected antimicrobial resistance pathogens, and it is predicted that AMR will be a leading cause of mortality by 2050, estimated at 10 million deaths (Rahman et al., 2022).

2.1.3. Antibacterial resistance mechanism

Bacterials and antibacterials are in the same ecological niche, so bacteria developed sophisticated resistance mechanisms to overcome the harsh antimicrobial effects. The primarily utilized resistance mechanisms by bacteria are drug uptake inhibition, drug target alteration, drug inactivation, and drug removal by the efflux system. So cell wall, cell membrane, nucleic acid synthesis, and protein synthesis are the four essential targets of antibiotics in bacterial cells, as shown in Figure 2.1. (Salam et al., 2023).

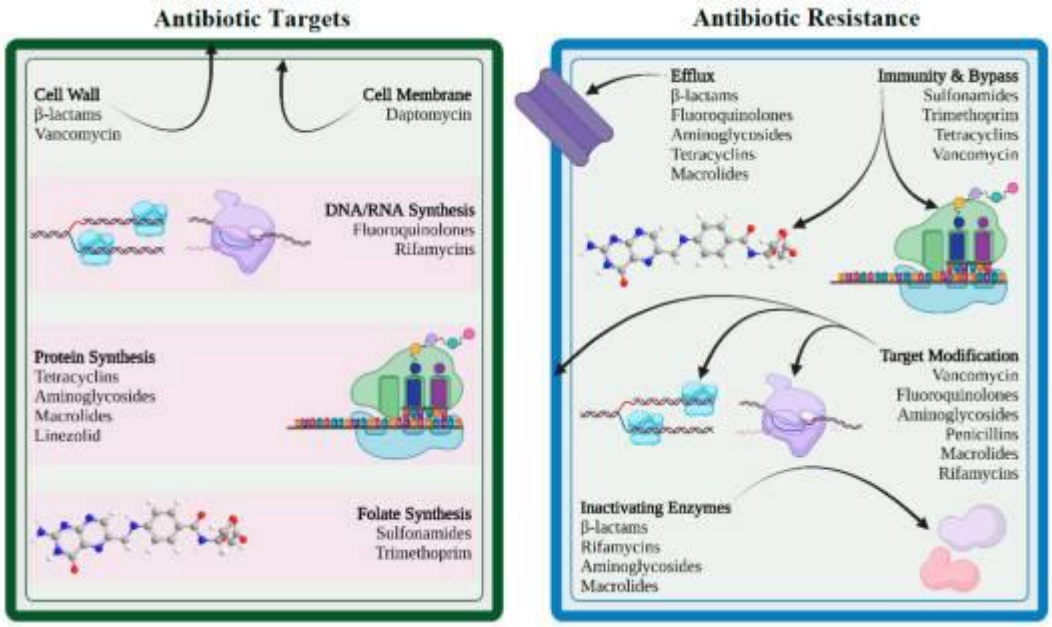

Figure 2.1. Drug targets and drug resistance mechanism (Salam et al., 2023).

Intrinsic, acquired, and adaptive are the three types of resistance in the bacterial population, all of the innate properties that particular bacteria show without any mutation to limit the effectiveness of a certain class of antibiotics are known as intrinsic resistances. Limited outer membrane permeability, in the case of gram negative bacteria such as vancomycin resistance in *E. coli*, and non-specific efflux pumping in many bacteria are referred to as intrinsic resistance (Reygaert, 2018).

Acquired resistance is a second type of resistance in bacteria that occurs through two main causes: Horizontal Gene Transfer (HGT) and bacterial chromosomal DNA mutations (Munita and Arias, 2016). For example, a previously vulnerable bacteria may become resistant through new genetic material incorporation (plasmids, transposons, integrons, and naked DNA, etc.), and this new genetic material acquisition occurs through many mechanisms such as transformation, transduction, and conjugation. For instance, antibiotic resistance strains of *Streptoccocus* spp. have occurred through transformation, and transduction may have contributed to the evolution of resistance in *Staphylococcus aureus*. While the most common mechanism for disseminating resistant genes against various types of antibiotics is plasmid-mediated conjugation, However, conjugation-based gene

transfer in both community and hospital settings contributes to the global dispersion of resistant determinants (Fernandez et al., 2012; Iskandar et al., 2022).

Adaptive resistance is a phenotype resistance that occurs through the acquisition of resistance ability by susceptible bacteria due to environmental changes without any genetic alteration. Also, phenotypic resistance can be described as a transient state in which a population of bacteria that is usually susceptible to antibiotics becomes resistant transiently under a sub inhibitory concentration of antibiotics, along with many environmental signals such as growth factors, pH, stress, nutrition, concentrations of ions, etc. (Olivares et al., 2013; Salam et al., 2023). This resistance type is linked with particular processes like biofilm formation, persistence, or a stationary growth phase (Corona and Martinez, 2013), and swarming (Olivares et al., 2013).

2.1.3.1. Antibacterial resistance via Drug uptake inhibition

For antibiotics or any medication to have a long-lasting effect, it must be inside a bacterial system at high concentrations for an extended period of time. One of the bacteria's most potent weapons to prevent the entry of or keep out any toxic substances, such as antibiotics, is an outer membrane barrier (Ray et al., 2017; Chio and Lee, 2019).

Naturally, limiting the antibiotic uptake by gram positive and gram negative bacteria is different due to their distinct structures in cell membranes. Lipopolysaccharide, a highly acylated glycolipid, is the main structure of gram negative bacteria that serves a significant role in drug passage and reducing into cells. This provides those bacteria with the innate resistance to a wide range of antimicrobial agents (Pagès et al., 2008).

Porin channels in gram negative bacteria, especially those with a larger outer membrane, are a selective gate for hydrophilic molecules to access cells, but due to changes in the permeability of porin channels through mutations and size reduction

in porin channels, the carbapenem resistance in Enterobacteriaceae members has been detected. Also, mutations within the porin channel in *E. aerogenes* have been seen that cause imipenem and certain cephalosporins resistant, and β-lactams and tetracycline resistance in Neisseria gonorrhoeae (Reygaert, 2018; Salam et al., 2023).

The name β-lactam antibiotics are due to the presence of β-lactam rings in their structures, which is a crucial structure in antibacterial activity.β-lactam antibiotics comprise a broad section of antibiotics with four major subclasses: penicillins, cephalosporins, carbapenems, and monobactams. The β-lactam target site is cell wall synthesis inhibition by binding to proteins in bacterial cell membranes (Cisneros-Farrar and C. Parsons, 2007). Resistance to β-Lactam has been observed in many bacteria strains, such as *Pseudomonas aeruginosa*, that is mediated by β-lactamases that destroy the amide bond of the β-lactam ring through reduction of cell membrane permeability, overexpression of efflux pumps, and the acquisition of resistance genes that encode porins and other proteins in the cell membrane (Breijyeh et al., 2020).

2.1.3.2. Antimicrobial resistance via Drug target alteration

Target site modification is a prevailing AMR approach (Lambert, 2005), in which pathogens alter antibiotic target sites, reducing or even eliminating drugs ability to bind to their target sites. Generally, this mechanism occurs via: ribosomal target site alterations, target enzyme modification, and cell wall precursor alterations (De Oliveira et al., 2020).

One of the target site alteration causes is the constant gene mutations in bacterial chromosomes in the presence of antibiotics. For instance, the emergence of rifamycin and quinolone resistance in previously susceptible bacteria is due to mutations in RNA polymerase and DNA gyrase (Ndagi et al., 2020).

According to the (Reygaert, 2018) experiment, one of the main cause of resistances in fluoroquinolones that target nucleic acid synthesis is topoisomerase

and DNA gyrase mutations, which reduce the drug's capacity to bind to these substances in both gram positive and gram negative bacteria. For example, quinolone resistance in *Acinetobacter baumannii* is related to modifications in GyrA (one subunit of DNA gyrase) (Lee at el., 2017).

One of the effective antibiotic groups against gram-positive cocci is macrolides, which were initially introduced in the early 1950s (Cisneros-Farrar and C. Parsons, 2007). On the other hand, *Streptococcus pneumoniae's* resistance to macrolides has been detected via demethylating of the target site by ermB gen, through ribosomal modification mechanism (Zahari et al., 2023).

2.1.3.3. Antimicrobial resistance via Drug inactivation

Inactivation of antimicrobial agents is considered one of the most efficient mechanisms acquired by microorganisms for protection (Peterson and Kaur, 2018). Three main enzyme classes are involved in the modification or destruction of active antimicrobial agents: hydrolases, group transferases, redox enzymes, and lysases. Antimicrobials could be physically altered by these enzymes, as well as by lowering the concentration of drugs in the local setting (Ray et al., 2017; Saha and Sarkar, 2021).

The most well-known instance of hydrolase enzymes is β-lactamases, which play a part in destroying the β-lactam ring (Bonomo, 2017), which is a crucial necessary component for bacterial cell wall inhibition, in the numerous classes of antibiotics, namely: penicillin, cephalosporins, carbapenems, and monobactams. The emergence of resistance in these antibiotics would be the cause of increasing resistance quickly because these are the most commonly prescribed groups of antibiotics (Nainu et al., 2021).

Apart from the β-lactam group, antibiotic inactivation by enzymes has also been identified for the tetracycline group in *E. coli*, despite the fact that antibiotic modification via redox enzymes is not a commonly utilized strategy by pathogens. In

addition, the widely recognized aminoglycoside resistance among ESKAPE pathogens is a result of transferase (Schroeder et al., 2017; De Oliveira et al., 2020).

2.1.3.4. Antimicrobial resistance via Efflux pump

Efflux pumps are one of the protective mechanisms employed by bacteria in situations where antimicrobials could successfully penetrate the cell membrane and easily attain the target site (Gaurav et al., 2023). Efflux pumps have been found in both pathogenic and non-pathogenic bacteria as a part of bacterial physiology, which are proteins in the cell membrane and involved in various functions like homeostasis. In addition to their vital role in raising antibacterial resistance, these bacterial proteins have the ability to expel numerous distinctive antimicrobial agents from the bacterial cytoplasm to the extracellular environment. As a result, the antibiotics intracellular concentration is decreased, reducing their effectiveness (Varela et al., 2021; Muteeb et al., 2023).

According to (Lorusso et al., 2022), in the 1980s, for the first time, the role of efflux pumps on antibiotic resistance was revealed in *Escherichia coli* for tetracycline. While efflux pumps are now one of the main causes of multidrug resistance in some bacteria, including tigecycline or imipenem resistance in *A. baumannii* (Breijyeh et al., 2020). The overexpression of the efflux pump in clinical isolates of *E. aerogenes* and *K. pneumoniae* was also reported. Also based on a publication by (Nikaido and Pagès, 2012), 90% of penetrating ciprofloxacin was pumped out by 92% of clinical isolates of *K. pneumoniae*. There are five categories of efflux pumps, namely: the resistance nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family transporters, and the ATP-binding cassete (ABC) superfamily.(Sharma et al., 2019).

2.1.4. Antimicrobial resistance drivers

Microorganism's inherent features and environmental factors are the main

drivers of AMR. Antimicrobial resistance factors can be divided into the following categories: Human-associated factors (e.g., inappropriate use, misuse, and overuse of antibiotics, lack of consciousness or inadequate education, self-medication), Animal-associated factors (e.g., broadly antibiotic use in agriculture and livestock sections, transmission of AMR via contact with animals).Environmental-associated factors (e.g., poor sanitation and waste systems, rapid spreading through mass travel). Other associated factors (e.g., fake drugs, inappropriate prescribing by physicians, outdated knowledge and instruction) (Endale et al., 2023; Salam et al., 2023).

Misuse of antibiotics contributes to being a strong cause of AMR in developed and undeveloped countries (World health organization, 2023). According to research conducted at a Chinese University hospital, out of 1025 antibiotic-prescribed cases, only 39 cases have undergone microbiological examination to determine the infection's origins. While misconceptions about antibiotics are the major causes of their misuse by individuals. For instance, for most viral infections in non-educational countries, antibiotics are prescribed as a therapy (Chokshi et al., 2019).

Overuse of antibiotics, including self-medication, over prescriptions by clinicians, and antibiotic consumption without prescriptions, is one of the strongest promoters of AMR, and there are interdependent associations between overuse of antibiotics and increased AMR continuously because widespread use of antibiotics in distinct sections such as medical, veterinary, and agricultural and for different purposes is a matter of concern according to the WHO's last warning. For instance, 0.1 to 0.2 million tons of antibiotics are consumed worldwide every year, and beta-lactam antibiotics like penicillins, cephalosporins, and carbapenems are the largest groups of antibiotics (50%–70%) used by humans (Iwu et al., 2020; Endale et al., 2023).

Antimicrobials are intensively utilized in the veterinary and agriculture sections around the world, in both developing and developed countries (Manyi-Loh et al., 2018), for prophylactic, therapeutic, growth, and animal product promotion

(Hosain et al., 2021). In numerous countries around the world, about 50% of the antibiotics used in human infection treatment are currently commercialized and used in agriculture. For instance, in the United States, approximately 80% of crucial antibiotic products are consumed in animal breeding. The excessive use of antibiotics in livestock and especially food-producing animals contributes to increasing AMR via resistance bacteria transmission to humans through direct contact with animals, ingesting animal products, or the environmental spread of animal feces (Manyi-Loh et al., 2018).

Poor quality of drug, incorrect storage technique of drug (e.g., storage medication in high temperature and humidity), and influx of counterfeit and non-standard drugs into the medication market are the major drivers of AMR in some countries by putting microbes under selection pressure. Based on the data obtained from an investigation that has been done in Cameroon, by collecting 284 antimalarials from 132 sellers, most of the antimalarial contained less than the required concentration of chemicals, and some of them had no effective ingredients in their structure (Ayukekbong et al., 2017; Iwu et al., 2020).

On the other hand, travel is one of the factors that elevate the prevalence of AMR. Due to the ease of travel in recent years, travel has increased broadly around the world, leading to the transmission of resistant bacteria genes by visitors in visiting regions to their own countries (Salam et al., 2023).

2.1.5. Strategies to counteract AMR

AMR was detected as a major worldwide challenge by the World Health Organization in April 2014. A year later, WHO adopted an ambitious scheme to confront the prevalence and transmission of AMR around the world under the name" Global Action Plan" (World Health organization, 2015; Irfan et al., 2022).The main five objectives of the global action plan are to: Boosting awareness about AMR, fortifying information through surveillance and investigation, diminish the incidence

of infections, optimal application of antimicrobials or medications, and continue to locate new techniques and drugs to overcome AMR (Inoue, 2019).

Ongoing collaborative effort, continual monitoring, and multifaceted strategies are required to struggle against AMR. There are seven vital strategies to prevent community-hospital-acquired infections, including hand hygiene, environmental hygiene, screening patients, surveillance, antibiotic stewardship, following guidelines, and patient safety (Dhingra et al., 2020; Haque et al., 2020).

Environmental situations like bad hygiene and overcrowded settings have played a role in dispensing resistant pathogens around the world. So, improving environmental hygiene, such as hand hygiene and the hygiene of medical equipment in hospitals and water systems, is an essential proposition to prevent pathogenic resistance exposure and transmission (Smith et al., 2004; Ayukekbong et al., 2017).

Another significant scheme that has been applied to overcome AMR is the Antimicrobial Stewardship Program (ASP). ASP is a strategy through some objective with the aim of eradicating AMR (Rahbarimanesh et al., 2019). Improving prescriber's education to select the right drug with the correct dose and duration for each patient is the first objective of ASP. In addition, keeping from overuse and irrational use of antibiotics is a second objective, and lowering the antimicrobial resistance advancement down to the minimum is the third objective of the stewardship program (Pinto Ferreira et al., 2022).

Vaccination is a basic method of preventing infections, while antimicrobials are used as a therapy. Therefore, increasing vaccination is considered a predictable way to reduce AMR. Although numerous alternatives are used to treat microbes, like probiotics, bacteriophages, bacteriocins and many others, uncovering and discovering a new potent antibiotic remains a crucial requirement (Ayukekbong et al.,2017; Singh et al., 2019; Salam et al., 2023).

2.2. Bacterial strains employed in our investigation

2.2.1. *Klebsiella pneumonia*e

K. pneumoniae, which belongs to the Enterobacteriaceae family, was for the first time identified in 1882 by Carl Friedlander from the lungs of a deceased patient with pneumonia (Bengoechea and Sa Pessoa. 2019). *K. pneumoniae* is a gram negative, rod-shaped, immobile, and mucoid capsule bacteria. It also pervasively resides in environments such as soil, water, medical devices, and animals (Paczosa and Mecsas. 2016; Muhsin et al., 2022).

K. pneumoniae is a major cause of nosocomial infections, including urinary and respiratory tract infections, blood stream infections, and infections in the intensive care unit (ICU), especially in immune-compromised patients. So the emergence of antibiotic resistance in *Klebsiella* strains is considered a vital global health crisis (Chen et al., 2011; Aires-de-Sousa et al., 2019). Overall, antibiotic resistance in *Klebsiella pneumoniae* occurs through two prime mechanisms: first, the expression of extended-spectrum β-lactamases (ESBLs), which confer resistance on bacteria to cephalosporins and monobactams. *Klebsiella pneumoniae* carbapenemases (KPCs) that give bacteria resistance against nearly all available β-lactams, including carbapenems, are considered the second mechanism, which is the most troubling resistance acquisition in *K. pneumoniae* (Parveen et al., 2011; Paczosa and Mecsas, 2016).

Li and Ni (2023) Globally, the death rate was estimated at 600,000 in 2019 due to antibiotic resistance in *K. pneumoniae*, and one of the traits that these bacteria use to protect their cells from antibiotics and any extracellular toxic substances is biofilm formation. In addition to that, it was identified that antimicrobial resistance in biofilm formation bacteria might be 10–1,000 times greater than that of bacteria that do not form biofilms (Planktonic bacteria). Certain factors serve in biofilm formation; one of them is type 3 fimbriae, which are identified as the main facilitators. Apart from the role of biofilm formation, type 3 fimbriae have a crucial

role in *K. pneumoniae* attachment to several cells, including tracheal epithelial cells, extracellular matrix proteins, renal tubular cells, and components of the basement membranes of lung tissue in humans (Chen et al., 2011; Seifi et al., 2016).

After the emergence of fluoroquinolone-resistant and (ESBL)-producing *K. pneumoniae* and Enterobacteriaceae in many countries, carbapenemases were widely used as a preferred treatment for many patients, leading to the appearance of carbapenemase resistance in *K. pneumoniae*. KPCs were detected for the first time in 1996 in one of the USA hospitals and submitted to the Centers for Disease Control and Prevention (CDC) as part of Project Intensive Care Antimicrobial Resistance Epidemiology (ICARE)((Girometti et al., 2014). Bacteria that have carbapenemase enzymes have reduced their sensitivity to numerous antibiotics. For instance, KPCs have acquired resistance to cephalosporins, monobactams, carbapenems, and even beta-lactamase inhibitors, and the only remaining treatment options for this strain are colistin, tigecycline, and one or more aminoglycosides; some are resistant even to these medications (Munoz-Price et al., 2013).

Numerous mechanisms contributed to *K. pneumoniae* resistance to carbapenems, namely: the alteration in outer membrane permeability through loss of Omps, overexpression of the active efflux pump, and acquisition of hydrolysis enzymes like β-lactamases and carbapenemases (Pitout et al., 2015; Elshamy and Aboshanab. 2020; Karampatakis et al., 2023). (Taha et al., 2023) demonstrated that blaKPC, blaOXA-48, and blaNDM-1 genes are responsible for carbapenem-resistant *K. pneumoniae*.

2.2.2. *Proteus mirabilis*

P. mirabilis is a gram negative, facultative anaerobic, heterotrophic, non-capsule, rod-shaped, and multi-host bacterium that infects various animals like chickens, ducks, turtles, cattle, and many companion animals. The *Proteus* genus, which belongs to the Morganellaceae family, was first described by German microbiologist Gustav Hauser in 1885 and includes many species like *Proteus*

mirabilis, Proteus vulgaris, Proteus penneri, and *Proteus hauseri* (Armbruster et al., 2018; Chakkour et al., 2024).

One of the *P. mirabilis* prominent features is their distinctive ability to form swarming motility on the solid surface and on agar plates, which has been identified as the bull's-eye pattern (Belas et al., 1998). Pyelonephritis, urolithiasis, prostatitis, however catheter-associated urinary tract infections (CAUTIs) are illnesses that *P. mirabilis* is considered to be partially responsible for. However, CAUTIs is a polymicrobial infection, and *P. mirabilis* is one of the main causes. *P. mirabilis* utilized numerous virulence factors to prevent their death from host immunity and antibiotics, as well as to colonize and ruin their host tissue, such as flagella, pili, urease, hemolysin, and metalloproteinase. Urease is primarily responsible accountable in CAUTIs through the decomposition of urea into carbon dioxide and ammonia, which increase the pH of urine and participate in crystalline biofilm formation. As a result, catheter blockage hinders urine flow, which may cause an infection in the kidneys (Drzewiecka. 2016; Armbruster et al., 2018; Liu et al., 2023).

bla- resistance determinants that are found in plasmids in bacteria display a key role in the appearance of and dissemination of multidrug resistance among the Enterobacteriaceae family, particularly *proteus* species. According to the (Shelenkov et al., 2020) investigation, intrinsic resistance to tetracyclines and polymyxins, such as colistin, has been recorded in *P. mirabilis*, in addition to acquired resistance to β-lactams. Mo et al., 2022, the substantial increase in antibiotic resistance, especially to cephalosporins, in *P. mirabilis* is a serious problem in the therapeutic management of UTIs.

2.2.3. *Streptococcus pyogenes*

S. pyogenes, or group A streptococci (GAS) is gram-positive, facultative anaerobic, catalase and oxidase-negative, and β-hemolytic streptococci according to hemolysis on blood agar (Gera et al., 2013). *S. pyogenes* is a host-adapted pathogen,

and it was identified that the ecological niche of GAS is quite narrow, and humans are the main host of these pathogens. In addition to some recorded cases of isolation *S. pyogenes* in a free-living European hedgehog (*Erinaceus europaeus*) with infection and other cases regarding dog conjunctivitis-related ocular discharge, there are no other reported cases or sources for GAS other than humans(Vela et al., 2017; Kanwal and Vaitla. 2023).

A vast variety of human infections belong to *S. pyogenes*, these may be superficial infections or quite severe illnesses, such as pharyngitis, impetigo, cellulitis, sepsis, STSS, endocarditis, and necrotizing fasciitis. Also, GAS infections may trigger serious autoimmune diseases, like rheumatic heart disease (RHD), acute rheumatic fever (ARF), and post-streptococcal glomerulonephritis (APSGN) (Brouwer et al., 2023). Globally, there are 616 million pharyngitis cases, 111 million skin infections (primarily impetigo), and 2 million invasive *S. pyogenes* disorder cases, with half a million deaths recorded annually. *S. pyogenes* is still one of the top 10 personal pathogens responsible for infections and death, and most of the death rate due to *S. pyogenes* is attributed to invasive and rheumatoid disease (Bessen. 2009; Beerens et al., 2021).

The only recognized biological host of *S. pyogenes* is humans, similar to a lot of other streptococci. Thus, transmission of these pathogens occurs from humans to humans directly through droplets of respiratory, hand contacts with nose secretions or any other contaminated place with bacteria, touch of skin with polluted lesions, or polluted food sources (Kanwal and Vaitla, 2023). Although the sensitivity of GAS to antibiotics according to geographic locations has changed, it is still universally sensitive to penicillin. In some cases, macrolides and first-generation cephalosporins were prescribed as treatments for GAS infections, but unfortunately, in some parts of the world, resistance to macrolides, cephalosporins, clindamycin and lincosamide increased, which is leading to a major global issue (Walker et al., 2014).

2.3. Schiff bases

2.3.1. Definition of Schiff bases and their synthesis mechanism

The term Schiff bases (SBs) is derived from German chemist Hugo Schiff, who is considered the first discoverer of Schiff bases in 1864 (Raczuk et al, 2022). SBs are a versatile group of organic compounds that contain the azomethine (-HC=N-) or imine (>C=N-) group in their structure (Tsantis et al, 2020). The general structure of Schiff bases is illustrated in Figure 2.2.

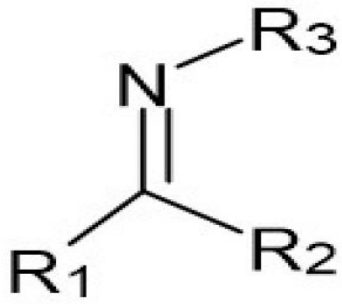

Figure 2.2. General structure of Schiff base (Raczuk et al., 2022)

SBs are the compounds recognized, even as imines or azomethines, as being formed by a reaction between a primary amine and a carbonyl group (aldehyde or ketone) under particular circumstances (Tsacheva et al, 2023). R3R2C=NR1 is the overall formula of SBs, in which the substituents R2 and R3 may be alkyl, aryl, heteroaryl, or hydrogen (Qin et al, 2013).

The Dean Stark apparatus is the most commonly applied technique in the formation of original SBs by condensation of an aldehyde or ketone with a primary amine by expulsion of an H2O molecule, as depicted in Figure 2.3. Lately, numerous newly constructed techniques have been applied by investors for Schiff base preparation purposes, namely: solvent-free, clay, or microwave irradiation, solid-state synthesis, molecular sieves, liquid crystals, water suspension medium, infrared, and ultrasound irradiation (Adesina, 2022).

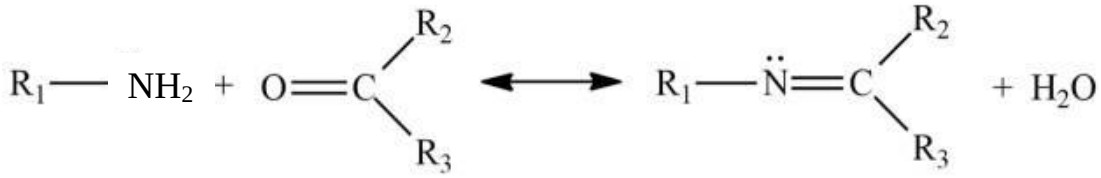

Figure 2.3. Overall scheme of Schiff base formation (Tsacheva et al., 2023)

"Green synthesis" considered a widespread applied procedure in various kinds of imines formation via utilizing aqueous medium, utilizing water as a solvent in the condensation reactions of aldehydes and amines, speeds up reaction time with high yield, and easier drying and simple filtration are required (Rao et al., 2010). Furthermore, SBs can be prepared through other solvent-based techniques, such as the conventional method of synthesizing Schiff bases by utilizing ethanol and methanol as solvents (Chinnasamy et al., 2010).

Infrared irradiation is a simple, effective, and environmentally safe method that is utilized in SB preparation, for instance, in the preparation of substituted N-benzylideneaniline derivatives in the absence of solvents (Vázquez et al., 2009). Furthermore, the synthesis of SBs is accelerated by microwave in the presence of montmorillonite K10 clay as an effective catalyst under solvent-free circumstances (Varma et al., 1997).

"Solid-state synthesis" is another appropriate technique that was used by (Schmeyers et al, 1998) to optimally form twenty azomethines without going through liquid stages by grinding solid anilines with solid benzaldehydes together in a mortar for two hours. Solid-solid condensation is a waste-free method in contrast to azomethine syntheses in solution (acid-catalyzed).

2.3.2. Antimicrobial activity of Schiff base with metal complexes

Over the past decades, numerous researchers have been investigating the components of SBs. Schiff bases are versatile groups of organic compounds that

have diverse pharmacological activities, including antimicrobial, antiproliferative, antimalarial, analgesic, anxiolytic, antidepressant, anti-inflammatory, antiviral, antipyretic, antibacterial, and antifungal activities (Krátký et al, 2017; Tsacheva et al.,2023). One of the Schiff bases characteristics is their capability to bind with numerous distinct metals and form triumphant metal complexes (Ay. 2016; Nagar et al., 2023).

Several studies displayed the pharmacological activities of numerous metals such Copper (Cu), Cobalt (Co), Manganese (Mn), Zinc (Zn), Nickel (Ni), Platinum (Pt), Palladium (Pd), Gold (Au), Argentum 'silver' (Ag) (Olar et al., 2022; Ashraf and Riaz, 2022). In addition, several biological activities of SBs with metal complexes have been demonstrated by (Sinicropi et al., 2022). Also, according to many investigations regarding antimicrobial activities, it was found that SBs-metals complexes have higher activities in comparison to SBs alone or initial Schiff bases (Ceramella et al., 2022).

2.3.2.1. Antibacterial activity of Schiff base

The antibacterial activity of SBs has been proven in numerous investigations. A series of SBs have been synthesized by (Nayak and Poojary, 2019) and screened for their biological activities through the broth dilution method against two Gram-positive (*S. aureus* and *E. faecalis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains, and Ciprofloxacin was used as a positive control. According to the minimum inhibitory concentration (MIC) that was recorded for each Schiff base, all of the newly synthesized compounds exerted excellent antibacterial activity on both gram positive and gram negative bacteria strains. Even some of the compounds displayed a stronger antibacterial action in comparison to the standard drug, with MIC values in the range of 1.56–12.5 μg/mL.

(Azab et al., 2015) reported an investigation about the preparation of a group of Schiff bases and their antibacterial activity compared to ampicillin and

streptomycin as a standard drug against different strains of bacteria, including *Staphylococcus aureus* (Gram positive), *Escherichia coli*, and *Pseudomonas aeruginosa* (Gram negative), by applying the agar diffusion method. The majority of compounds displayed moderate to strong antibacterial activity when measuring the inhibition zone of any of the chemicals. Further, the most powerful activity recorded by 7a, 7b, and 9a compounds is either higher or almost equal to that of reference drugs (ampicillin and Streptomycinon).

The pharmacological potential of metals has been demonstrated in many studies, and recently, the combination of Schiff bases metal complexes has been intensively investigated in various fields. Kumar and coworkers evaluate the antibacterial activity of fluorinated benzaldehydes and their copper (II) complexes against *E. coli* and *S. aureus* with ciprofloxacin as a positive control. As a result, in six Schiff base ligands and four SBs-metal complexes, all of the ligands showed no to moderate effect, while all of the complexes showed good to excellent antibacterial activity, and among the complexes, the best effect was recorded by Cu-L6, which displayed a MIC of 0.76 mM against *S. aureus* (Habala et al., 2016). In addition, the antibacterial activity of Schiff base-cupper and palladium complexes was checked in the study of Alyar et al. by the micro dilution method against *S. maltophilia*, *S. aureus*, *K. pneumonia*, and *E. coli* with the standard drugs Sulfisoxazole and Sulfamethoxazole, and the results demonstrated that synthesized metal complexes have better inhibition effects against bacteria than free ligands. Also, Cu (II) complexes exhibited further inhibition zones in contrast to Pd (II) complexes against evaluated bacteria (Kumar et al., 2023).

2.3.2.2. Antifungal activity of Schiff base

In the study of Magalhães et al., they checked the in vitro antifungal activity of twenty-three cinnamyl-schiff bases against strains of *Candida*, *Aspergillus*, *Fonsecaea*, and specifically, *Cryptococcus* species. Among the compounds, the outstanding antifungal action was recorded by compounds 1 and 23 cinnamyl SBs, whose structures have been shown in Figure 2.4. In comparison to fluconazole that

was used as a reference drug, compounds 1 and 23 displayed minimum inhibitory concentrations against all the *Cryptococcus neoformans* strains (MIC = 1.33 µg/mL and 1.4 µg/mL, respectively) and against *Cryptococcus gattii* strains (MIC = 5.3 µg/mL and 2.8 µg/mL, respectively), which were more than two-fold lower than those of fluconazole. In the case of fluconazole, the MIC of each *Cryptococcus neoformans* and *Cryptococcus gattii* strain was recorded to be 5.2µg/mL and 9.2 µg/mL, respectively. Also, cinnamyl-SB compound 11 was as strong as FCZ in opposition to all strains from both *Cryptococcus species* (Magalhães et al., 2020; Ceramella et al., 2022).

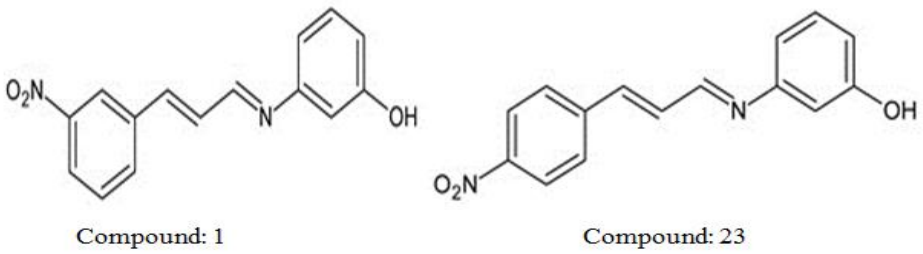

Figure 2.4. Chemical structure of two cinnamyl SBs (Magalhães et al., 2020).

In the era of prevalent infectious disease, uncovering and developing a new and robust antibiotic is considered a great accomplishment of modern science and technology (Chinemerem Nwobodo et al., 2022). For this purpose, two Schiff base ligands L1 and L2 and their complexes with Zn (II) were synthesised and evaluated for their antifungal activity by (Joseyphus and Nair, 2008), against *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataicola,* and *Candida albicans* through the disc diffusion method. In outcomes, ligands exhibited less to moderate activity against all utilised fungals in comparison to the ZnL1 complex, which showed high activity against *A. niger* and *R. stolonifer*, while the best activity against *A. niger, A. flavus*, and *C. albicans* was reported by the ZnL2 complex.

Another attempt to find a new antibiotic was made by (Jesmin et al., 2008),

by screening the antibacterial and antifungal capabilities of three prepared Schiff bases by the disc diffusion method on (*A. flavus, A. fumigatus, A. niger, Candida albicans*, and *Mucor sp*.) and some bacteria strains. The activity of each compound and the standard drug (nystatin) was determined by measuring the inhibition zone of each of them. According to the consequences, *Aspergillius flavus* displayed resistance to all of the chemicals, while each of the compounds showed moderate to good effects against other fungal strains.

2.3.2.3. Antimalarial activity of Schiff base

The urgent requirement for finding a new antimalarial has increased due to the development of drug resistance among malaria parasites and the toxicity of current antimalarial drugs. The antimalarial activities of a series of fifteen Schiff bases derived from sulphonamide was evaluated in comparison to acetazolamide clinical drugs against the carbonic anhydrase enzyme of *Plasmodium falciparum*, which is responsible for metabolic activity in malaria parasites. The antimalarial ability of each compound was determined by detecting the ability of compounds to bind and inhibit the carbonic anhydrase enzyme. Among compounds, the sixth chemical displayed good malarial activity, which was four times more effective than acetazolamide (Krungkrai et al., 2001; Fonkui et al., 2018).

Another effort was reported by Jarrahpour et al. With the intention of developing a new antimalarial by synthesizing the novel Schiff bases bearing morpholine scaffold and assessing against the chloroquine-resistant *P. falciparum* K14 strain through the broth micro dilution assay. As a result, the most effective antimalarial activity was recorded by compound 13, which displayed an IC_{50} of 2.28 μg/mL (Jarrahpour et al., 2015; Fonkui et al., 2018). Malaria disease affects 500 million people annually, with 1-3 million deaths, and the main cause of human malaria is *Plasmodium falciparum* (Da Silva, et al., 2011). Another study showed the antimalarial activity of Azines Schiff base ligands, and their palladium complexes against *P. falciparum*. The remarkable results were reported by L4 and C4 with (IC50s of 0.83 and 0.42 μg/mL, respectively) (Meena and Baroliya, 2023).

3. MATERIAL and METHODS

3.1. Materials

3.1.1. Chemical Compounds

Six fluorinated Schiff base ligand and, palladium and copper complexes utilized in this study have been obtained by Harran University, Faculty of Arts and Science, Chemistry Department. Gentamicin has been used as a positive control and also to compare the antibacterial effects of chemicals.

C1: Ortho-florophenyl-3,5-di-tert-butilsalisilaldimine
M.W: 326 g/mol**.**

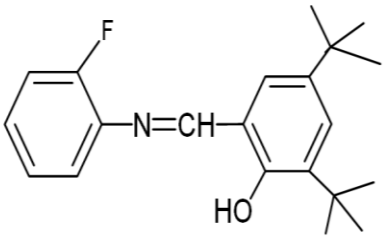

Figure 3.1. The open formula of compound 1.

C2: Bis (ortho-florophenyl-3,5-di-tert-butilsalisilaldiminato)Pd.
M.W: 758.4 g/mol.

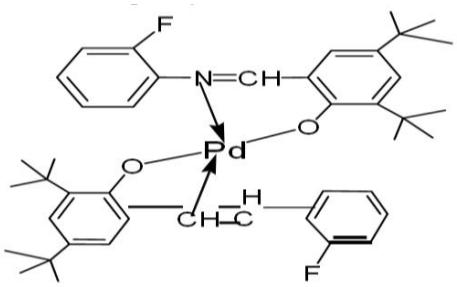

Figure 3.2.The open formula of compound 2.

C3: Bis (ortho-florophenyl-3,5-di-tert-butilsalisilaldiminato)Cu.
M.W: 715,5 g/mol**.**

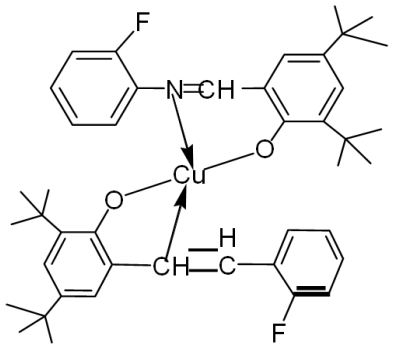

Figure 3.3. The open formula of compound 3**.**

C4: Para-florophenyl-3,5-di-tert-butilsalisilaldimine.
M.W: 326 g/mol.

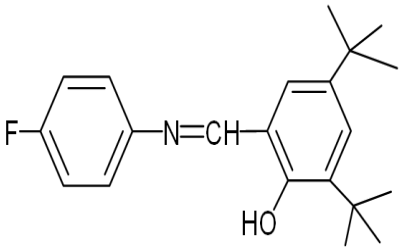

Figure 3.4. The open formula of compound 4**.**

C5: Bis (para-florophenyl-3,5-di-tert-butilsalisilaldiminato)Pd.
M.W: 758.4 g/mol.

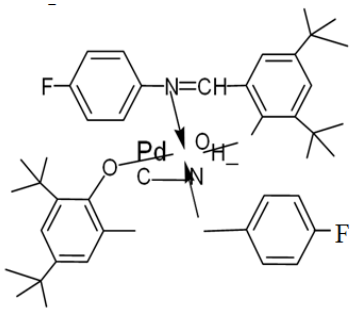

Figure 3.5. The open formula of compound 5.

C6: Bis (para-florophenyl-3,5-di-tert-butilsalisilaldiminato)Cu.
M.W: 715,5 g/mol.

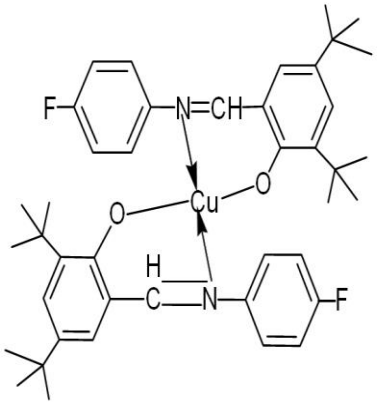

Figure 3.6. The open formula of compound 6.

3.1.2. MTT Stain

MTT (Dimethyl thiazol Diphenyl-tetrazolium Bromide) dye prepared as 250 μM to detect the cytotoxicity and antibacterial activity of our 6 Schiff base compounds on, *K pneumonia, P mirabilis*, and *Streptococcus pyogens.*

3.1.3. Bacteria Strains

P. mirabilis (ATCC 14153), *K. pneumoniae* (ATCC 13883) and *S. pyogens* (ATCC 19615), are the three strains of bacteria that were used in this study.

3.1.4. Bacteria Media

Müller Hinton agar and Nutrient Broth have been used to revive and reproduce the lyophilized bacteria. For future use, the reactivated bacteria are also stored on the side of the petri dish.

3.1.5. McFarland Standard

For all bacterial strains, (0.5) turbidity according to the McFarland turbidity

standards has been prepared by spectrophotometer that is equal to ($1.5x10^8$ CFU / ml) in the medium.

3.2. Method

3.2.1. Compounds preparation

All Schiff base compounds were weighted on a precision balance (Sartorius) to a concentration of 10mM and then dissolved in absolute alcohol. After dissolving each compound in 100 percent ethanol, the sterilization process has been performed by passing each compound through a (20µm pore diameter) syringe type filter (Minisart®, Biotech, USA) (Sani et al., 2018).

3.2.2. Preparation of culture plates

1 µM, 10 µM, 100 µM, and 1000 µM concentrations of SB compounds with gentamicin as a positive control will be added into 96-well micro plates in a triple pattern in 10µl volumes.

3.2.3. Bacteria culture preparation

P. mirabilis, K. pneumonia, and *S. pyogens* were activated in 7 ml of sterile NB medium and incubated for 24 h at 37°C. At the end of incubation, the number of bacteria was adjusted to 0.5 McFarland turbidity, which is equal to 1.5 x 10^8 CFU/ml (Dietvorst et al., 2021), by diluting in sterile NB medium. 100µl of each bacterial strain at the amount of 1.5 x 10^8 CFU/ml was seeded in all wells and incubated at 37°C for 18 h, in which chemicals were planted before. In contrast, for the purpose of stocking bacteria for subsequent application, a portion of activated bacteria from cultured NB medium was transferred to sterile solid medium (Müller Hinton agar and incubated for 24h at 37°C. After the incubation, petri dish stored at 4°C. The method (Mohammed et al., 2022) applied with slight conversion.

3.2.4. MTT assay

After incubation of chemicals with cultured bacteria for 18 hours, 10µl of MTT solution at a dose of 250 mM was applied to all wells for 4 hours at 37°C. During the incubation period, the chemical structure of MTT (yellow- colored) converted to formazan crystal (purple- colored) by living bacteria in culture wells. Ultimately, to dissolve formazan crystals, 50 µl of DMSO (dimethylsulfoxide, Sigma) was added to each well (Benov. 2021).

The optimal optical density (OD) was achieved by putting culture plates into an Enzyme-Linked immunosorbent assay (ELIZA) plate reader (Thermo Scientific, Country) and reading at 570 nm wavelength (Hansen et al., 1989; Benov. 2021) according to color changes in the culture plate. Acquired optical density values were utilized to detect the minimum Inhibitory concentration (MIC_{50}) of every chemicals and antibiotic as well.

3.2.5. Statistical Analysis

Each compound's OD values are displayed as mean and standard deviation (SD) according to the data collected from the triple pattern. Homogeneity of variance was used to analyse all statistical data and ensure consistey amongst all genta dosages and compounds. The results were evaluated using the SPSS version 16.0 application. After the homogeneity of variance test, MIC_{50} values were detected in nonlinear regression analysis by utilizing OD data.

4. RESULTS and DISCUSSION

4.1. Result

4.1.1. Antibacterial activity on *K. pneumoniae*

4.1.1.1. Antibacterial activity of gentamicin on *K. pneumonia*e

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of gentamicin are presented in Table 4.1.

Table 4.1. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with gentamicin.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Gentamicin	OD Values	1.136±0.060	0.567±0.005	0.284±0,011	0.203±0.020	0.120±0.015	P=0.007
				MIC_{50}:2.09			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumoniae* with gentamicin is presented in Figure 4.1.

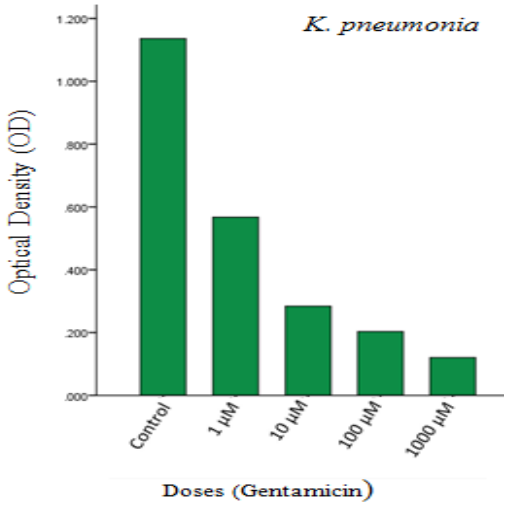

Figure 4.1. Antibacterial activity of gentamicin on *K. pneumoniae*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Gentamicin on *K. pneumoniae* was found to be 2.09.

4.1.1.2. Antibacterial activity of Compound 1 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of compound 1 are presented in Table 4.2.

Table 4.2. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with compound 1.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 1	OD Values	1.136±0.060	0.808±0.044	0.730±0.015	0.659±0.036	0.580±0.087	P=0.054
				MIC_{50}:6.06			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with Compound 1 is presented in Figure 4.1.

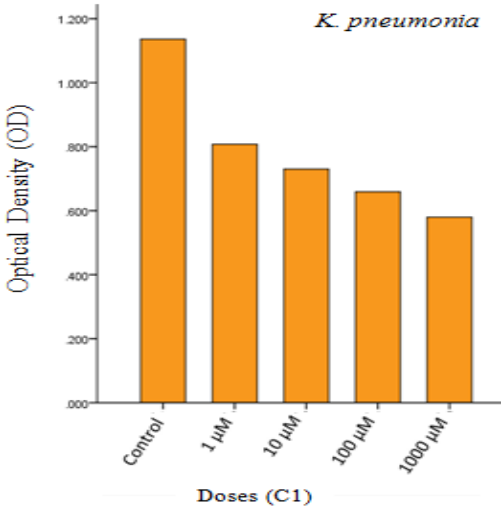

Figure 4.2. Antibacterial activity of Compound 1 on *K. pneumoniae.*

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 1on *K. pneumonia* was found to be 6.06.

4.1.1.3. Antibacterial activity of compound 2 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 2 are presented in Table 4.3.

Table 4.3. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with compound 2.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound2	OD Values	1.136±0.060	0.833±0.226	0.725±0.036	0.831±0.025	0.879±0.030	P=0.098
				MIC_{50}:16.73			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with compound 1 is presented in Figure 4. 3.

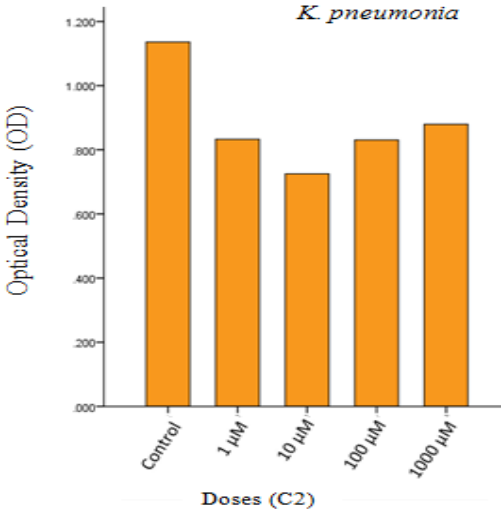

Figure 4.3. Antibacterial activity of Compound 2 on *K. pneumoniae.*

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 2 on *K. pneumonia* was found to be 16.73.

4.1.1.4. Antibacterial activity of compound 3 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 3 are presented in Table 4.4.

Table 4.4. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with compound 3.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 3	OD Values	1.136±0.060	0.963±0.014	0.810±0.039	0.686±0.015	0.695±0.266	P=0.001
				MIC_{50}:7.15			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with compound 3 is presented in Figure 4. 4.

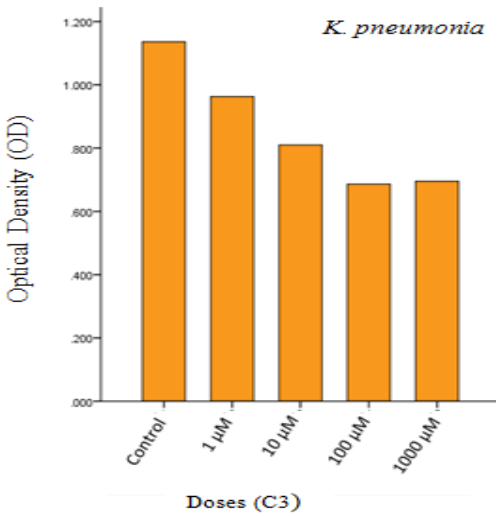

Figure 4.4. Antibacterial activity of Compound 3 on *K. pneumoniae*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 3on *K. pneumoniae* was found to be 7.15.

4.1.1.5. Antibacterial activity of compound 4 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 4 are presented in Table 4.5.

Table 4.5. MIC_{50} was obtained according to OD from the incubation *K. pneumoniae* with compound 4.

		Doses (µM)					Homogeneity Of
		Control	1	10	100	1000	variance
Compound4	OD Values	1.136±0.060	1.000±0.053	0.876±0.010	0.841±0.045	0.747±0.047	P=0.241
				MIC_{50}:8.87			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with compound 4 is presented in Figure 4. 5.

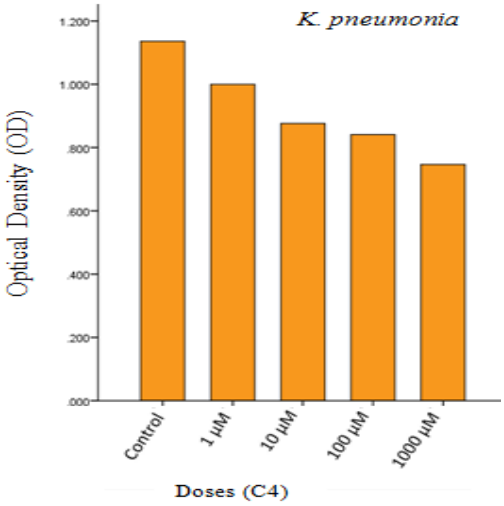

Figure 4.5. Antibacterial activity of Compound 4 on *K. pneumoniae.*

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 4 on *K. pneumoniae* was found to be 8.87.

4.1.1.6. Antibacterial activity of compound 5 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 5 are presented in Table 4.6.

Table 4.6. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with compound 5.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound5	OD Values	1.136±0.060	0.977±0.011	0.886±0.009	0.854±0.032	0.799±0.015	P=0.010
				MIC_{50}:10.34			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with compound 5 is presented in Figure 4. 6.

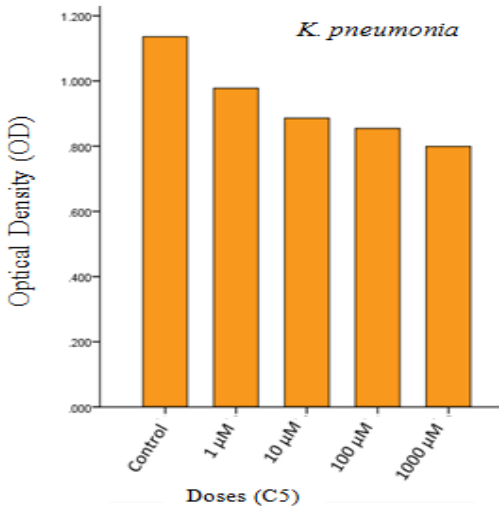

Figure 4.6. Antibacterial activity of Compound 5 on *K. pneumoniae*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 5on *K. pneumoniae* was found to be 10.34.

4.1.1.7. Antibacterial activity of compound 6 on *K. pneumoniae*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 6 are presented in Table 4.7.

Table 4.7. MIC_{50} was obtained according to OD from the incubation *K. pneumonia*e with compound 6.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound6	OD Values	1.136±0.060	0.980±0.015	0.867±0.010	0.817±0.009	0.774±0.026	P=0.008
				MIC_{50}:9.35			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *K. pneumonia*e with compound 6 is presented in Figure 4. 7.

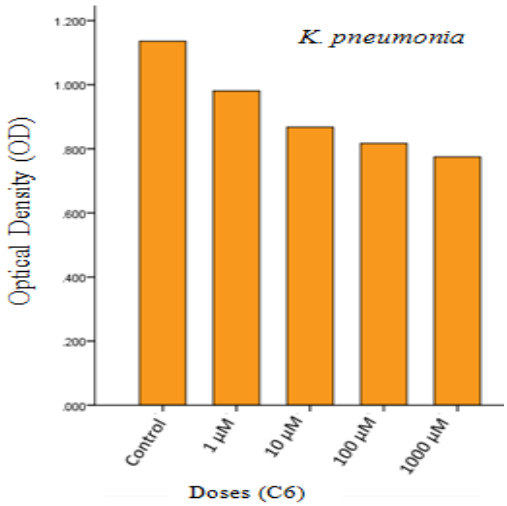

Figure 4.7. Antibacterial activity of Compound 6 on *K. pneumoniae*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 6 on *K. pneumoniae* was found to be 9.35.

4.1.2. Antibacterial activity on *Proteus miriabilis*

4.1.2.1. Antibacterial activity of gentamicin on *P. mirabilis*

The mean±SD values of OD data obtained from the MTT test. Homogeneity of variance analysis results (P values) and MIC_{50} values of gentamicin are presented in Table 4.8.

Table 4.8. MIC_{50} was obtained according to OD from the incubation *P. mirabilis* with gentamicin.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Genta	OD Values	1.114±0.051	0.429±0.030	0.289±0.009	0.155±0.021	0.123±0.023	P=0.239
				MIC_{50}:1.87			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with gentamicin is presented in Figure 4. 8.

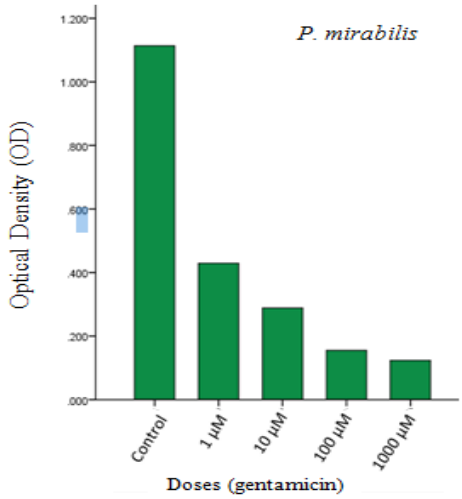

Figure 4.8. Antibacterial activity of gentamicin on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Gentamicin on *P. mirabilis* was found to be 1.87.

4.1.2.2. Antibacterial activity of compound 1 on *P. mirabilis*

The mean±SD values of OD data obtained from the MTT test. Homogeneity of variance analysis results (P values) and MIC50 values of compound 1 are presented in Table 4.9.

Table 4.9. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 1.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 1	OD Value	1.114±0.051	0.913±0.019	0.688±0.009	0.463±0.030	0.449±0.047	P=0.261
				MIC_{50}:5.38			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 1 is presented in Figure 4.9.

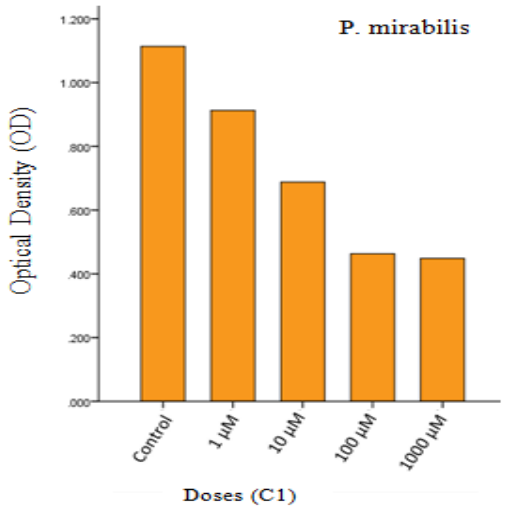

Figure 4.9. Antibacterial activity of Compound 1 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 1 on *P. mirabilis* was found to be 5.38.

4.1.2.3. Antibacterial activity of compound 2 on *P. mirabilis*

The mean±SD values of OD obtained from the MTT test. Homogeneity of variance analysis results (P values) and MIC_{50} values of compound 2 are presented in Table 4.10.

Table 4.10. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 2.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 2	OD Values	1.114±0.051	0.552±0.036	0.503±0.013	0.452±0.012	0.416±0.008	P=0.072
				MIC_{50}:11.99			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 2 is presented in Figure 4.10.

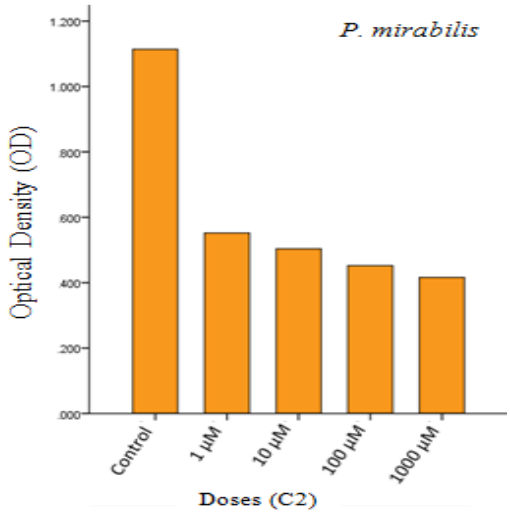

Figure 4.10. Antibacterial activity of Compound 2 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 2 on *P. mirabilis* was found to be 11.99.

4.1.2.4. Antibacterial activity of Compound 3 on *P. mirabilis*

The mean±SD values of OD obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} values of compound 3 are presented in Table 4.11.

Table 4.11. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 3.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 3	OD Values	1.114±0.051	0.858±0.049	0.783±0.061	0.498±0.018	0.433±0.023	P=0.289
				MIC_{50}:5.62			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 3 is presented in Figure 4.11.

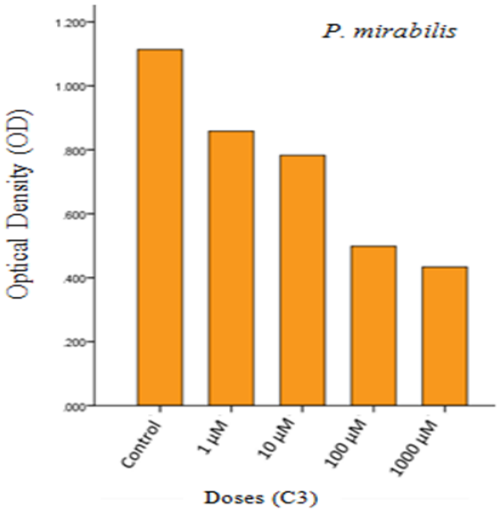

Figure 4.11. Antibacterial activity of Compound 3 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 3 on *P. mirabilis* was found to be 5.62.

4.1.2.5. Antibacterial activity of compound 4 on *P. mirabilis*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of compound 4 are presented in Table 4.12.

Table 4.12. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 4.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 4	OD Values	1.114±0.051	0.951±0.047	0.691±0.018	0.471±0.026	0.462±0.032	P=0.364
				MIC_{50}:5.31			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 4 is presented in Figure 4.12.

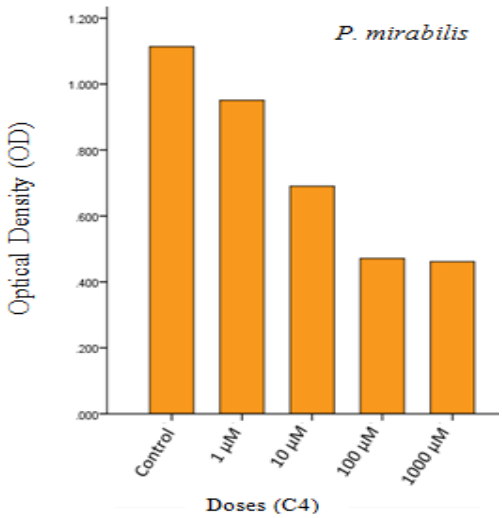

Figure 4.12. Antibacterial activity of Compound 4 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 4 on *P. mirabilis* was found to be 5.31.

4.1.2.6. Antibacterial activity of compound 5 on *P. mirabilis*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of compound 5 are presented in Table 4.13.

Table 4.13. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 5.

	Doses (µM)					Homogeneity Of variance
	Control	1	10	100	1000	
Compound 5 OD Values	1.114±0.051	0.649±0.043	0.575±0.024	0.487±0.035	0.476±0.020	P=0.580
			MIC_{50}:10.07			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 5 is presented in Figure 4.13.

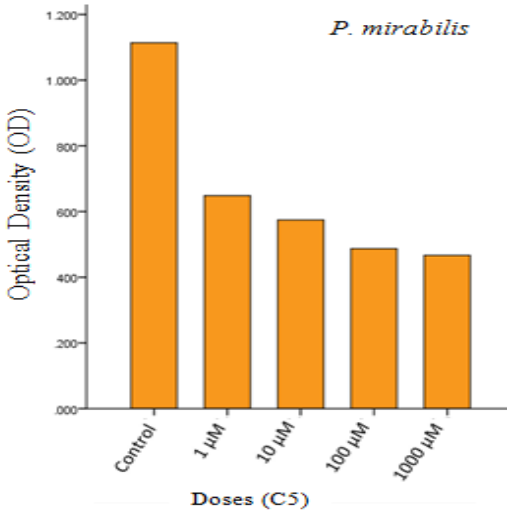

Figure 4.13. Antibacterial activity of Compound 5 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 5 on *P. mirabilis* was found to be 10.07.

4.1.2.7. Antibacterial activity of compound 6 on *P. mirabilis*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of compound 6 are presented in Table 4.14.

Table 4.14. MIC50 was obtained according to OD from the incubation *P. mirabilis* with compound 6.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 6	OD Values	1.114±0.051	0.858±0.092	0.693±0.064	0.478±0.030	0.460±0.027	P=0.393
				MIC_{50}:5.91			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *P. mirabilis* with compound 6 is presented in Figure 4.14.

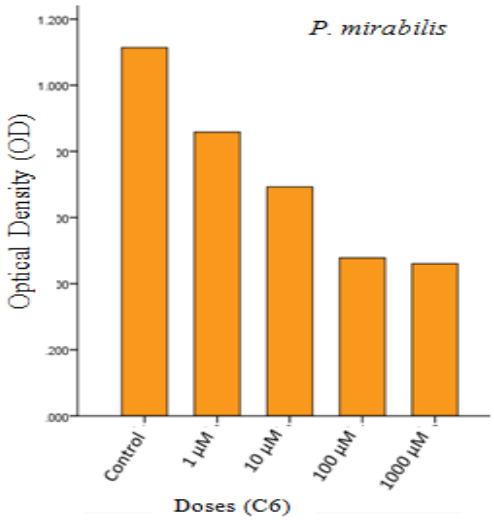

Figure 4.14. Antibacterial activity of Compound 6 on *P. mirabilis*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 6 on *P. mirabilis* was found to be 5.91.

4.1.3. Antibacterial activity on *Streptococcus pyogenes*

4.1.3.1. Antibacterial activity of gentamicin on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of gentamicin are presented in Table 4.15.

Table 4.15. MIC_{50} was obtained according to OD from the incubation *S. pyogenes* with gentamicin.

	Doses (µM)					Homogeneity Of variance
	Control	1	10	100	1000	
Gentamicin OD Values	0.893±0.024	0.349±0.005	0.312±0.010	0.181±0.010	0.077±0.006	P=0.029
			MIC_{50}:1.68			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with gentamicin is presented in Figure 4.15.

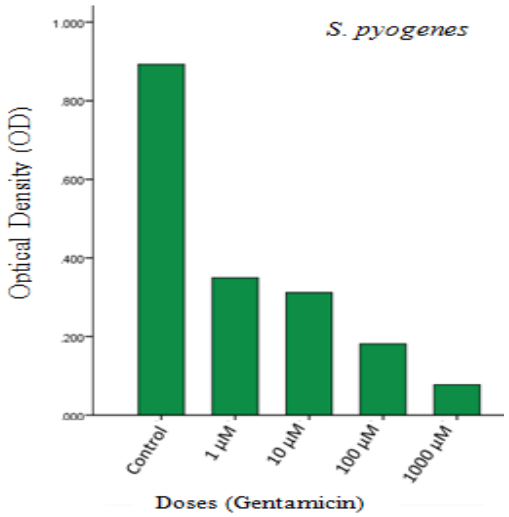

Figure 4.15. Antibacterial activity of gentamicin on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Gentamicin on *S. pyogenes* was found to be 1.68.

4.1.3.2. Antibacterial activity of compound 1 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of compound 1 are presented in Table 4.16.

Table 4.16. MIC_{50} was obtained according to OD from the incubation *S. pyogenes* with compound 1.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound1	OD Values	0.893±0.024	0.414±0.013	0.322±0.019	0.310±0.057	0.228±0.014	P=0.066
				MIC_{50}:4.21			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 1 is presented in Figure 4.16.

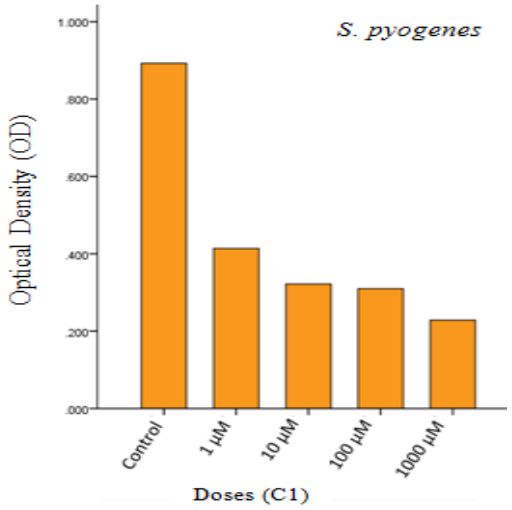

Figure 4.16. Antibacterial activity of Compound 1 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 1on *S. pyogenes* was found to be 4.21.

4.1.3.3. Antibacterial activity of compound 2 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 2 are presented in Table 4.17.

Table 4.17. MIC_{50} was obtained according to OD from the incubation of *S. pyogenes* with compound 2.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound 2	OD Values	0.893±0.024	0.363±0.111	0.323±0.038	0.245±0.013	0.127±0.034	P=0.151
				MIC_{50}:3.87			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 2 is presented in Figure 4.17.

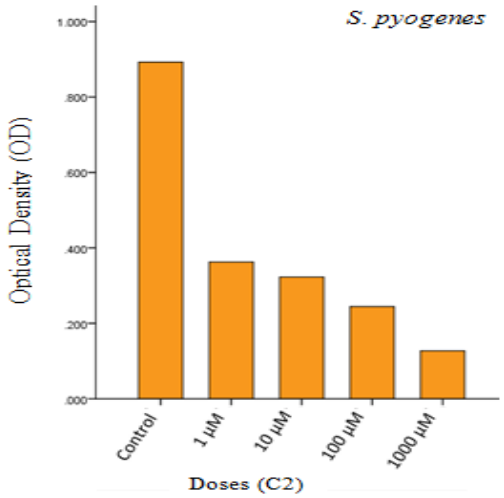

Figure 4.17. Antibacterial activity of Compound 2 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 2 on *S. pyogenes* was found to be 3.87.

4.1.3.4. Antibacterial activity of compound 3 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 3 are presented in Table 4.18.

Table 4.18. MIC_{50} was obtained according to OD from the incubation of *S. pyogenes* with compound 3.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound3	OD Values	0.893±0.024	0.296±0.013	0.211±0.044	0.181±0.006	0.163±0.004	P=0.046
				MIC_{50}:3.72			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 3 is presented in Figure 4.18.

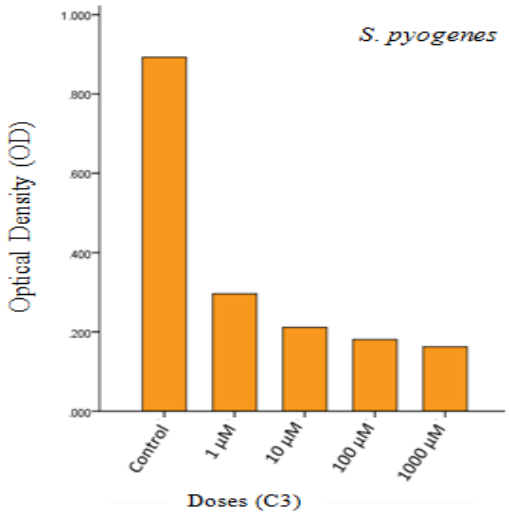

Figure 4.18. Antibacterial activity of Compound 3 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 3 on *S. pyogenes* was found to be 3.72.

4.1.3.5. Antibacterial activity of compound 4 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 4 are presented in Table 4.19.

Table 4.19. MIC_{50} was obtained according to OD from the incubation of *S. pyogenes* with compound 4.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound4	OD Values	0.893±0.024	0.568±0.031	0.310±0.006	0.216±0.002	0.193±0.019	P=0.017
				MIC_{50}:4.03			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 4 is presented in Figure 4.19.

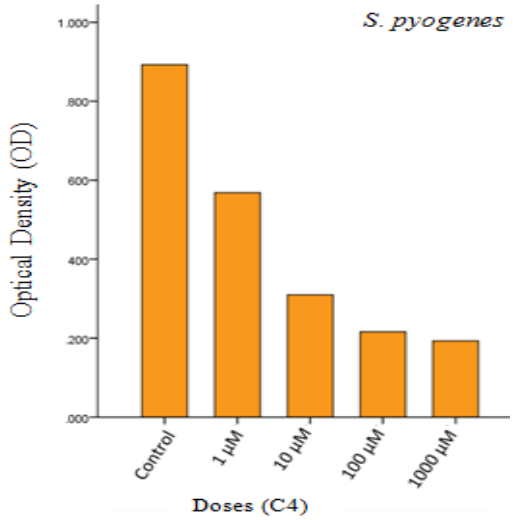

Figure 4.19. Antibacterial activity of Compound 4 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 4 on *S. pyogenes* was found to be 4.03.

4.1.3.6. Antibacterial activity of compound 5 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC50 value of compound 5 are presented in Table 4.20.

Table 4.20. MIC_{50} was obtained according to OD from the incubation of *S. pyogenes* with compound 5.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound5	OD Values	0.893±0.024	0.264±0.013	0.235±0.023	0.178±0.027	0.163±0.021	P=0.595
				MIC_{50}:3.73			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 5 is presented in Figure 4.20.

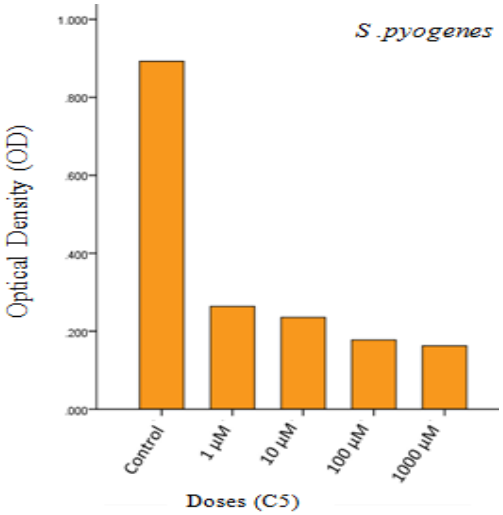

Figure 4.20. Antibacterial activity of Compound 5 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 5 on *S. pyogenes* was found to be 3.73.

4.1.3.7. Antibacterial activity of compound 6 on *S. pyogenes*

The mean±SD values of OD data obtained from the MTT test, homogeneity Of variance analysis results (P values) and MIC_{50} value of compound 6 are presented in Table 4.21.

Table 4.21. MIC_{50} was obtained according to OD from the incubation of *S. pyogenes* with compound 6.

		Doses (µM)					Homogeneity Of variance
		Control	1	10	100	1000	
Compound6	OD Values	0.893±0.024	0.327±0.026	0.249±0.030	0.200±0.009	0.168±0.001	P=0.029
				MIC_{50}:3.80			

The chart graphic of OD values attained after the application of the MTT test and after the incubation period of the culture process of *S. pyogenes* with compound 6 is presented in Figure 4.21.

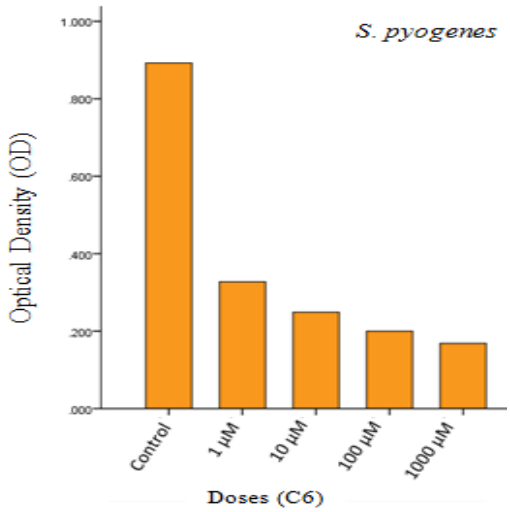

Figure 4.21. Antibacterial activity of Compound 6 on *S. pyogenes*.

By using the OD values attained from the MTT assay according to regression analysis, the MIC_{50} of Compound 6 on *S. pyogenes* was found to be 3.80.

4.2. Discussion

In the absence and deficit of effective pharmaceuticals for illness battles and pathogen eradication, dealing with infectious disease is still a challenging and significant global issue because the emergence and development of antimicrobial resistance are more rapid than the discovery and uncovering of new effective and powerful antibiotics with minimum inhibitory concentrations and low side effects. Therefore, recently, one of the chemical groups that has attracted the attention of scientists and researchers and is constantly worked on as antimicrobial agents is the Schiff bases and related complexes due to the great majority of their biological efficacies (Frieri et al., 2017; Abdel-Rahman et al., 2023). In this case, in our investigation, we looked at the effects of six Schiff bases and their metal complexes on some resistant acquired bacteria.

According to the results obtained by using the disc diffusion method, ten synthesized Schiff bases demonstrated a strong effect on the majority of tested bacteria, especially *Listeria monocytogenes*, which displayed an 18-mm inhibition zone similar to ciprofloxacin that was used as a positive control. On the other hand, *Staphylococcus aureus* showed the least sensitivity to the newly tested molecule (Nastasa et al., 2018) .While in the recent experiment that was carried out by (Hassan et al., 2020). The activity of evaluated Schiff bases ascertained using micro dilution by detecting MIC and Minimum Bactericidal concentration (MBC). The evaluated Schiff bases showed potent to excellent activity against *B. subtilis* and minimal inhibitory concentrations exhibited by compound 8a against *E. coli* (0.97 µg/mL), which were sixteen times more active than the standard drug Tetracycline.

In order to get an accurate and precise result in our investigation, we applied micro dilution instead of the disc diffusion method. On the other hand, rather than using MIC, we calculated MIC_{50}, which displayed a minimum concentration that inhibited 50 percent of the growth of bacteria.

The antibacterial activity of a series of Schiff bases derived from 2-acetylpyridine and their metal complexes was screened against resistance-acquired nosocomial pathogenic bacteria within hospital settings. Including *P. aeroginosa, A. baumanii*, methicillin-resistant *Staphylococcus aureus*, and *K. pneumonia*. At first, to determine the antibacterial action and inhibition zone of each compound, the disc diffusion assay was applied to eight clinical strains, which included two strains for each bacterial species. As a consequence, strong inhibitive action was recorded by all seven transition metal-bonded Schiff-base compounds against Methicillin-resistant *Staphylococcus aureus* (MRSA). On the other hand, none of the compounds exhibited an inhibitory zone against *K. pneumonia*, while every compound showed low antibacterial efficacy against *A. baumanii* and *P. aeroginosa*. Then, to ascertain the achievable result, the MIC and MBC of all transition metal-bonded Schiff bases were determined by applying a broth micro-dilution assay. As a result, it was concluded that only MRSA showed high sensitivity to the tested compounds (Gwaram et al., 2012).

Despite the proven effects of Schiff bases as antimicrobial agents, presently, to enhance the activity of Schiff bases and prevail over the antimicrobial resistance, SBs-metal complexes are intensively working on, as described by (Sinicropi et al., 2022). The consequences obtained by (Alshater et al., 2023) illustrated this as well. By assessing the in vitro activity of ligand H2L and some metal complexes as Ni2+, Cu2+, Ag+, and Hg2+ against Gram positive (*S. aureus* and *Streptococcus* mutants) and Gram negative (*E. coli* and *K. pneumonia*),and gentamicin was used as a reference drug, the findings revealed that complexes, because of their chelation capacity, showed greater efficacy than ligands. Also, the most potent activity against different bacterial strains has achieved by Ni complexes.

Ejidike IP (2018), in his recent study, investigated the antibacterial and antifungal activity of Schiff base (BEB) and the corresponding heterocyclic copper complexes against Gram-negative bacteria: *K. pneumoniae* and *P. aeruginosa*, Gram-positive bacteria; *S. aureus* and *E. faecalis* and fungi; *C. albicans* and *C. neoformans*. Among the compounds, Cu (II)-bearing compounds showed higher

activity than ligands and lower activity in comparison to neomycin as a standard drug. Also, the results obtained in our investigation revealed the antibacterial ability of complexes. Among six fluor-bearing SBs with copper and palladium complexes regarding *S. pyogenes*, the best activity was recorded by the SB complexes (C3 and C6) with MIC_{50} values of 3.72 and 3.80, respectively. While C1 Schiff base ligands with MIC_{50} values of 6.06 showed better activity on *K. pneumonia* in comparison to complexes.

The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) is a widely applied assay to determine cell viability in the presence of and test new chemicals, as supported by (Ghasemi et al., 2021). Furthermore, (Janowska et al., 2023) performed an MTT assay to ascertain cell survival after evaluating the activity of newly synthesized fluorephenyl Schiff base on some bacteria and fungi, as well as on metastatic breast cancer (MDA-MB-231) and prostate cancer (PC3). The findings showed a strong effect against *Candida* spp., including *C. albicans*, with minimal antibacterial and cytotoxic activity. While, based on current research among eight chemicals, Cu2 (L2)4 has demonstrated potent anticancer activity against A375 malignant melanoma cells (Corte-Real et al., 2023).

Likewise, in our experiment, we evaluated the antibacterial activity of six fluorophenyl Schiff bases with Cu and Pd complexes. The achieved result display entirely different effects of Schiff bases and complexes on various strains of bacteria. While Cu and Pd complexes showed more effect than ligands against *S. pyogenes*. On the other hand, the result data for both gram negative bacteria, *K. pneumoniae* and *P. mirabilis*, could be arranged in the following order: C1ligand > C3-cupper > C4ligand > C6-cupper > C5-palladium > C2-palladium, and C1, C4 ligand > C3, 6-Cupper > C2 and C5-palladium, while the MIC_{50} obtained from our positive control on *K. pneumoniae*, *P. mirabilis*, and *S. pyogenes* was 2.09, 1.87, and 1.68, respectively. Obtaining distinct outcomes with Schiff bases and complexes on gram-negative *P. mirabilis*, *K. pneumoniae*, and gram-positive *S. pyogenes* is attributed to their distinct structural characteristics.

5. Conclusion and Recommendation

5.1. Conclusion

Discovering a new alternative medication with numerous targets, a low concentration, and minimum adverse effects is an urgent request of the WHO due to ongoing, increasing antibiotic resistance. For these purposes, we assessed six Schiff base compounds with capper and palladium complexes that were obtained by Harran University on three acquired resistance bacteria, *K. pneumoniae*, *P. mirabilis*, and *S. pyogenes*, by using the MTT reagent method.

In vitro screening is the initial stage for ascertaining the antimicrobial effect of newly synthesized chemicals. In addition, MTT is one of the most widely applied methods for determining MIC by researchers. In our investigation, to quantify the vitality of cells, we also performed a modified MTT assay based on the reduction of yellow tetrazolium MTT to purple formazon dye by the dehydrogenase enzyme. The amount of formazon indicates the number of metabolically active, viable cells. Furthermore, we have applied the micro plate reader ELIZA to obtain OD and calculate the MIC_{50} for each compound, with gentamicin as a positive control as well.

Based on the consequences that we achieved, great effectiveness against *K. pneumoniae* and *P. mirabilis* was recorded by C1 and C4, with MIC_{50}s of 6.06 and 5.31., respectively. *S. pyogenes* was more susceptible to nearly all of the compounds, and the most effective MIC_{50} was 3.72 by C3. We observed that each chemical has exhibited distinct effects on various bacterial strains due to the differences in the composition of each bacterium. On the other hand, for *K. pneumoniae, P. mirabilis*, and *S. pyogenes*, the gentamicin MIC_{50} values were 2.09, 1.87, and 1.68, respectively.

5.2. Recommendation

Our study is part of a larger body of work being done by researchers in the sense of responsibility across the globe to identify and develop a robust chemical with great effect to overcome the struggles of antibiotic-resistant microbes.

Besides the fact that we obtained them by evaluating the six fluorinated Schiff bases with copper and palladium complexes, they were less efficacious when compared to the positive control (gentamicin). But the minimum inhibitory concentration we determined for each of the compounds is very sufficient to take our compounds into consideration, particularly with regard to gram-positive *S. pyogenes*.

Ultimately, further investigation is required to check the efficacy of each of our compounds and evaluate their effects on other bacteria strains, particularly those that exhibit the lowest inhibitory concentrations that might be selected as alternative medications.

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