An-Najah National University

Faculty of Graduate Studies

Novel cationic water-soluble polynitrogen/copper (II) complexes as a new antimicrobial therapy

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Dedication

I dedicate my thesis to my beloved wife Dana Bdair. To my precious, my little handsome angel, Jad.To my parents Rabah and Heyam Amer. My brothers and sisters. To my parents in law lecturer Sami and Eman Bdair. To my brothers and sisters in law. To a special person Khalid Amin Abu-Khater.

Acknowledgment

First of all, I thank Allah for giving me the strength that I needed to finish my thesis.

I would like to express my profound gratitude to Dr. Ashraf Sawafta and Prof. Ismail Warrad for their valuable supervision, encouragement and patience throughout the entire period of research.

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Thanks are also to the technicians in Department of Biology and Biotechnology at An-Najah National University.

Last, but not least, special thanks from all my heart to my wife Dana Sami Bdair for her patience, encouragement and support through out this work.

الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Novel cationic water solublepolynitrogen/copper (II) complexes as a new antimicrobial therapy

أقر بأن ما شملت عليه هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:** معاذ رباح جمال عامر

Signature: **التوقيع:**

Date: **التاريخ:**

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List Of Abbreviation

K.pneumonia	*Klebsiellapneumoniae*
K.variicola	*Klebsiellapneumoniae*
E. coli	*Escherichia coli*
EPEC	Eenteropathogenic*E.coli*
EHEC	Enterohemorrhagic *E. coli*
EAEC	Enteroaggregative *E. coli*
ETEC	Enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
HC	Haemorrhagic colitis
HUS	HaemolyticUraemic Syndrome
Stx	Shiga toxin
STEC	*E. coli* serotypes that produce Shiga toxins
P.	*Proteus*
S.aureus	*Staphylococcus aureus*
UTI	urinary tract infections
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
C1	Complex one
C2	Complex two
C3	Complex three
C4	Complex four
C5	Complex five
C6	Complex six
TSB	Trypticase soy broth
MHA	Muller Hinton agar
Cm	Centimeter
Mm	millimeter
Mg	Milligram
µg	Microgram
g	Gram
ml	Millileter
µl	Microleter

°C	°Celsius
CN(30)	Cephalexin (30)
Cu	Copper
dipn	Dipropylenetriamine
UV	Ultra Violet
En	Ethylenediamine
pn	Propylenediamine
TG	Thermogravimetry
en	Ethylenediamine
EV	ElementarVarrio
TG	Thermogravimetry
DTA	differential thermal analysis
TA	Thermal analyses
RT	room temperature
API20	Analytical profile index20
CLSI	The Clinical and Laboratory Standard Institute

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Abstract

The six dicationic water soluble copper(II) complex1, complex2 complex3, complex4, complex5 and complex6, of general formula [CuNNN(NN)]Br_2 [NNN = triamine and NN is *chda* = 1,2-diamiocyclohexane or Me2*pn* = 2,2dimethyl-1,3diamino propane] were prepared under ultrasonic mode with very good yield. These complexes were characterized by elemental microanalysis, UV-visible and IR spectroscopy.

It was found that four complexes (complex1, complex3, complex4, complex5) of the six complexes have a high antimicrobial activity against *Klebsiella*, *Escherichia coli*, *Proteus* and *Staphylococcus aureus*. Complex2 have a little antimicrobial activity. Complex6 have not any antimicrobial activity at any kind of the used bacteria, this could be because the complex is unstable.

The antibiotic Cephalexin was used as a standard to compare the antibacterial activity of the six complexes against the bacteria. Cephalexin used as antibacterial drug to kill Gram-positive and Gram-negative bacteria. The antibacterial activity of the complexes1,3,4,5 against the bacteria were better than Cephalexin. Also the complexes 1,3,4,5 showed a high antimicrobial activity against *Klebsiella* compared with Cephalexin

that have not any antibacterial activity against this kind of bacteria, which encourages to use these complexes as a topical treatment instead of Cephalexin.

Chapter One
Introduction

1. Introduction

1.1 General Background

Bacteria are living organisms that have only one cell. Under a microscope, they look like balls, rods, or spirals. They are so small that a line of 1,000 could fit across a pencil eraser. Most bacteria won't hurt you - less than 1 percent of the different types make people sick. Many are helpful. Some bacteria help to digest food, destroy disease-causing cells, and give the body needed vitamins. Bacteria are also used in making healthy foods like yogurt and cheese. Infectious bacteria can make you ill. They reproduce quickly in your body. Many give off chemicals called toxins, which can damage tissue and cause sickness. Examples of bacteria that cause infections include *Staphylococcus aureus, Klebsiella pneumonia, Proteus,* and *Escherichia coli*[1].

The human health effects caused by waterborne, airborne, milk borne, and soil borne transmission vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhea, dysentery, hepatitis and typhoid fever. Contaminated water can be the source of large outbreaks of disease, including cholera, dysentery and cryptosporidiosis; for the majority of waterborne pathogens, however, there are other important sources of infection, such as person to personcontact and food [1].

Antibiotic therapy is one of the most important therapies used for fighting infectious diseases and has tremendously enhanced the health aspects of

human life since its introduction. Despite the advancements in this therapy, we still live in an era where incidents of antibiotic resistant infections are alarmingly on rise [2]. The significance role of antibiotics in nature remains unfounded due to the responses of bacteria through the manifestation of various forms of resistance following the introduction of a new antibiotic for clinical use. The most important factor influencing the emergence and spread of antibiotic resistance is the excessive bacterial exposure to antibiotics [3]. Indiscriminate and over use of antibiotics causes selective pressure, allowing only the fittest genotype to thrive. Despite of the fact that evolution is inevitable, the intensive use of antimicrobial agents in the community, hospital and agriculture is undeniably responsible for fuelling this crisis. Today, bacteria, which are resistant not only to a single drug but simultaneously to many drugs are rampantly spread in the community and clinically due to the improper use of antibiotics in the past decade[3, 4].

1.2 Microbial Strains (Bacterial Strains)

1.2.1 *Klebsiella*

The *Klebsiella* genus consists of diverse organisms that are capable of colonizing and causing disease in humans and animals or existing as endophytes that colonize plants. *Klebsiella pneumoniae* isolates associated with human disease have been linked to pneumonia, meningitis, bacteremia, and urinary tract infections[5]. Additional *Klebsiella* species capable of nitrogen fixation have been isolated from the roots of plants, where they occur in a mutualistic relationship as endophytes[6, 7].

The *Klebsiella* genus originally included the species *Klebsiella planticola*, *Klebsiella terrigena* and *Klebsiella ornithinolytica*, which have been recently described as a new genus, *Raoultella*[8]. Furthermore, there are phylogenetically and phenotypically diverse *K.pneumoniae* isolates that likely represent distinct species, such as the recently described *Klebsiella variicola* [9]. This highlights the fact that the nomenclature and identification of *Klebsiella* species are complex. For the purposes of this study, the *Klebsiella* species investigated are the human disease-associated *K.pneumoniae* and the nitrogen-fixing *Klebsiella* species typified as *K.variicola*. *K.pneumoniae* was previously thought of predominantly as a community-acquired agent of infection but recently has become more prevalent as a nosocomial pathogen of infections such as pneumonia, meningitis, septicemia, and urinary tract infections [5, 10, 11].

1.2.2 *Escherichia coli*

Escherichia coli (*E.coli*) is a rod-shaped, Gram-negative bacterium found in high numbers in the gut of warm-blooded animals. For over 100 years, it has been used to detect faecal contamination in water and as an indicator of waterborne disease risk[12]. *E.coli* and other members of the coliform group remain the most widely used microbial indicators of water safety globally.Despite its documented limitations as an indicator of health risk, *E.coli* is not normally present in waters that are not faecallycontaminated [13].*E.coli* strains that cause diarrhea in humans have been divided into different pathotypes according to their virulence

attributes and the mechanisms involved in the disease process[14, 15].Five major groups of intestinal pathogenic strains have been established, such as enteropathogenic *E. coli* (EPEC),enterohemorrhagic *E .coli* (EHEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E.coli* (ETEC) and enteroinvasive *E.coli* (EIEC).While EPEC is a major cause of infantile diarrhea in the developing world, EHEC is associated with foodborne outbreaks in the developed world and can cause bloody diarrhea, haemorrhagic colitis (HC) and the HaemolyticUraemic Syndrome (HUS) due to the elaboration of Shiga toxin (Stx). More than 400 *E.coli* serotypes that produce Shiga toxins (STEC) have been described[16]. A small number of these have been to have a crucial to be role in severe disease such as HC and HUS in humans. A classification scheme has been established to group STEC strains into the five seropathotype groups A-E depending on the severity of disease, the incidence of human infections and the frequency of their involvement in outbreaks[17]. *E.coli 0157:H7* is one of hundreds strains of the *E.coli*, a bacterium that is belonged to the to the *Enterobacteriaceae*family. It was recognized in 1982 as a human pathogen. *E.coli 0157:H7* is gram negative, facultative anaerobic, rod shaped bacterium and have the ability of adaptation for long time and survival in disinfection process. Most *E.coli* strains are harmless and can be lived in the intestines of healthy humans and animals, but *E.coli 0157:H7* strain can cause severe illness due to toxin production and outbreak associated bloody diarrhea. The cell wall contains the "O" antigen, and the "H" represents flagellar antigen. It is most common as a causative agent of (HUS)[18].

1.2.3*Proteus (P)*

Gram-negative bacteria of the genus *Proteus* belong to the Enterobacteriaceae family. These microorganisms were described by Hauser in 1885 and originally had two species *P.mirabilis* and *P.vulgaris*. The biochemical classification of the genus *Proteus* has been changing. Currently, the genus consists of five species *P.mirabilis*, *P.vulgaris*, *P.penneri*, *P.hauseri*, and *P.myxofaciens*, as well as three unnamed *Proteus*genomo species 4, 5, and 6[19]. Proteus bacilli are widely distributed in the natural environment, where they are involved in decomposing organic matter of animal origin. They are also present in the intestines of humans and animals[20] and are opportunistic pathogens, which, under favorable conditions, cause mainly wound and urinary tract infections (UTI)[21]. Their importance in rheumatoid arthritis has also been shown[22]. *Proteus* rods are a frequent cause of UTI in patients with a urinary catheter in place or with structural and/or functional abnormalities in the urinary tract or who have had surgical intervention in the urogenital system. Strains of *P. mirabilis* cause UTI with the highest frequency among the *Proteus* species, including complicated infections and infections in long catheterized patients. In addition, *Proteus* bacteria may be associated with nosocomial infections[23] and can cause hematogenous and ascending infections, the latter being more common for these microorganisms. *Proteus* bacteria are dimorphic being able to display two types of behavior. When grown in liquid media they are motile, peritrichously flagellated short rods called ‘swimmer cells’. When transferred to a solid medium, the

short rods differentiate into elongated forms called ‘swarmer cells’, which are multinucleated, non-septated, and highly flagellated. Populations of swarmer cells can migrate in a coordinated way on solid media and then disintegrate into short rods. This process is cyclic and is known as the swarming phenomenon or swarming growth. Both morphologically and physiologically, different short swimmer rods and swarmer cells are important for pathogenesis, although their significance in particular stages of infection remains to be clarified[24].

1.2.4 *Staphylococcus aureus* (S.aureus)

Staphylococcus aureus is a Gram-positive human pathogen of increasing significance, mainly due to its high incidence and the increasing spread of antibiotic resistances[25]. *S.aureus* has advanced to a main problem in hospital settings since effective treatment options for infections caused by this pathogen are limited[26]. Multiple virulence factors allow *S.aureus* to cause a broad spectrum of infectious diseases, ranging from superficial abscesses of the skin to severe diseases such as endocarditis, osteomyelitis, toxic shock syndrome or sepsis. Furthermore, *S. aureus* is particularly important in healthcare settings, where it is causing up to 40% of nosocomial infections. Vancomycin and related antibiotics form the drugs of last resort against resistant strains. Therefore, the recent emergence of vancomycin/methicillin-resistant *S.aureus*strains represents a major threat for the health care system, requiring the development of new therapeutic options against *S.aureus* infections[25].

1.3 Cephalexin Antibiotic

Cephalexin is the first generation of cephalosporins, this medication is active against gram–positive cocci, including staphylococci and streptococci. Also this drug has minimal activity against gram-negative cocci, enterococci, methicillin-resistant *S.aureus*, and most gram-negativerods[27]. It's mechanism of action by preventing bacteria from forming their cell wall, and so bacteria cannot able to survive and stop the spread of infection in the body [28].

1.4 Copperand Copper II Complexes as Antimicrobial Agent

Copper sulfate is a fungicide used to control bacterial and fungal diseases of fruit, vegetable, nut and field crops. Some of the diseases that are controlled by this fungicide include mildew, leaf spots, blights and apple scab. It is used in combination with lime and water as a protective fungicide, referred to as Bordeaux mixture, for leaf application and seed treatment. It is also used as an algaecide, an herbicide in irrigation and municipal water treatment systems, and as a molluscicide, a material used to repel and kill slugs and snails. Copper sulfate is a naturally occurring inorganic salt and copper is an essential trace element in plant and animal nutrition [29,30,31,32,33].

Copper is one of 26 essential trace elements occurring naturally in plant and animal tissue. The usual routes by which humans receive toxic

exposure to copper sulfate are through skin or eye contact, as well as by inhalation of powders and dusts [33].

Danish bacterial isolates from livestock so far have not or have only to a limited degree developed resistance to antimicrobial compounds commonly used for disinfection. Acquired copper resistance was only found in enterococci. There were large differences in the intrinsic susceptibility of the different bacterial species to these compounds, and *Salmonella* especially seems intrinsically less susceptible than the other bacterial species, which might have human health implications[34].

Lactic acid, in combination with copper sulfate, could be used to inhibit the growth of pathogens. Natural ingredients, such as lactic acid and low dose of copper ions, can be used to improve the safety of food products[35].

Mixed-ligand copper (II) complexes with nitrogen-amino ligand have been investigated in pharmaceutical field due to their anticancer, antioxidant and antimicrobialpotential [36-48].

Amine-Copper(II) complexes exhibit prominent antimicrobial and anticancer potential activity by inducing apoptosis [47-51]. In general, redox-active agents that damage DNA in vitro are thought to exhibit apoptotic activities in live cells by inducing oxidative stress and/or DNA damage [52].

Triamine ligands with tridentate N-donor ligands are suitably placed for forming two 5 or 6-membered chelate metal complexes [53, 54].Although

metal complexes with such tridentate amine ligands have been thoroughly investigated, only one example combining both bidentate and tridentate amine for preparation of mononuclear [Cu(II)/triamine/diamine]2X complexes have been isolated and characterized by X-ray single crystal diffraction up to date [54].The authors have recently investigated the spectroscopic and the biological activity of $[Cu(dipn)(N\text{-}N)]_2Br$ [dipn = dipropylenetriamine, N-N = ethylenediamine (en) and propylenediamine (pn)] [54]; the structure of $[Cu(dipn)\ (pn)]Br_2$ was resolved by X-ray single crystal analysis. Herein, it is reported the synthesis and the spectroscopic properties of two new dicationiccopper (II) complexes of general formula $[Cu(dien)(NN)]Br_2$ complexes.

1.5 The aim of the study

1. To screen the antibacterial properties of a range of new copper (II) complexes with different ligand.

2. To compare their performance against multidrug resistant bacteria strains.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Chemical Material

Chemical complexes were prepared in the chemical laboratory of An-Najah National University under the supervision of Prof. Ismail warad and his team [54]. The prepared complexes were solid blue powder in 6 vials named complex 1 to complex 6 and ready to be used in the study.

2.2 General Synthesis of The Desired [copper (II) L1L2] Br2 Complexes

An ethanolic solution (10 mL) of 1,2diaminocyclohexan ligand (1.5 mmol) and 2,2dimethyl-1,3diamino propane ligand (1.5 mmol) were mixed together and added drop-wise to CuBr2 (1.5 mmol), dissolved in 10 mL of (50%) ethanol. The resultingreaction mixture was stirred for 30 min. The reaction mixture wassubjected to ultrasound waves for 5–20 min until the blue precipitates appeared. The solid was filteredand carefully washed withdichloromethane, then dried undervacuum.

2.3 General procedure for complexes (1-2) synthesis

1 mmolof $CuBr_2.2H_2O$ was dissolved in 20 ml of methanol, 1 mmol oftriamine and 1 mmoldiamine mixture dissolved in 2 ml of methanol then added to the Cu(II) solution. The mixture was left under stirring and ultrasound waves for about 30 min until the solution turned to deep blue. The solvents were then removed under vacuum and the remaining solid

was washed with isopropanol, dichloromethane and then dried under vacuum. Crystals of 2 suitable for X-ray diffraction structural analysis were obtained by slow evaporation of water from the solution of the complexes.

2.4 Physical Measurements

Microanalyses (C, H, N) were performed using an ElementarVarrio EL analyzer. The FT-IR spectra (4000–400 cm^{-1}) were obtained using a Perkin–Elmer 621 spectrophotometer with KBr discs. Thermal analyses, thermogravimetry (TG) and differential thermal analysis (DTA) were carried out with TA Instruments SDT-Q600 in air. Electronic spectra were recorded in water at room temperature (RT) on Pharmacia LKB-Biochrom 4060 spectrophotometer. The electrochemical properties of the complex 1 were investigated by cyclic voltammetry in DMF solutions containing 0.1 M of TBAPF6 as supporting electrolyte. Cyclic voltammograms were recorded at different scan rate 0.1V vs Ag/AgCl.

2.5 Preparation of Dried Filter Paper Discs

Whatman filter papers are used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven or by autoclaving. To each disk add 50 µl of chemical complexes and wait until all disks become dried [55].

2.6 Preparation of Müeller-Hinton Agar

Müeller-Hinton agar was prepared using a commercially available dehydratedbase according to the manufacturer's instructions.Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath. Then it was used to prepare the plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. The agar medium was allowed to cool to room temperature and it was stored in a refrigerator (2 to 8°C) [56].

2.7 Antimicrobial Assays

The invitro antibacterial activities of new copper complexes were evaluated against four selected bacteria clinical isolates, which includes three Gram negative bacteria; *Proteus*, *Escherichia coli* and *Klebsiella pneumonia* and one a Gram-positive bacterium *Staphylococcus aureus*. The isolates were obtained from the research laboratory of the science faculty at Najah University, and their identities were confirmed by gram stain, catalase, coagulase for *staphylococcus aureus*, and by gram stain and API20 E for *Escherichia coli*, *Proteus*, and *Klebsiella pneumonia*.

The susceptibility of each bacterium isolate to copper complex were tested by disk diffusion susceptibility test (Bauer-Kirby) and quantitative dilution susceptibility tests [69].

2.8 Disk Diffusion Assay

In disk diffusion method, few colonies from the tested bacterium, which werecultured for 18 hours at 35˚C, were used to inoculate 5ml of trypticase soy broth (TSB) and the broth turbidity will be adjusted to 0.5 MacFarland standard (which is approximately equal to $1.5x10^8$ CFU/ml). Then a sterile swab was dipped into the standardized broth and used to streak the surface of Muller Hinton agar (MHA) to obtain uniform inoculums for each of *Staphylococcus aureus*, *Proteus*, *Escherichia coli*, and *Klebsiella pneumonia*. Within 15 minutes of inoculation, a sterile filter paper disk (6mm in diameter) containing 50 µl of different concentration of copper complex that has been prepared previously[70]. which was dried previously in hot air oven, were placed onto the surface of inoculated plate. The plate was incubated at room temperature for 30 minutes to allow diffusion of copper complex to agar before incubated at 35˚C for 18 hours for *Staphylococcus aureus,Proteus,Escherichia coli*, and *Klebsiella pneumonia*. After that, the plate was examined for development of complete inhibition zone around the disk, and the zone was measured to the nearest whole millimeter. Cephalexin and distilled water were used as positive and negative control, respectively[57].

2.9 Minimum Inhibitory Concentration (MIC)

MIC is the lowest concentration of drug that inhibits the growth of microorganisms[58]. Theagar dilution method was used fortesting MICas the following. For each chemical complex a serial dilution waspreparedin

sterile distilled water to achieve a decreasing concentration ranging from 20 to 5mg/ml in labeled sterile tubes. Sterile tips were used to bore well in the presolidified MHA plates. Then the plates were inoculated with bacteria that adjusted previously to 0.5 McFarland standard.After that 50µl from each dilution was added into the wells. All plates were incubated at 37°C for 24 hrs. MIC was determined as the lowest concentration of a chemical complex shows a clear zone of inhibition [59].

2.10 Minimum Bactericidal Concentration (MBC)

To determine the concentration of drug that inhibits at least 99.9% of the bacterial colonies. An aliquot of 100 µl from the tube showing MIC were inoculated into a MHA antibiotic free plate. Then plates were incubated at 37C for 24hrs. Each plate was examined for the growth of a bacterium and determined the concentration of the complex at which 99.9% of bacteria were killed [60].

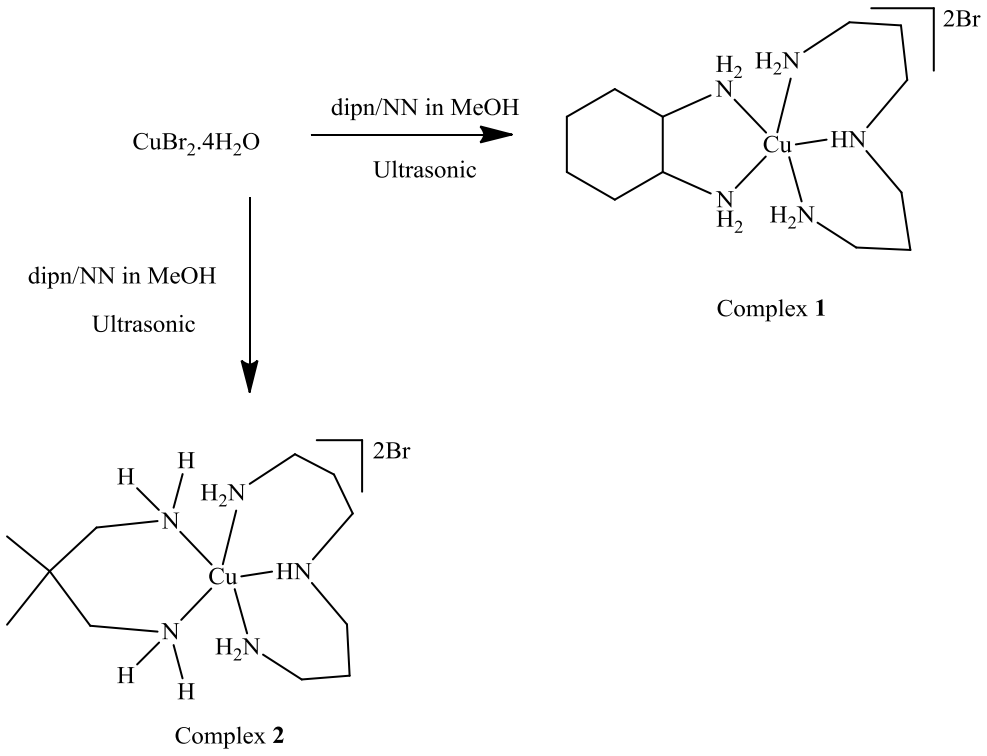
Chapter Three

Result, Discussion and Conclusion

3. Resulta nd discussion

3.1 Synthesis of complexes 1-2

Mixed triamine-diamine ligand copper(II) complexes of the general formula [Cu(dien)(NN)]Br_2(1-2) were synthesized in good yields under open ultrasonic atmosphere. The reaction can be easily monitored by eye sight, due to the colour change from brown to blue upon addition of the N-donor ligands. These complexes were characterized using elemental analysis and spectral methods. Based on water solubility and conductivity, these complexes appeared to be dicationhalide salt. The X-ray crystal structure of complex2 confirmed such suggestion and showed a distorted square pyramidal geometry Cu(II) dication ion [54].



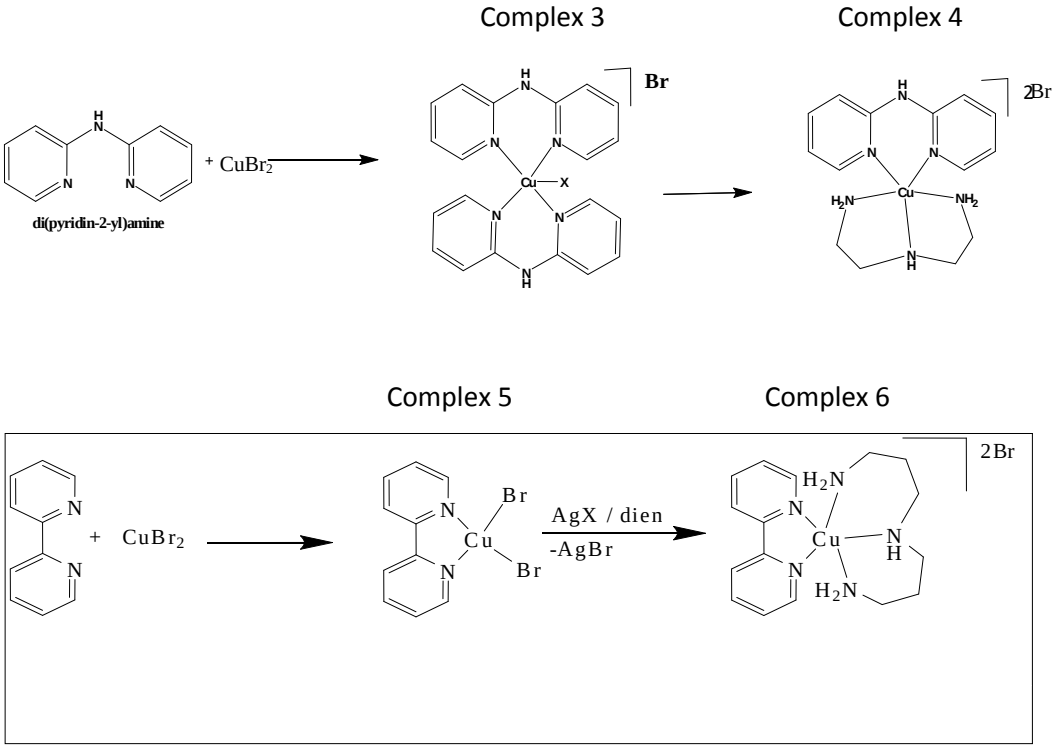
Scheme 3.1 Synthesis of desired complexes (1-2).

Complex 1: $CuBr_2 4H_2O$ with 1,2diaminocyclohexan.

Complex 2: $CuBr_2 4H_2O$ with 2,2dimethyl-1,3diamino propane.

3.2 Synthesisof complexes 3-6

These family of Cu(II) complexes were prepared in chemistry lab [70]. The structure and the complexes lable are represented in Scheme 3.2.



Scheme 3.2 Synthesis of desired complexes **(3-6)**

3.3 Visible and ultraviolet spectral data

The electronic absorption spectra behaviours for the desired complexes were measured in distilled water at room temperature. The spectra of the two complexes in water exhibit high intense π to π electron transitions in the UV region, around 250 nm (for complex1) 255 nm (for complex2),

along with a low intensity d to d electron transitions band around 610 nm (for complex1) and 625 nm (for complex2)**,**as seen in Fig. 3.3.

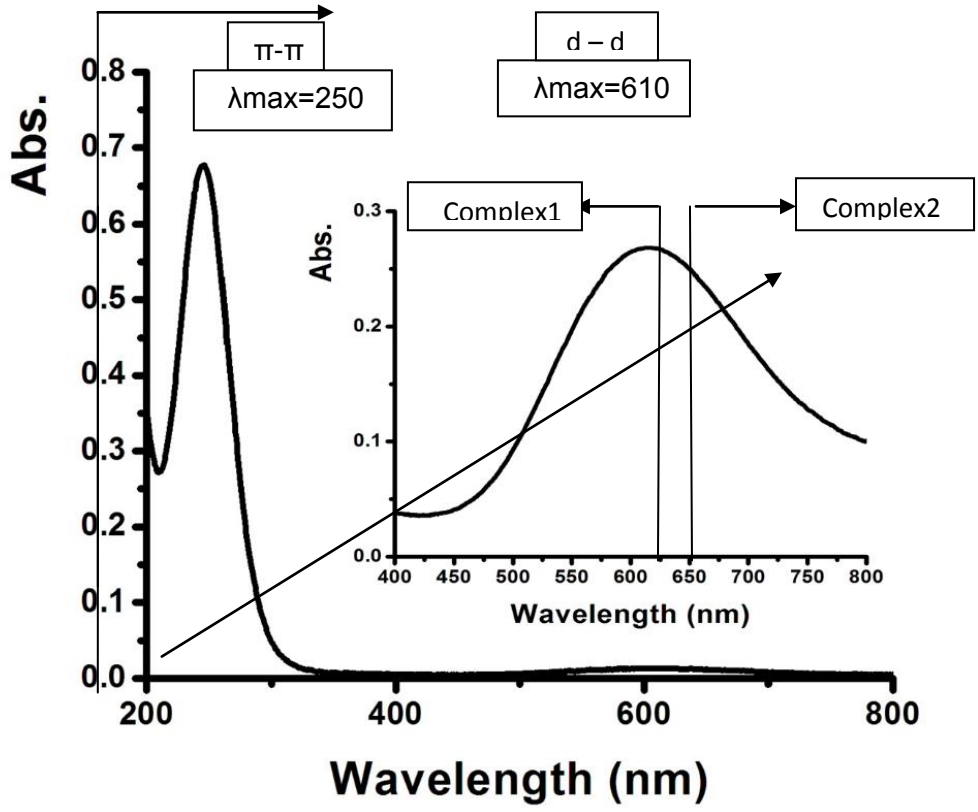


Figure 3.1UV–Vis spectrum of complex 2 dissolved in water at RT.

3.4 IR Spectral investigation

Several main peaks were detected due to function group vibrations. Peaks at ~ 3380cm^{-1}and 1480 cm^{-1}, assigned, to $\nu_{(O\text{-}H)}$ and $\nu_{(bend)}$, respectively, are the characteristic bands of H_2O, which indicates the existence of molecular lattice water. The three bands at 3100–3340 and 1500–1600 cm^{-1} assigned to ν_s(N–H), ν_{as}(N–H) and ν (N–H), respectively, are shifted to wave numbers lower than those encountered in the free ligand, confirming the coordination of the N-donor groups with copper [61, 62]. The strong bands at around 2800-2900 cm^{-1}are indexed to the stretching vibration of C-H of

CH_2 in the dien and CH_3-group of Me_4en diamine ligands [63]. The appearance of a broad bands at ~ 500-600 cm^{-1}may be attributed to $\nu_{(Cu-N)}$ bond vibrations [64, 65]. Bands that appear in the ~ 250-290 cm^{-1} region were assigned to the $\nu_{(Cu-Br)}$ vibration [66].

IR spectrumof C1andC2are given in Fig. 3.4.

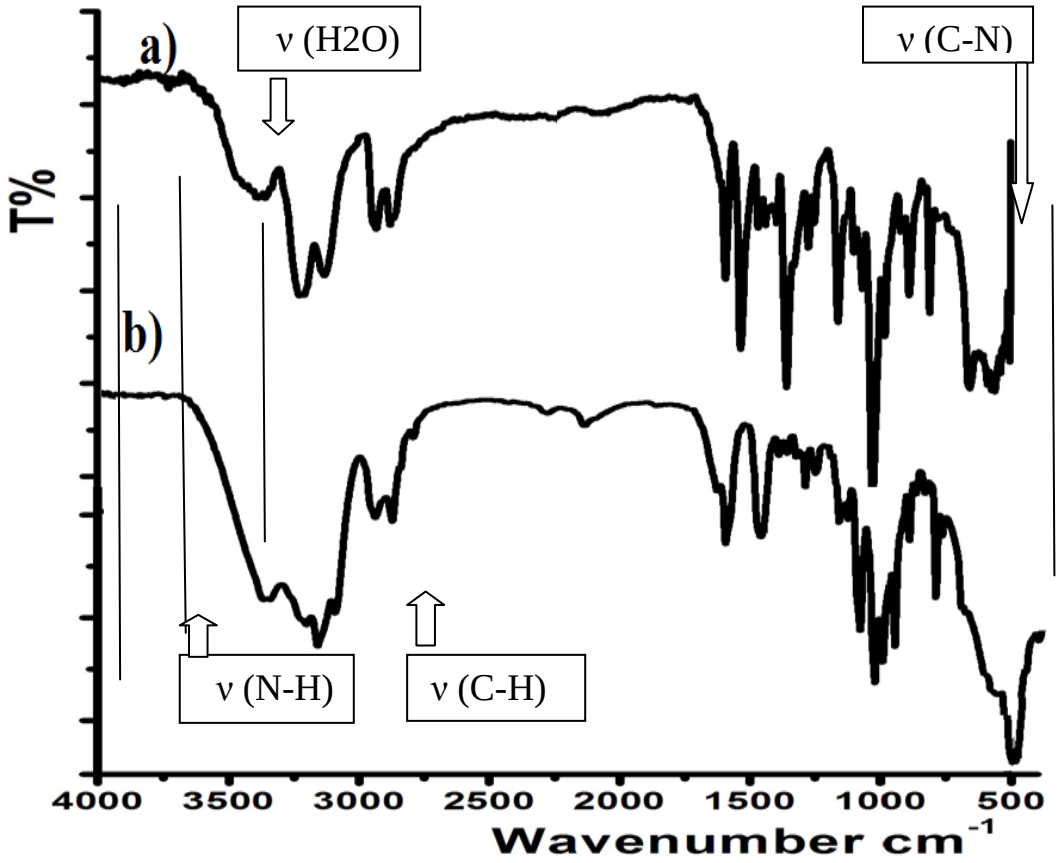


Figure 3.2 FT-IR spectrum of the Complex1 a) and Complex2 b).

3.5 [Cu (dien) NN]Br_2(1)

Yield 90%, M.p. = 140 °C. MS m/z 465.2 [M+] Calculated: C, 18.64; H, 5.47; N, 18.11. Found C, 18.31; H, 5.25; N, 18.02%, IR (KBr, νcm^{-1}): 3360 (ν_{H2O}), 3380 and 3280 and 31 20(ν_{H-N}), 2930 (ν_{C-H}), 1580 (ν_{N-H}), 1160 (ν_{N-C}), 540 (ν_{Cu-N}).UV–Vis in water: 250 and 605 nm.

3.6 [Cu(dien)Me_2pn]Br_2(2)

Yield 85%, M.p. = 145 °C. MS m/z 282.2 [M+] for $C_{10}H_{29}Br_2CuN_5$ Calculated: C, 27.13; H, 6.60; N, 15.82. Found C, 27.02; H, 6.44; N, 15.38%, IR (KBr, νcm^{-1}): 3380 (ν_{H2O}), 3340, 3270 and 3120, (ν_{H-N}), 2920 (ν_{C-H}), 1560 (ν_{N-H}), 1180 (ν_{N-C}), 505 (ν_{Cu-N}). UV–Vis inwater: λ_{max}255 and 625 nm.

3.7Antimicrobial assay/C1

Table 3.1Antimicrobial activity (cm inhibition zone diameter) of C1 at different concentrations (mg/ml).

C1	A	B	C	D
5	0.0	0.0	0.0	0.0
10	0.8	0.7	0.9	0.6
15	0.9	0.8	1.0	0.6
20	0.9	0.8	1.1	0.7
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

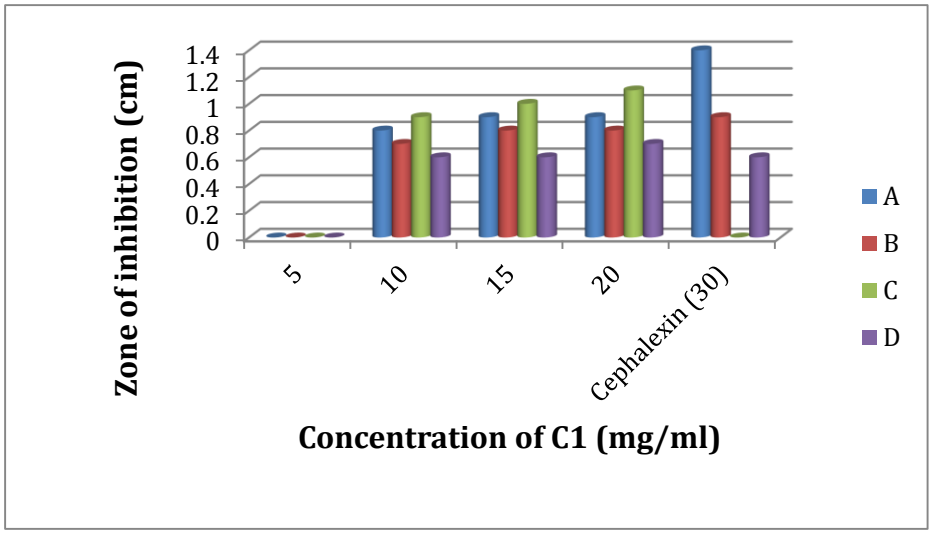


Figure 3.3 The effect of the complex C1 on different strains of bacteria.

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C1 was tested against several microbial strains (*E.coli, S.aureus, K.pneumoniae, proteus*). The highest activity of C1 was against *klebsiella pneumonia* with inhibition zone of 1.1 cm. The same complex exhibited moderate activity against *E.coli*, *Staphylococcus aureus and Proteus*; with inhibition zones of 0.9, 0.8 and 0.7 cm, respectively. Weak activity of the tested compound was observed against *Proteus* with inhibition zone 0.7 cm (Table 3.7). C1 was able to target Gram positive and Gram-negative bacteria indicating a broad-spectrum antimicrobial activity for this C1. Such broad-spectrum activity could be mediated by targeting essential steps in microbial growth or by causing metabolic toxicity [67].

3.7.1 Minimum inhibitory concentration (MIC)

Table 3.2Minimum inhibitory concentration (MIC) of synthesized C1 against growth of bacteria expressed in mg/mL.

	A	**B**	**C**	**D**
C1	10	10	10	10
Zone of inhibition (cm)	0.8	0.7	0.9	0.6

A):*E.coli*; (B): *Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D): *Proteus.*

Table 3.7.1 shows the MICs against the bacterial strains. C1 has higher antibacterial activity against *klebsiella pneumonia* (zone of inhibition 0.9 cm), while the activity of C1 is lower against *Proteus* (zone of inhibition 0.6 cm). But C1 shows promising results as broad-spectrum antibacterial agents.

3.8 Antimicrobial assay/C2

Table 3.3 Antimicrobial activity (cm inhibition zone diameter) of C2 at different concentrations.

C2	A	B	C	D
5	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0
15	0.0	0.5	0.0	0.0
20	0.6	0.6	0.6	0.0
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

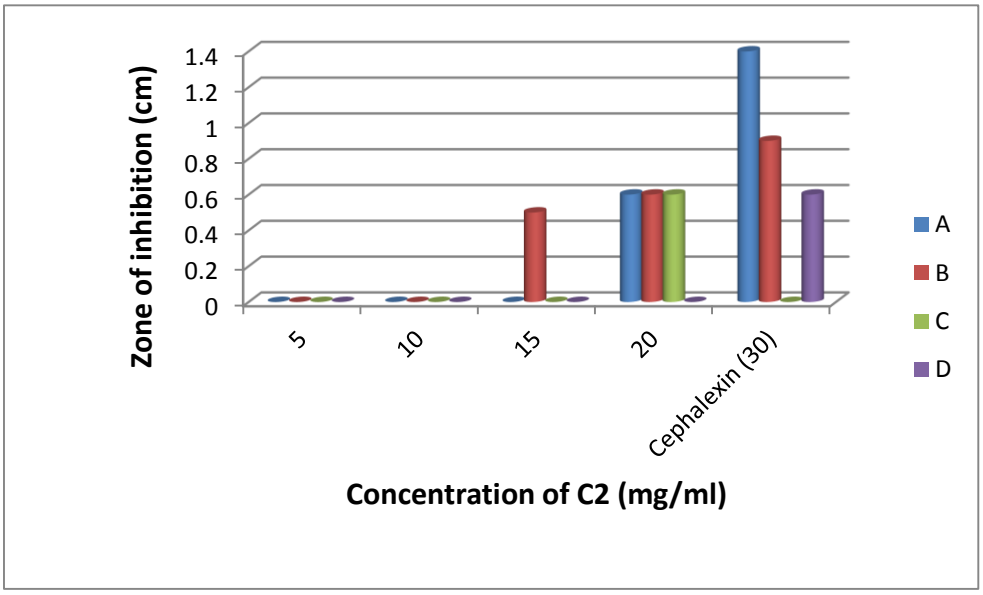


Figure 3.4 The effect of the complex C2 on different strains of bacteria.

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C2 was tested against several microbial strains (*E.coli, S.aureus, K.pneumoniae, proteus*). The highest activity of C2 was against *K.pneumonia* and *S.aureus* with inhibition zone of 0.6 cm. The same complex has no effect against *E.coli,and Proteus* (Table 3.8).

3.8.1 Minimum inhibitory concentration (MIC).

Table 3.4 Minimum inhibitory concentration (MIC) of synthesized C2 against growth of bacteria expressed in mg/mL.

	A	B	C	D
C2	20	15	20	No effect
Zone of inhibition(cm)	0.6	0.5	0.6	0.0

A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D):*Proteus.*

Table 3.8.1 shows the MICs against the bacterial strains. C2 has higher antibacterial activity against *Staphylococcus aureus* (MIC is 15 mg/ml with zone of inhibition 0.5 cm) , while the activity of C2 is lower against*klebsiella pneumonia* and *E.coli* (MIC is 20 mg/ml with zone of inhibition 0.6 cm), in contrast, C2 has no effect on *Proteus*.

3.9 Antimicrobial assay/C3

Table 3.5 Antimicrobial activity (cm inhibition zone diameter) of C3at different concentrations.

C3	A	B	C	D
5	0.0	0.0	0.0	0.0
10	0.7	1.3	0.6	0.6
15	0.9	1.7	0.8	0.9
20	1.9	2.0	1.5	1.1
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

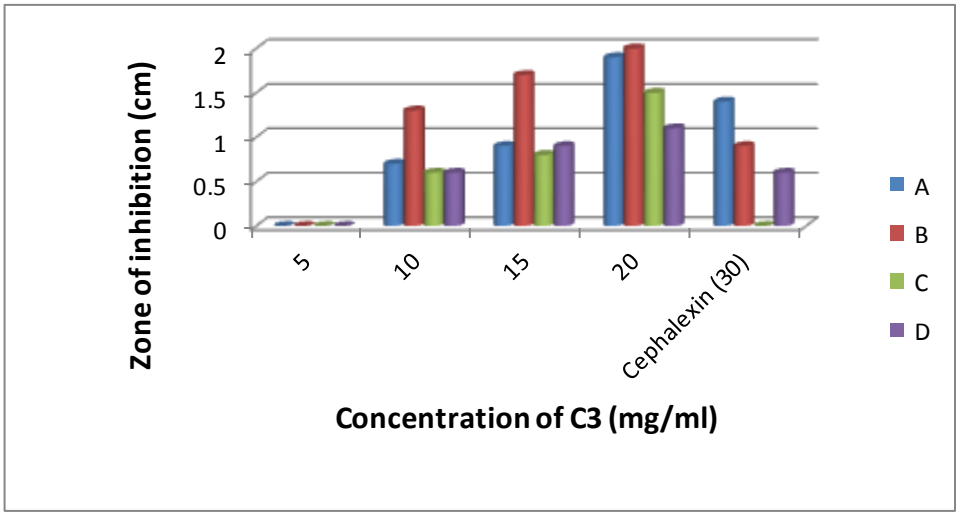


Figure 3.5 The effect of the complex C3 on different strains of bacteria.

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C3 was tested against several microbial strains (*E.coli, S.aureus, K.pneumoniae, proteus*). The highest activity of C3 was against *Staphylococcus aureus*with inhibition zone of 2.0 cm. The same complex exhibited moderate activity against *E.coli;k.pneumoniaand Proteus*; with inhibition zones of 1.9, 1.5 and 1.1 cm, respectively. Weak activity of the tested compound was observed against *Proteus* with inhibition zone1.1 cm (Table 3.9). C3 was able to target Gram positive and Gram-negative bacteria indicating a broad-spectrum antimicrobial activity for this C3. Such broad-spectrum activity could be mediated by targeting essential steps in microbial growth or by causing metabolic toxicity [67].

3.9.1 Minimum inhibitory concentration (MIC).

Table 3.6 Minimum inhibitory concentration (MIC) of synthesized C3 against growth of bacteria expressed in mg/mL.

	A	**B**	**C**	**D**
C3	10	10	10	10
Zone of inhibition(cm)	0.7	1.3	0.6	0.6

A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D):*Proteus.*

Table 3.9.1 shows the MICs against the bacterial strains. C3 has higher antibacterial activity against *Staphylococcus aureus* (zone of inhibition 1.3 cm) , while the activity of C3 is lower against*klebsiella pneumonia* and *Proteus* (zone of inhibition 0.6 cm). But C3 show promising results as antibacterial agents.

3.10 Antimicrobial assay/C4

Table 3.7 Antimicrobial activity (cm inhibition zone diameter) of C4 at different concentrations.

C4	**A**	**B**	**C**	**D**
5	0.0	0.0	0.0	0.0
10	0.7	1.1	1.0	1.0
15	1.2	1.2	1.1	1.1
20	1.4	1.3	1.2	1.2
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

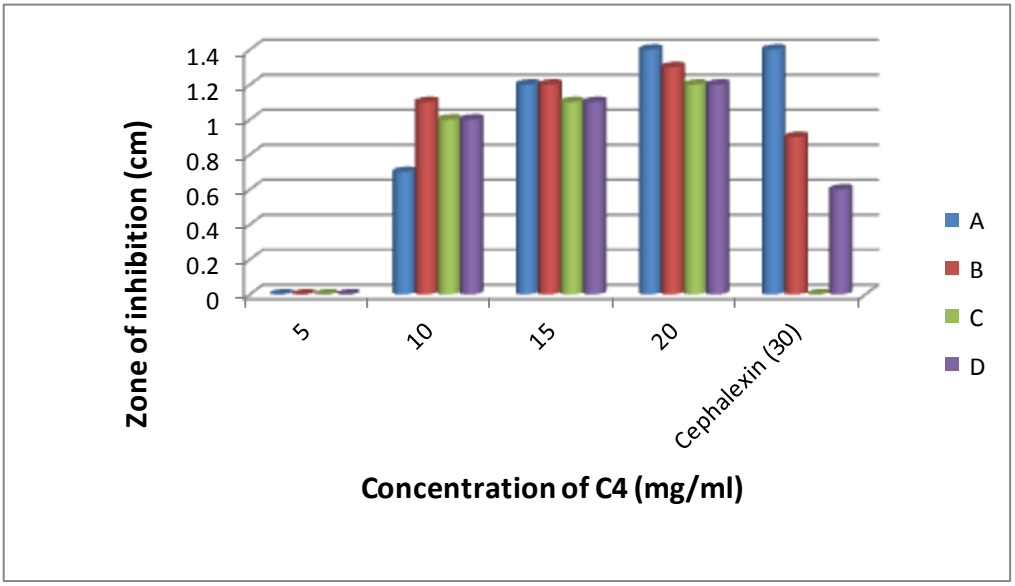


Figure 3.6 The effect of the complex C4 on different strains of bacteria.
(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C4 was tested against several microbial strains (*E.coli, S.aureus, K.pneumoniae, proteus*). The highest activity of C4 was against *E.coli*with inhibition zone of 1.4 cm. The same complex exhibited moderate activity against *Staphylococcus aureus; Klebsiella pneumonia and Proteus*; with inhibition zones of 1.3, 1.2 and 1.2 cm, respectively. Weak activity of the tested compound was observed against *klebsiella pneumonia* and *Proteus* with inhibition zone1.2 cm (Table 3.10). C4 was able to target Gram positive and Gram-negative bacteria indicating a broad-spectrum antimicrobial activity for this C4. Such broad-spectrum activity could be mediated by targeting essential steps in microbial growth or by causing metabolic toxicity [67].

3.10.1 Minimum inhibitory concentration (MIC).

Table 3.8 Minimum inhibitory concentration (MIC) of synthesized C4against growth of bacteria expressed in mg/mL.

	A	B	C	D
C4	10	10	10	10
Zone of inhibition(cm)	0.7	1.1	1.0	1.0

A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D):*Proteus.*

Table 3.8.1 shows the MICs against the bacterial strains. C4 has higher antibacterial activity against *Staphylococcus aureus* (zone of inhibition 1.1 cm) , while the activity of C4 is lower against*E.coli*(zone of inhibition 0.7 cm). But C4show promising results as antibacterial agents.

3.11Antimicrobial assay/C5

Table 3.9 Antimicrobial activity (cm inhibition zone diameter) of C5 at different concentrations.

C5	A	B	C	D
5	0.0	0.0	0.0	0.0
10	0.8	1.0	0.7	0.6
15	1.4	1.3	1.0	1.0
20	1.6	2.0	1.4	1.3
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

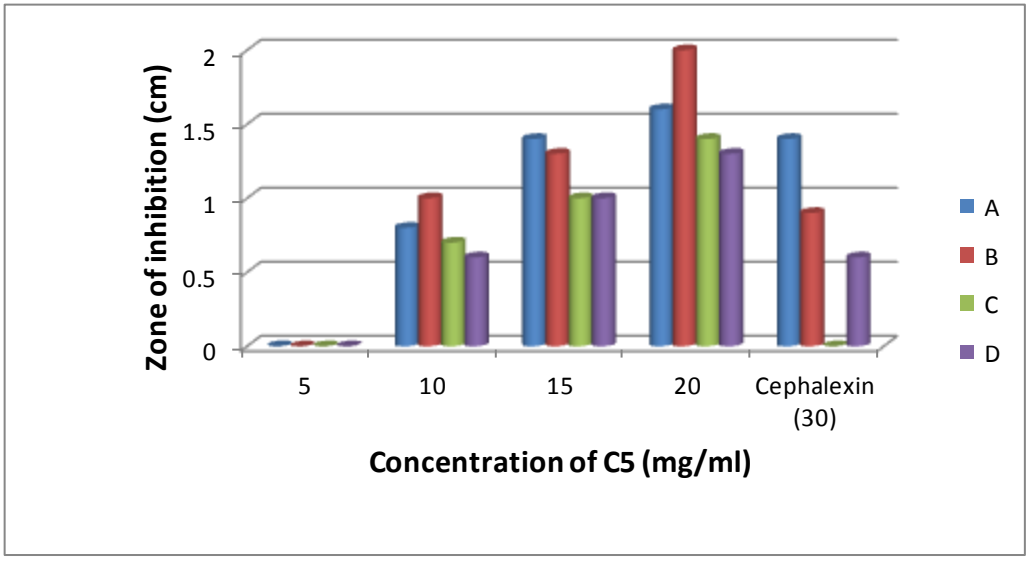


Figure 3.7 The effect of the complex C5 on different strains of bacteria.
(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C5 was tested against several microbial strains (*E.coli, S.aureus, K.pneumoniae, proteus*). The highest activity of C5 was against *S.aureus* with inhibition zone of 2 cm. The same complex exhibited moderate activity against *E.coli, K.pneumonia and Proteus*; with inhibition zones of 1.6, 1.4 and 1.3 cm, respectively. Weak activity of the tested compound was observed against *Proteus* with inhibition zone1.3 cm (Table 3.11). C5 was able to target Gram positive and Gram-negative bacteria indicating a broad-spectrum antimicrobial activity for this C5. Such broad-spectrum activity could be mediated by targeting essential steps in microbial growth or by causing metabolic toxicity [67].

3.11.1 Minimum inhibitory concentration (MIC).

Table 3.10 Minimum inhibitory concentration (MIC) of synthesized C5 against growth of bacteria expressed in mg/mL.

	A	B	C	D
C5	10	10	10	10
Zone of inhibition(cm)	0.8	1.0	0.7	0.6

A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D):*Proteus.*

Table 3.11.1 shows the MICs against the bacterial strains. C5 has higher antibacterial activity against *Staphylococcus aureus* (zone of inhibition 1.0 cm), while the activity of C5 is lower against*Proteus* (zone of inhibition 0.6 cm). But C5show promising results as antibacterial agents.

3.12 Antimicrobial assay/C6

Table 3.11 Antimicrobial activity (cm inhibition zone diameter) of C6 at different concentrations.

C6	A	B	C	D
5	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0
Cephalexin (30)	1.4	0.9	0.0	0.6

(A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

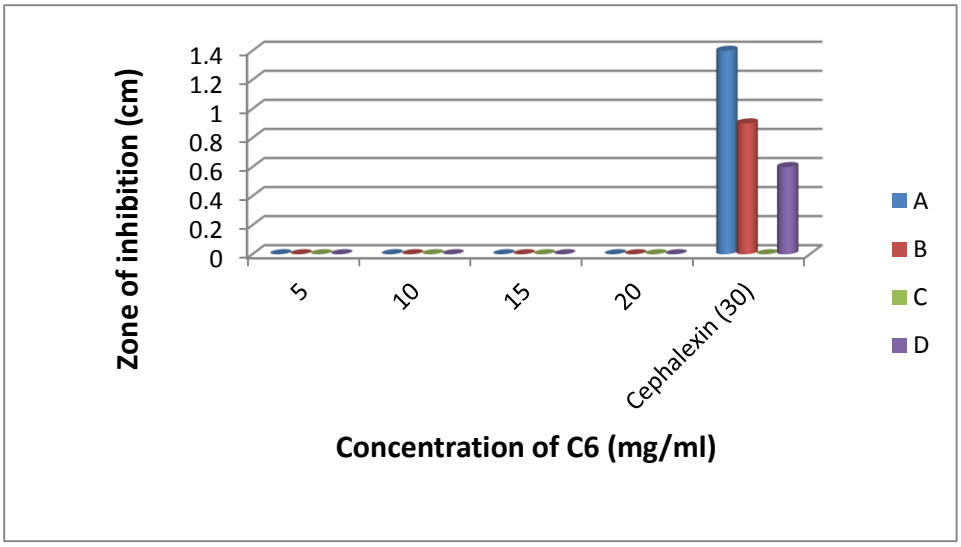


Figure 3.8 The effect of the complex C6 on different strains of bacteria.

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

In order to evaluate the antimicrobial activity of the chemical compound, the synthesized C6 was tested against several microbialstrains (*E.coli, S.aureus, K.pneumoniae, proteus*). C6 has no effect at any of the bacterial strains (Table 3.12).

3.13 Minimum Inhibitory Concentration of The Best TwoCopper II Complexes.

The minimum inhibitory concentration of the best two copper II complexes was determined using broth microdilution method. The applied protocol was similar to that of CLSI [68]. Briefly, C3&C5, were dissolved in 100% Ethanol to achieve a concentration of 1000 µg/ml. These solutions were serially diluted (2-fold) 10 times with nutrient broth (HIMEDIA, India). Well number 11 was considered negative control of bacterial growth, while well number 12 contained nutrient broth only and was the positive control of bacterial growth. The achieved 10 concentrations of C3&C5 were 0.97, 1.95, 3.90, 7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/ml. Overnight

grown bacterial isolates were applied to all wells except negative control. The final standard bacterial concentration in each well was adjusted to 1µl of 5×10^7 (CFU/ml) of each. After inoculation of bacteria, the plates were covered and incubated at 37 °C for 24 hours. Broth microdilution method was performed in duplicate for each isolate.

The lowest compound concentration (highest dilution) that inhibited the growth of tested microorganisms and did not show any visible growth in the test media was considered as minimum inhibitory concentration MIC.

Table 3.12 Minimum inhibitory concentration (MIC) of synthesized C3&C5 against growth of bacteria expressed in µg/mL.

Compound	A	B	C	D
C3	62.5	15.62	31.25	31.25
C5	125	62.5	250	125

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

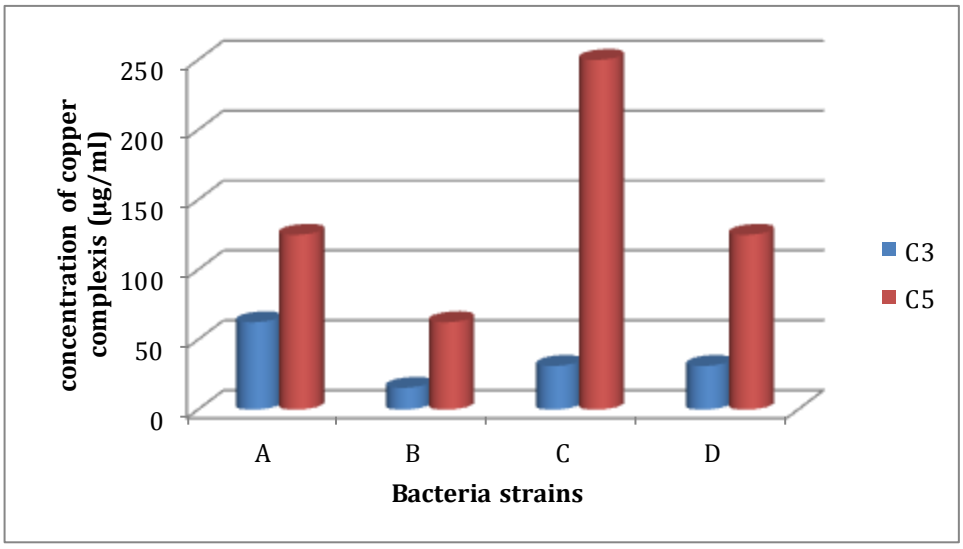


Figure 3.9 Minimum inhibitory concentration (MIC) of synthesized C3&C5 against growth of bacteria expressed in µg/mL.

A):*E.coli*; (B):*Staphylococcus aureus*; (C):*klebsiella pneumonia*; (D):*Proteus.*

Table 3.13 shows the MICs against the bacterial strains. The compound C3 has higher antibacterial activity against the studied microorganisms while the activity of C5 is lower. But both compounds show promising results as antibacterial agents. C3 and C5were able to target Gram positive and Gram-negative bacteria indicating a broad-spectrum antimicrobial activity for this both complexes. This broad-spectrum activity could be happened by targeting essential steps in microbial growth or by causing metabolic toxicity. Although both compounds show an effective results as antibacterial agents[67].

Table 3.13 Minimum Bactericidal Concentration (MBC) of synthesized complexes 1&2 against growth of bacteria expressed in µg/mL.

Compound	**A**	**B**	**C**	**D**
M3	125	31.25	62.5	62.5
M5	250	125	500	250

(A):*E.coli*; (B): *Staphylococcusaureus*; (C):*klebsiella pneumonia*; (D*):Proteus.*

3.14 Conclusion

The six dicationic water soluble copper (II) complex1, complex2 complex3, complex4, complex5 and complex6, of general formula [CuNNN(NN)]Br_2 [NNN = triamine and NN is *chda* = 1,2-diamiocyclohexane or Me2*pn* = 2,2dimethyl-1,3diamino propane] were prepared under ultrasonic mode with very good yield. These complexes were characterized by elemental microanalysis, UV-visible and IR spectroscopy, thermal and electrochemical techniques. The antibacterial activity of the complexes1,3,4,5 against the bacteria were better than Cephalexin, unlike complex 2 that showed a little antimicrobial activity and complex 6 that have not any antimicrobial activity. Also the complexes 1,3,4,5 showed a high antimicrobial activity against *Klebsiella* compared with Cephalexin that have not any antibacterial activity against this kind of bacteria, which encourages to use these complexes as a topical treatment instead of Cephalexin.

References

[1]. Chandar, P., Vennela, S., Samyuktha, V. R., Ramana, V., Ch, D., &Srinivas, B.(2014).**In-Vitro Screening For the Different Medicinal Plants**.

[2]. Yap, P. S. X., Yiap, B. C., Ping, H. C., & Lim, S. H. E. (2014). ***Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance*. The open microbiology journal**, *8*, 6.

[3]. Cantón, R., &Morosini, M. I. (2011).**Emergence and spread of antibiotic resistance following exposure to antibiotics.** FEMS microbiology reviews, *35*(5), 977-991.

[4]. van den Bogaard, A. E., &Stobberingh, E. E. (1999). **Antibiotic usage in animals.** Drugs, *58*(4), 589-607.

[5]. Podschun, R., &Ullmann, U. (1998).**Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors.** Clinical microbiology reviews, 11(4), 589-603.

[6]. Pinto-Tomás, A. A., Anderson, M. A., Suen, G., Stevenson, D. M., Chu, F. S., Cleland, W. W., ... & Currie, C. R. (2009).**Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants.** Science, 326(5956), 1120-1123.

[7]. Fouts, D. E., Tyler, H. L., DeBoy, R. T., Daugherty, S., Ren, Q., Badger, J. H., ... &Methé, B. A. (2008). **Complete genome sequence of**

the N2-fixing broad host range endophyteKlebsiellapneumoniae 342 and virulence predictions verified in mice. PLoS Genet, 4(7), e1000141.

[8]. Drancourt, M., Bollet, C., Carta, A., &Rousselier, P. (2001). ***Phylogenetic analyses of Klebsiella species delineate Klebsiella and Raoultella gen. nov., with description of Raoultellaornithinolytica comb. nov., Raoultellaterrigena comb. nov.andRaoultellaplanticola comb. nov***. **International Journal of Systematic and Evolutionary Microbiology,** 51(3), 925-932.

[9]. Rosenblueth, M., Martínez, L., Silva, J., &Martínez-Romero, E. (2004). **Klebsiellavariicola, a novel species with clinical and plant-associated isolates.** Systematic and applied microbiology, 27(1), 27-35.

[10]. Ko, W. C., Paterson, D. L., Sagnimeni, A. J., Hansen, D. S., Von Gottberg, A., Mohapatra, S., ... & Yu, V. L. (2002). **Community-acquired Klebsiellapneumoniae bacteremia: global differences in clinical patterns.** Emerging infectious diseases, 8(2), 160-166.

[11]. Carpenter, J. L. (1990).**Klebsiella pulmonary infections: occurrence at one medical center and review.** Review of Infectious Diseases, 12(4), 672-682.

[12].Brown, J., Stauber, C., Murphy, J. L., Khan, A., Mu, T., Elliott, M., &Sobsey, M. D. (2011). ***Ambient- temperature incubation for the field detection of Escherichia coli in drinking water***. **Journal of applied microbiology,** 110(4), 915-923.

[13].Reynolds, K. A., Mena, K. D., &Gerba, C. P. (2008). **Risk of waterborne illness via drinking water in the United States.**In Reviews of environmental contamination and toxicology (pp. 117-158).Springer New York.

[14].Donnenberg, M. S., &Whittam, T. S. (2001). ***Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic Escherichia coli.*** **Journal of Clinical Investigation**, 107(5), 539.

[15].Robins- Browne, R. M., & Hartland, E. L. (2002). ***Escherichia coli as a cause of diarrhea.*** **Journal of gastroenterology and hepatology**, 17(4), 467-475.

[16].Scheutz F, Strockbine NA: **Genus I. Escherichia. In Bergey's Manual of Systematic Bacteriology.** 2nd edition. Edited by Garrity GM, Brenner DJ, Krieg NR, Staley JT. Springer; 2005:607-624

[17].Karmali, M. A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., ... &Kaper, J. B. (2003). ***Association of genomic O island 122 of Escherichia coli EDL 933 with verocytotoxin-producing Escherichia coli seropathotypes that are linked to epidemic and/or serious disease.*** **Journal of Clinical Microbiology**, 41(11), 4930-4940.

[18]. Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., ... & Cohen, M. L. (1983). **Hemorrhagic colitis associated with a rare Escherichia coli serotype.** New England Journal of Medicine, 308(12), 681-685.

[19]. O'Hara, C. M., Brenner, F. W., Steigerwalt, A. G., Hill, B. C., Holmes, B., Grimont, P. A., ... & Brenner, D. J. (2000). ***Classification of Proteus vulgaris biogroup 3 with recognition of Proteus hauseri sp. nov., nom. rev. and unnamed Proteus genomospecies 4, 5 and 6.*** **International journal of systematic and evolutionary microbiology**, 50(5), 1869-1875.

[20]. Manos, J., &Belas, R. (2006).**The genera Proteus, Providencia, and Morganella.**In The prokaryotes (pp. 245-269).Springer New York.

[21]. Coker, C., Poore, C. A., Li, X., & Mobley, H. L. (2000).**Pathogenesis of Proteus mirabilisurinary tract infection.** Microbes and infection, 2(12), 1497-1505.

[22]. Ebringer, A., & Rashid, T. (2006). ***Rheumatoid arthritis is an autoimmune disease triggered by Proteus urinary tract infection.*** **Journal of Immunology Research,** 13(1), 41-48.

[23]. Knirel, Y. A., Perepelov, A. V., Kondakova, A. N., Sof'ya, N. S., Sidorczyk, Z., Rozalski, A., &Kaca, W. (2011). **Structure and serology of O-antigens as the basis for classification of Proteus strains.** Innate immunity, 17(1), 70-96.

[24]. Rather, P. N. (2005). **Swarmer cell differentiation in Proteus mirabilis.**Environmental microbiology, 7(8), 1065-1073.

[25].Becher, D., Hempel, K., Sievers, S., Zühlke, D., Pané-Farré, J., Otto, A., ... &Hecker, M. (2009). **A proteomic view of an important human**

pathogen–towards the quantification of the entire Staphylococcus aureus proteome. PLoS One,4(12), e8176.

[26].Hecker, M., Becher, D., Fuchs, S., & Engelmann, S. (2010). ***A proteomic view of cell physiology and virulence of Staphylococcus aureus.*** **International Journal of Medical Microbiology**, 300(2), 76-87.

[27]. Tenover, F. C. (2006).***Mechanisms of antimicrobial resistance in bacteria.*** **The American journal of medicine**, 119(6), S3-S10.

[28]. Mateus, A., Brodbelt, D. C., Barber, N., &Stärk, K. D. C. (2011). ***Antimicrobial usage in dogs and cats in first opinion veterinary practices in the UK.*** **Journal of Small Animal Practice**, 52(10), 515-521.

[29]. Wauchope, R. D., Buttler, T. M., Hornsby, A. G., Augustijn-Beckers, P. W. M., & Burt, J. P. (1992). **The SCS/ARS/CES pesticide properties database for environmental decision-making**. In *Reviews of Environmental Contamination and Toxicology* (pp. 1-155). Springer New York.

[30]. Kutz, F. W., Wood, P. H., & Bottimore, D. P. (1991). **Organochlorine Pesticides and Polychlorinated Biphenyls in Human Adipose Tissue*.** In *Reviews of Environmental Contamination and Toxicology* (pp. 1-82). Springer New York.

[31]. Edwards, C. A., & Thompson, A. R. (1973). **Pesticides and the soil fauna.** In*Residue Reviews* (pp. 1-79). Springer New York.

[32]. Wani, A. A., Sikdar-Bar, M., & Khan, H. A. (2013). **Acute toxicity of copper sulphate to African catfish, Clarias gariepinus**. *GERF Bulletin of Biosciences*,*4*(1), 14-18.

[33]. Roychoudhury, S., & Massanyi, P. (2008). ***In vitro copper inhibition of the rabbit spermatozoa motility.*** **Journal of Environmental Science and Health, Part A,***43*(6), 651-656.

[34]. Aarestrup, F. M., &Hasman, H. (2004). **Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection**. *Veterinary microbiology*, *100*(1), 83-89.

[35]. Ibrahim, S. A., Yang, H., &Seo, C. W. (2008). **Antimicrobial activity of lactic acid and copper on growth of Salmonella andEscherichia coli O157: H7 in laboratory medium and carrot juice.** *Food Chemistry*, *109*(1), 137-143.

[36]. Balasubramanian, K. P., Parameswari, K., Chinnusamy, V., Prabhakaran, R., & Natarajan, K. (2006). **Synthesis, characterization, electro chemistry, catalytic and biological activities of ruthenium (III) complexes with bidentate N, O/S donor ligands.** Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 65(3), 678-683.

[37]. Balasubramanian, K. P., Karvembu, R., Prabhakaran, R., Chinnusamy, V., & Natarajan, K. (2007). **Synthesis, spectral, catalytic and antimicrobial studies of PPh 3/AsPh 3 complexes of Ru (II) with**

dibasic tridentate O, N, S donor ligands. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 68(1), 50-54.

[38]. Rosu, T., Pahontu, E., Maxim, C., Georgescu, R., Stanica, N., & Gulea, A. (2011). **Some new Cu (II) complexes containing an ON donor Schiff base: synthesis, characterization and antibacterial activity.** Polyhedron, 30(1), 154-162.

[39]. Sathyadevi, P., Krishnamoorthy, P., Alagesan, M., Thanigaimani, K., Muthiah, P. T., & Dharmaraj, N. (2012). **Synthesis, crystal structure, electrochemistry and studies on protein binding, antioxidant and biocidal activities of Ni (II) and Co (II) hydrazone complexes.** Polyhedron, 31(1), 294-306.

[40]. Vyas, K. M., Joshi, R. G., Jadeja, R. N., Prabha, C. R., & Gupta, V. K. (2011). **Synthesis, spectroscopic characterization and DNA nuclease activity of Cu (II) complexes derived from pyrazolone based NSO-donor Schiff base ligands.** Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 84(1), 256-268.

[41]. Karvembu, R., Hemalatha, S., Prabhakaran, R., & Natarajan, K. (2003). **Synthesis, characterization and catalytic activities of ruthenium complexes containing triphenylphosphine/triphenylarsine and tetradentate Schiff bases.** Inorganic Chemistry Communications, 6(5), 486-490.

[42]. Frey, G. D., Bell, Z. R., Jeffery, J. C., & Ward, M. D. (2001). **Complexes of ruthenium (III) and chromium (III) with a new tetradentate N 2 O 2-donor ligand: crystal structures, redox properties and spectroelectrochemistry.** Polyhedron,20(26), 3231-3237.

[43]. Keskioğlu, E., Gündüzalp, A. B., Cete, S., Hamurcu, F., & Erk, B. (2008). **Cr (III), Fe (III) and Co (III) complexes of tetradentate (ONNO) Schiff base ligands: synthesis, characterization, properties and biological activity.** Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 70(3), 634-640.

[44]. Sigman, D. S., Mazumder, A., & Perrin, D. M. (1993). **Chemical nucleases.**Chemical Reviews, 93(6), 2295-2316.

[45]. Stanton, M. F., & Otsuka, H. (1963). ***Morphology of the oncogenic response of hamsters to polyoma virus infection.*** **Journal of the National Cancer Institute,**31(2), 365-409.

[46]. Krishnamurti, C., Saryan, L. A., & Petering, D. H. (1980). Effects of ethylenediaminetetraacetic acid and 1, 10-phenanthroline on cell proliferation and DNA synthesis of Ehrlich ascites cells. Cancer research, 40(11), 4092-4099.

[47]. KoÈpf-Maier, P., & Koepf, H. (1987). **Non-platinum group metal antitumor agents. History, current status, and perspectives**. Chemical Reviews, 87(5), 1137-1152.

[48]. Takamiya, K. (1960). Anti-tumour activities of copper chelates.

[49]. Ng, C. H., Kong, K. C., Von, S. T., Balraj, P., Jensen, P., Thirthagiri, E., ... & Chikira, M. (2008). **Synthesis, characterization, DNA-binding study and anticancer properties of ternary metal (II) complexes of edda and an intercalating ligand.** Dalton Transactions, (4), 447-454.

[50]. Barve, A., Kumbhar, A., Bhat, M., Joshi, B., Butcher, R., Sonawane, U., & Joshi, R. (2009**). Mixed-ligand copper (II) maltolate complexes: synthesis, characterization, DNA binding and cleavage, and cytotoxicity.** Inorganic chemistry, 48(19), 9120-9132.

[51]. Zhang, S., Zhu, Y., Tu, C., Wei, H., Yang, Z., Lin, L., ... & Guo, Z. (2004). ***A novel cytotoxic ternary copper (II) complex of 1, 10-phenanthroline and L-threonine with DNA nuclease activity.*** **Journal of inorganic biochemistry,** 98(12), 2099-2106.

[52]. Woo, S. H., Park, I. C., Park, M. J., An, S., Lee, H. C., Jin, H. O., ... & Hong, Y. J. (2004). ***Arsenic trioxide sensitizes CD95/Fas-induced apoptosis through ROS-mediated upregulation of CD95/Fas by NF-κB activation.*** **International journal of cancer**, 112(4), 596-606.

[53]. Al-Noaimi, M., Nafady, A., Warad, I., Alshwafy, R., Husein, A., Talib, W. H., & Hadda, T. B. (2014). **Heterotrimetallic Ru (II)/Pd (II)/Ru (II) complexes: Synthesis, crystalstructure, spectral Characterization, DFT calculation and antimicrobial**

study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 122, 273-282.

[54]. Mousa, A. N., Choudhary, M. I., Awwadi, F. F., Talib, W. H., Hadda, T. B., Yousuf, S., ... & Warad, I. (2014). **Characterization and biological activities of two copper (II) complexes with dipropylenetriamine and diamine as ligands.** Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 127, 225-230.

[55]. Vincent, J. G., Vincent, H. W., & Morton, J. (1944). **Filter Paper Disc Modification of the Oxford Cup Penicillin Determination**. Experimental Biology and Medicine, 55(3), 162-164.

[56]. Suffredini, I. B., Paciencia, M. L., Nepomuceno, D. C., Younes, R. N., &Varella, A. D. (2006). **Antibacterial and cytotoxic activity of Brazilian plant extracts-Clusiaceae.** Memorias do InstitutoOswaldo Cruz, 101(3), 287-290.

[57]. Wanger, A., Mills, K., Nelson, P. W., & Rex, J. H. (1995). **Comparison of Etest and National Committee for Clinical Laboratory Standards broth macrodilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant Candida isolates.** Antimicrobial Agents and Chemotherapy, 39(11), 2520-2522.

[58]. Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli, A. S., &Esimone, C. O. (2001). ***Evaluation of extracts of the root of Landolphiaowerrience for antibacterial activity.*** **Journal of ethnopharmacology**, 78(2), 119-127.

[59]. Wiegand, I., Hilpert, K., & Hancock, R. E. (2008).**Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances.** Nature protocols, 3(2), 163-175.

[60]. Taylor, P. C., Schoenknecht, F. D., Sherris, J. C., &Linner, E. C. (1983). **Determination of minimum bactericidal concentrations of oxacillin for Staphylococcus aureus: influence and significance of technical factors.**Antimicrobial Agents and Chemotherapy, 23(1), 142-150.

[61]. Spackman, M. A., & McKinnon, J. J. (2002). **Finger printing intermolecular interactions in molecular crystals.** Cryst Eng Comm, 4(66), 378-392.

[62]. Patel, R. N., Singh, N., Shukla, K. K., Niclós-Gutiérrez, J., Castineiras, A., Vaidyanathan, V. G., & Nair, B. U. (2005). **Characterization and biological activities of two copper (II) complexes with diethylenetriamine and 2, 2′-bipyridine or 1, 10-phenanthroline as ligands.** Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 62(1), 261-268.

[63]. Nagaraj, K., Ambika, S., Rajasri, S., Sakthinathan, S., & Arunachalam, S. (2014). **Synthesis, micellization behavior, antimicrobial and intercalative DNA binding of some novel surfactant copper (II) complexes containing modified phenanthroline ligands**. Colloids and Surfaces B: Biointerfaces, 122, 151-157.

[64]. Tabassum, S., Amir, S., Arjmand, F., Pettinari, C., Marchetti, F., Masciocchi, N., ... & Pettinari, R. (2013). **Mixed-ligand Cu (II)–vanillin Schiff base complexes; effect of coligands on their DNA binding, DNA cleavage, SOD mimetic and anticancer activity**. European journal of medicinal chemistry, 60, 216-232.

[65]. González-Álvarez, M., Pascual-Álvarez, A., del Castillo Agudo, L., Castiñeiras, A., Liu-González, M., Borrás, J., & Alzuet-Piña, G. (2013). **Mixed-ligand copper (ii)–sulfonamide complexes: Effect of the sulfonamide derivative on DNA binding, DNA cleavage, genotoxicity and anticancer activity**. Dalton Transactions,42(28), 10244-10259.

[66]. Manikandamathavan, V. M., Rajapandian, V., Freddy, A. J., Weyhermüller, T., Subramanian, V., & Nair, B. U. (2012). ***Effect of coordinated ligands on antiproliferative activity and DNA cleavage property of three mononuclear Cu (II)-terpyridine complexes***. **European journal of medicinal chemistry,** 57, 449-458.

[67]. Srinivasan, D., Nathan, S., Suresh, T., & Perumalsamy, P. L. (2001). ***Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine.*** **Journal of Ethnopharmacology**, 74(3), 217-220.

[68]. Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli, A. S., & Esimone, C. O. (2001). ***Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity.*** **Journal of ethnopharmacology**, 78(2), 119-127.

[69]. Barry, A. L., Coyle, M. B., Thornsberry, C., Gerlach, E. H., & Hawkinson, R. W. (1979). ***Methods of measuring zones of inhibition with the Bauer-Kirby disk susceptibility test***. **Journal of clinical Microbiology,** 10(6), 885-889.

[70]. Fuqha,Muheeb. (2015). **Complexes design, spectral, structural and biological activities of novel dicationic [copper (II)/1,10-phenanthroline/ N-tridentate]2 bromide, chemical research,** (1)(2), 27,43.

جامعة النجاح الوطنية

كلية الدراسات العليا

تحضير معقدات نحاس أيونية مبتكرة واستخدامها كمضادات للبكتيريا المقاومة للمضادات الحيوية

إعداد

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا، في جامعة النجاح الوطنية، في نابلس – فلسطين.

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الملخص

تم تحضير ستة معقدات من النحاس الثنائي الذائب في الماء المركب 1، المركب2، المركب3، المركب4، المركب5 والمركب 6 ذو الصيغة العامة [CuNNN (NN)] Br2 [NNN = triamine and NN is chda = 1,2−diamiocyclohexane or Me2pn = 2,2dimethyl−1,3diamino propane]، باستخدام الأشعة فوق الصوتية بحاصل جيد. هذه المعقدات تم تحليلها بواسطة التحليل الأولي، امتصاص الاطياف الضوئية، الأشعة تحت الحمراء. تم تقييم نشاط هذه المعقدات ضد أنواع مختلفة من البكتيريا وهي *Klebsiella*, *Escherichia coli*, *Proteus*, *Staphylococcus aureus*.

ووجد أن أربعة مركبات من الستة مركبات لها نشاط عالي وواعد وهي المركبات 1،3،4،5 والمركب 2 كان له تأثير قليل في قتل البكتيريا، أمّا المركب 6 لم يكن له أي تأثير يذكر على البكتيريا وقد يكون السبب في ذلك أن هذا المركب غير مستقر. تم استخدام مضاد حيوي وهو السيفاليكسين كمعيار لتتم مقارنة فعالية المركبات على قتل المركبات، ويستخدم هذا المضاد كدواء لقتل البكتيريا من نوع +Gram و−Gram. وبشكل عام المركبات 1،3،4،5 كان تأثيرها أفضل من السيفاليكسين في قتل البكتيريا على الرغم من أنه تم استخدامها بتراكيز أقل من السيفاليكسين. ولوحظ أيضا أن المركبات 1،3،4،5 لها تأثير عالي في قتل البكتيريا من نوع *Klebsiella* مقارنة بالسيفاليكسين الذي لا يوجد له أي فعالية في قتل هذا النوع من البكتيريا مما قد يشجع على استخدامه كبديل موضعي لهذا المضاد.