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Synthesis and study of N, N` -(ethane-1,2-diyl)bis(1-phenyl methanimine) and their complex derivative with *in-vivo* and *in-vitro* Bacterial biological study

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Abstract

In this research, the synthesis and biological activity evaluation of N, N`-(ethane-1,2-diyl)bis(1-phenyl methanimine) as Schiff base with Zinc (II) dithiocarbamate complex are successfully reported. The suggested geometry around zinc ion centre was determined using selected unique physicochemical and spectroscopic techniques. These include melting point, conductance, FTIR, UV-Vis, mass spectroscopy and ^{1}H-NMR spectroscopy. The analytical as well as the spectroscopic characterization data proves the formation of zinc complex with general formula $[Zn(L)]_2$ and the complex of this ion is adopting tetrahedral geometry about the metal centre. Furthermore, the present study was carried out to evaluate antibacterial activity of obtained Schiff base ligand and Zn complex against five strains of bacteria (*Staphylococcus aureus, Escherichia coli, Lacto bacillus, Klebsiella Pneumonae,and Salmonella typhi*). The results of antimicrobial screening suggest that Schiff bases can show a considerable activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Salmonella typhi while Zn-complex was reported to be more active against all bacterial strains which is used in this test. The antibacterial activity of each compound has been examined practically (*in vivo*) by treating burns rats infected with tested bacteria (*Staphylococcus aureus and Salmonella typhi*), the results have shown signs of good recovery at short time if it compared with antibiotic Ofloxacin (OFX) used at the same time. And the number of bacteria was reduced significantly ($P \leq 0.001$) during the treatment days. Furthermore, the results showed that Zn-complex is effective in treating various skin conditions; it is preferable to use it in the treatment of pathological injuries and against other skin infections.

Keywords: Dithiocarbamate, Schiff base ligand, Zinc complexes, Biological activity, Rats.

Introduction

Drug resistance against antibacterial agents can be problematic specifically when used for medical purposes. This problem can be avoided through taking some procedures such as the preparation of new metal complexes by using chelation process coordination of transition metal ions. As a matter of fact, Schiff bases have N atoms as a part of their basic scaffold and that will lead to the ability of its derivatives to contain donor atom that can act as perfect chelating agents for the process of metal ions transition. Therefore, Schiff bases and their metal complexes have been widely investigated because of the significant properties they have such as: the antiparasitic, the fungicidal-bactericidal, and the anticancer [1]. Dithiocarbamates. are organosulphur compounds. Their metal complexes have highly attracted in research attention due to some reasons such as: Its diverse uses and its fascinating biological, structural, magnetic, electrochemical and thermal properties [1-7]. Dithiocarbamates can be used to serve many functions, for example, they are used as accelerators in vulcanization, they are used as high-pressure lubricants, they are also used as fungicides, pesticides [2] and medicinal chemistry [3]. Furthermore, Dithiocarbamate species are considered potential drug candidates that have been shown to be anti-cancer [4] anti-bacterial and antifungal activities [5] with good examples of resistance in industry. For instance, the formation of Zn-dithiocarbamate complexes showed resistance for increasing temperature and conductivity. Also, these complexes are useful for the manufacture of electrical wires [6].

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Therefore, they have application in manufacturing of a nanoparticle sorbent composed or metal [7]. An example of the compounds which play an important role in agriculture is the formation of Mancozeb fungicides, an ethylene-bis-dithiocarbamate with manganese and zinc. These are the most commonly commercial used fungicides across the globe [8]. The complexes of Zn-dithiocarbamate are used also to increase the number of small nodes in the plant, increase the weight of dry roots, the concentration of chlorophyll in the leaves of the plant as well as the eradication of agricultural pests and used in biomedical application [9]. Complexes of dithiocabamate with bipyridine Au(III), are of a particular interest in biomedical field as they are used as anti-cancer agents used as anti-cancer agents for prostate, breast, ovarian cancer cell lines and in Hodgkin lymphoma cells [10]. Concentrations of zinc ions are necessary for optimal bacterial growth of most in vitro microorganisms. However, high concentrations of zinc ions may show some antimicrobial activity against various bacterial and fungal strains [11]. As soon as, zinc is used as nonbactericidal and at specific concentrations can block or hinder the biofilm formation by several Gram- positive or Gram-negative bacterial swine pathogens [12]. Antimicrobial resistance (AMR) can be widely seen as a growing problem. Without effective antimicrobials, infections can become more difficult to treat. Medical and surgical procedures can be at high-risk interventions that may cause a prolonged sickness or lead to disability or cause death. It already has been proved that certain pathogens are already resistant to most of antimicrobials in the market. Resistance maybe grow as a result of multi-various reasons such as: the wrong use of medicine, low-quality of medicines, incorrect prescriptions, and many issues related with infection prevention and control. Therefore, new adapted medicines compounds can be target different pathogens [13].

Material and Methods

Materials

Ethylenediamine, Benzaldehyde, zinc chloride, Methanol, Carbon disulphide, Potassium hydroxide, and Dimethyl sulfoxide (DMSO) manufactured by Sigma-Aldrich company. These reagents are commercially available and they are used in our research without purifying them.

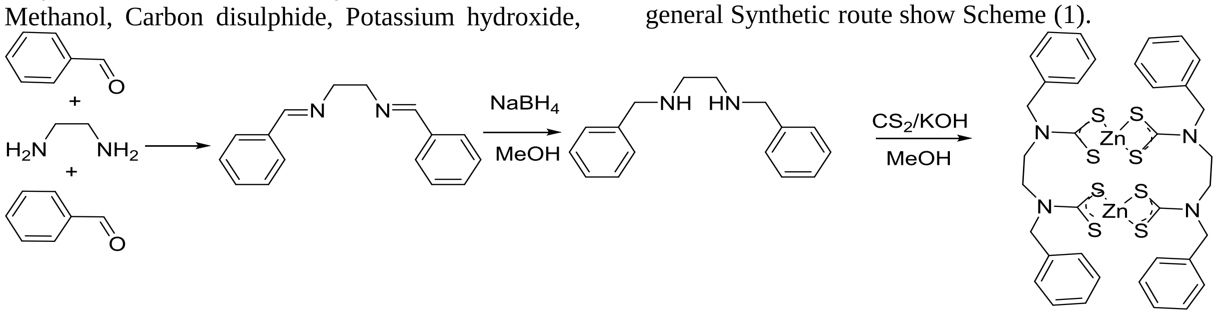
Physical measurements

^{1}H NMR spectra were recorded on a Bruker 500 MHz Avance III. Fourier transform infrared spectra (FT–IR) Were given on a Shimadzu.–8400S spectrophotometer within a range of. (4000 –200 cm^{-1}) by preparation of KBr discs. Melting point (MP) was recorded using an Electro-Thermal Stuart apparatus. Using Euro Vector 3000, the elemental analysis (C, H, N, and S) for the compounds were obtained. The electronic spectra is measured by using a Shimadzu UV-Visible 160A Spectrophotometer by dissolving synthesised compounds in DMSO at concentration of (10^{-3} M). A Shimadzu 620 G atomic absorption spectrophotometer was used to determine metal content in the prepared complexes. Finally, the conductivity measurements for each compound at (25 ºC) dissolved in (10^{-3} mole L^{-1}) of DMSO were recorder by using Jenway meter model 4070.

Synthesis

Synthesis of Schiff base (N, N`-(ethane-1,2-diyl) bis (1-phenylmethanimine).

Ethylenediamine (0.5 g, 0.008 mol) was dissolved in toluene (25 mL), then the solution was added with stirring to a mixture of benzaldehyde (1.76 g, 0.016 mol) in (25 mL) of toluene with few drops of HBr. Using Dean- Stark equipment, the reflux phase of the mixture under N_2 gas atmosphere was then performed for 45 min. In addition, under low pressure the solvent was removed completely to give a yellow solid powder; yield (1.71g, 87.03%), m.p 115-116ºC. (FT IR cm^{-1}) (Fig. 1): 3002 ν(C-H, aro), 2985 and 2850 ν(C-H, aliph), 1639 ν(C=N), 1600 ν(C=C), 1430 and 1396 νas,s (C-N) table (1). NMR data (ppm), δH (500 MHz, DMSO-d$_6$) (Fig. 2): 3.83 (4H, t, -CH2-CH$_2$-), 7.39- 7.72 (10H, m, aromatic-H), 8.29 (2H, s, -CH=) [15]. The Elemental Analysis for Chemical Formula $C_{16}H_{16}N_2$ has: C, 81.32%; H, 6.82%; N, 11.85. mass spect m/z equal 236. The general Synthetic route show Scheme (1).



Scheme (1): Synthetic route of binuclear Zn- complex

Table 1. FTIR forSynthesis of Schiff base and Zn- complex

Symbol	ν(C-H) Arom.	ν(C-H). alipha	ν(C=N).	ν(C=C). Arom.	ν(C-N).	ν (C-S).	ν(Zn-S).
Pre-ligand	3002	2985 2850	1639	1600	1430 1396	-	–
Zn- complex	3002	2981	-	1525	1382 1372	1035 1099	453

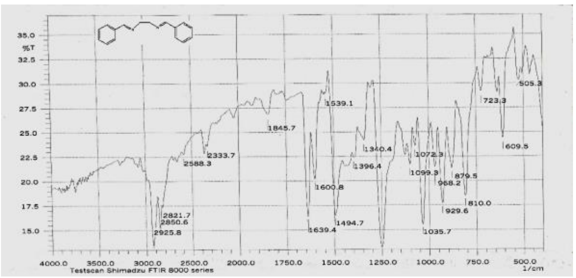


Fig. 1. FTIR Spectra of Schiff base (N, N` -(ethane-1,2-diyl)bis(1-phenylmethanimine)

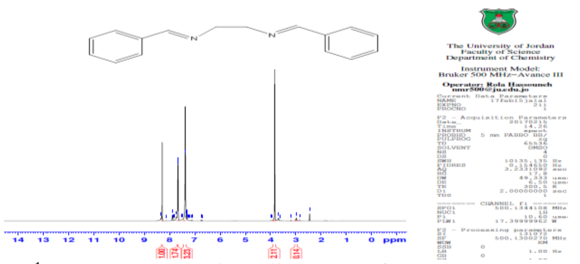


Fig. 2. ^{1}H NMR spectrum of Schiff base (N, N` -(ethane-1,2-diyl)bis(1-phenylmethanimine) in DMSO-d_6 solution

Via a one pot reaction was synthesized of macrocyclic complexes

A general method for the preparation and application of the necessary bimetallic dithiocarbamate-based zinc macrocyclic complexes as shown in the following [14, 15].

In a suitable reaction solution, two moles of secondary amine which obtained from Schiff base reaction was a stirred with excess of 5 moles of $NaBH_4$ as reduce agent in methanolsolution. Next, two moles of KOH (eq) was added to obtained potassium secondary amine salt. At 0 °C, carbon disulphide (2,50 equivalents) was then added to the mixture. Until this step, the compound is prepared (Ligand salt has not yet been isolated) through adding zinc ion in one equivalent. The mixture was kept stir overnight and water was add to the solution if the precipitation do not appear. Finally, the mixture is filtered and dried to achieve zinc complex as white solid; yield (0.81g, 83%), m.p 172-174°C. (FT IR cm^{-1}) (Fig. 3): 3002 ν(C-H, aro), 2091 ν(C-H, aliph), 1525 ν(C=C), 1382 and 1372 $\nu_{as,s}$ (C-N), 1035 and 1259 $\nu_{as,s}$ (CS_2), 453 ν(Zn-S). NMR data (ppm), δH (500 MHz, DMSO-d_6) (Fig. 4): 3.70(4H, t, -CH_2-CH_2-), 3.85 (4H, s, N-CH2-), 7.00- 8.40 (10H, m, aromatic-H). The Elemental Analysis for Chemical Formula: $C_{36}H_{40}N_4S_8Zn_2$: C, 47.21%; H, 4.40%; N, 6.12%; S, 28.00%; Zn, 14.28%.

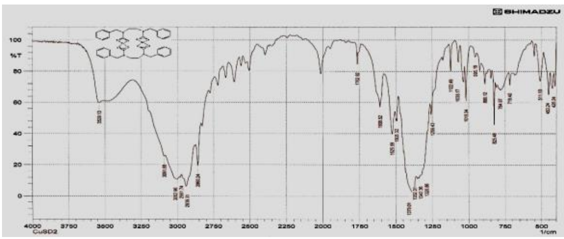


Fig. 3. FTIR spectra of L and its complex with Zn(II) metal ion

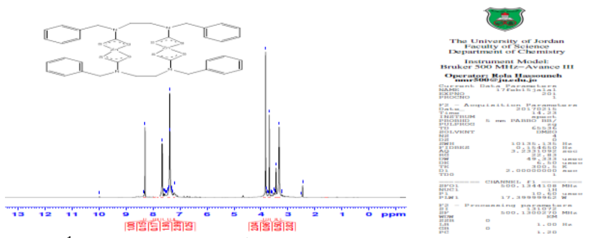


Fig. 4. ^{1}H NMR spectrum of [Zn (L)]$_2$ in DMSO_d_6 solution.

Biological testing

Diagnosis bacteria

All bacterial species were isolated from patients with burn infection (*Staphylococcus aureus* , *Escherichia coli, Klebsiella Pneumoniae and Salmonella typhi except lactobacillus*), while all respectively selective and differential isolated media defined on the basis of the morphological, colonial, gram stain and biochemical tests. Biochemical tests used for identification of bacteria include: Urease, catalase, oxidase, indole, Methyl red, Voges Proskauer, Simmons citrate utilization, TSI, and finally API 20E used for diagnosis [16].

Test of antibiotic susceptibility

The already modified Kirby-Bauer method was used to determine the susceptibility of obtained isolates against some antibiotics such as Cipfloxacin. (CPX) 5 µg, Ceftazidime. (CAZ) 30 µg, Lincomycine. (L) 2 µg, Clindamycine. (CD) 2 µg, Chloramphenicol. (C) 30 µg, Cefixime. (CFM) 5 µg, Amoxycillin. (AMX) 10 µg, Cotrimoxazole. (COT) 5 µg, Erythromycin. (ERY) 15 µg, Augmentin. (AUG) 12.5 µg, Ofloxacin. (OFX), and Nitroforantoin. (NIT) 300 µg antibiotics as described by reference [17].

Bacterial susceptibility testing

Agar diffusion test

Muller Hinton Agar (MHA) obtained from (HiMedia, India) is adopted to evaluate the inhibition zone diameter (ID) through using basic well diffusion methods. The plates were inoculated with the standardized suspension (comparing with McFarland tube) of the test isolates. The plates were placed to dry in incubator for 30 minutes at 37 ºC with aiding of a sterile standard allowed core borer, 5 wells with 5mm in diameter were bored at equidistant. Each of the Schiff base and Zinc complex dissolved in Dimethyl sulfoxide (10% DMSO), final consternation (50, 100, 150, 200) mg/mL and 25µl of each compound dropped into the appropriate well in the inoculated plate. Next, to a sterile water and DMSO used as negative controls, the prepared plates were incubated at 37 °C for 24h (aerobic and an aerobic). The resulting zones of inhibition are measured by using calibrated ruler in millimeters. Finally, the average of three readings is considered to be the inhibition zone of the bacterial isolates at each specific concentration [18].

Determination of minimum inhibitory concentration (MIC)

The above mentioned compounds (Schiff base and Zinc complex), this was later tested for antimicrobial activity to evaluate the MIC for each bacterial isolate. Five bacterial species *Staphylococcus aureus, Escherichia coli, Lacto bacillus, Klebsiella Pneumonae and Salmonella typhi* were grown in nutrient broth tubes separately for 6 h. Afterwards, 100 µL of 106 cells/mL of growth culture were inoculated in nutrient broth tubes containing different concentrations of the Schiff base and Zinc complex (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 mg/ml) for 24 h at 37 °C separately for each compound and each bacteria in addition to control samples, then the bacterial growth was evaluated on the basis of the turbidity of the suspension and all tubes were read by spectrophotometer (LKB- PYEUNICAM-SP 6.55, England) at 620 nm. [19].

Inhibitory effect of both compounds on Staphylococcus aureus and Salmonella typhi (In vivo).

Twenty-four male rats (150-180 g) aged between 12 and 18 weeks were used for all in vivo experiments. They were kept in a light, food and temperature-controlled room and all rats were acclimatized for at least 1 week prior to beginning the experiments. The dorsal back skins of the mice were shaved and ethanol (70%) was used as antiseptic for the shaved region and then burned by using inflamed knife and then contaminated with both bacteria *Staphylococcus aureus and Salmonella typhi* (1.5×108 bacteria /mL), After two days of injury the inflammation, redness and suppuration region were observed and the experimental mice were randomly separated into the following groups (n = 30): Group 1: untreated rats (control). Group 2: burn rats treated with ointment composed of antibiotic for *Staphylococcus aureus bacteria* (OfX). Group 3: burn rats gently treated with ointment composed of antibiotic for *Salmonella typhi bacteria* (OfX). Group 4: burn treated with ointment for *Staphylococcus aureus bacteria* (concentration 50 mg/mL) consisting of 0.5 g of Schiff base ligand added to 9.5 g of Vaseline. Group 5: burn treated with ointment for *Salmonella typhi* (concentration 50 mg/mL) consisting of 0.5 g of Schiff base and Zinc complex added to 9.5 g of Vaseline. Group 6: burn treated with ointment for *Staphylococcus aureus* each (concentration 50 mg/mL) consisting of 0.5 g of Zn- complex added to 9.5 g of Vaseline.Group 7: burn treated with ointment for each bacteria (concentration 50 mg/mL) consisting of 0.5 g of Zn- complex added to 9.5 g of Vaseline. The treatment continued twice each day during 15 consecutive days and the numbers of bacteria was calculated [20, 21].

Results and Discussion

Chemistry

The free Schiff base pre-ligand was synthesis by the reaction of ethylenediamine with benzaldehyde in ethanolic solution reaction. Next, zinc macrocyclic complexes obtained from the reaction of zinc chloride with ligand in a methanolic solution reaction.

The synthesis of dithiocarbamate-based macrocyclic complex was achieved by the reaction of the pre-ligand (Schiff base) with the corresponding zinc ion in a ratio of 1:1 mole. In this step the metal ion is major role of the desired dinuclear macrocyclic self-assembly as shown in Scheme 1. The properties of yielded complex are solid, stable, and completely soluble in DMSO. A lot of techniques have successfully characterized the new synthesized ligand and its macrocyclic dinuclear complex, such as: melting point, electrical conductance, elemental analysis, FTIR spectroscopy, UV electronic spectra, and ^{1}H-NMR. The analytical characterization data provided the successfully formation of all compounds as shown in Table 1. In addition, the non-electrolytic nature of these complexes is indicated by the molar conductance of the synthesized complexes dissolved in DMSO solutions.

FTIR spectra

The FTIR spectra of ligand and each corresponding complex are collected together in Figure (1 and 3). FTIR spectra of ligand and its corresponding complexes are exhibited obvious two bands in the region of 1602-1565 cm^{-1} that made reference to ν(C=N) and ν(C=C) groups of the pyridyl ring. The clear strong band in the range of 1460-1500 cm^{-1} in each complexes spectra is assigned to ν($N-CS_2$) group stretching vibrations. Interestingly, the band spectra show double bond character increase (carbon-nitrogen) compared to the free-ligand observed at 1462 cm-1 [22]. The νas (CS_2) that shows two bands at 1111 and 1049 cm^{-1} in the ligand [23, 24] is shifted and appeared in the complexes spectra around 1046 and 999 cm^{-1}. Moreover, a new band was observed at 945 cm^{-1} which refers to νs(CS_2) stretching [25] and these bands indicate the anisobidentate chelation mode of ligand to the metal atoms [26, 27, 28]. The assignment details of complexes prominent bands are listed in Table (1).

Electronic spectra

The UV-Vis spectral data for synthesized new compound has shown obvious peaks around 266-368 nm refer to the intra-ligand field in the complex. Moreover, defined peaks of visible region at 375-435 nm was refer to charge transfer bands spectra related to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$(3). As expected, Zn ion which has d10 electron is diamagnetic and normally prefers to tetrahedral coordination formation.

^{1}H-NMR spectra

The proton NMR spectrum for [Zn-complex] displays chemical shifted to up field compare to pre-ligand chemical shift. This can be approving the formation of Zn- complex by the signal appearance observed around δ=2.52 and 3.36 ppm assign to the NMR solvents like DMSO-d_6, and water residual in solvents [30].

Measurements of Conductivity

The molar conductance measurements of Zn-complex in DMSO solution appear at 12.00 Ω-$1cm^2mol^{-1}$ which indicates their nonelectrolyte behavior [31].

Biological Activity

All bacterial isolates were tested for antimicrobial susceptibility testing using disk diffusion methods and the results were interpreted according to standard values provided by Clinical and Laboratory Standard Institute of anti-microbial susceptibility testing [32]. Table (2) illustrate the susceptibility profile for all isolates by using 12 antimicrobial agents. Isolated bacteria showed a variation in their sensitivity and resistance to used antibiotics. On other hand, the high interesting resistance of the bacterial isolates may be related to the presence and disseminations of the plasmids within heterogeneous populations of these bacteria [33]. The other possible reason can be attributed to the fact that the a huge number of the populace purchasable antibiotics in open markets without appropriate medical prescription which will lead to wrong use of drug concentration for wrong diseases. The emergence of drug resistance in human pathogens to widely used antibiotics has needed a search for new antimicrobials [34].

The result of antibacterial activity of Zn- complex and Schiff bases for selected bacteria revealed that the inhibition zone (10-25) mm and (8-20) respectively, while Schiff base was no effect up to Lactobacillus as shown in Table (3) and Figure(5).

The results of antimicrobial. screening indicate that Schiff bases can provide significant activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Salmonella typhi while Zn- complex was proved to be more active against all tested bacterial strains due to Gram-negative bacteria species. In addition, Zinc ion can be bind to the proteins that were inserted into the layer of lipopolysaccharides and could act as channels (porins) filled with water. In Gram-positive bacteria, which contain structurally thicker cell walls compared to Gram-negative bacteria, the effect of zinc on Gram-negative bacteria can be less than the effect [35]. Antibacterial activity of these compounds can show ascending order when the concentration is increased, the area of inhibited growth increased too. The MIC of both compounds presented in table (4). It found that Zn- complex was effective against both tested bacteria with the MIC ranged between (60-50 mg/mL) .and the more effective against two bacteria. Moreover, Schiff bases were the MIC ranged between (100- 80 mg/mL). On the other hand, Medeiros and his group [36], studies many potential mechanisms methods where the Many pathogenetic events in the bacterium, such as the reduction of biofilm formation, adherence to epithelium, or expression of the virulence factor, can be changed by physiological zinc levels. Moreover, the special bacterium’s effect on the epithelium (cytokine response to exposure of EAEC) can modify the EAEC pathogenesis in both in vivo and in vitro. These effects can be useful to explain the benefits of zinc in childhood diarrhoea or malnutrition. The increasing of complexes activity can be demonstrating on the basis of chelation theory and Overtone’s model theory [37]. The complex formation could, according to the chelation theory, lead to the crossing of a microorganism cell membrane. This is because chelation greatly decreases the polarity of the metal ion and allows the positive charge of metal to be partially shared with the donor groups, thus eventually improving the lipophilic existence of the chelated metal system, which performs its penetration ability through the lipid layer of microorganism cell membranes [38]. Furthermore, dithiocarbamate (dtc) and its complexes exhibit huge importance applications effect in biological system [39]. Nowadays the materials and industrial application of dithiocarbamate (dtc) species in environmental chemistry are considered promising candidates that could show multi-biological activity such as antibacterial, anticancer and antifungal [40].

Table 2. Antibiotic activity against bacterial species under study

Bacterial Species	CAZ	C	CFM	CD	L	AMX	ST Ŕ	COT	AUG	ERY	NIT	OFX
Staphylococcus aureus	Ŕ	Ŕ	S	S	Ŕ	S	S	Ŕ	Ŕ	Ŕ	Ŕ	S
E. coli	Ŕ	Ŕ	S	Ŕ	Ŕ	Ŕ	S	S	Ŕ	Ŕ	S	S
Lactobacillus	Ŕ	S	Ŕ	Ŕ	S	Ŕ	Ŕ	Ŕ	S	S	S	S
Klebsiella pneumonaei	Ŕ	Ŕ	S	Ŕ	Ŕ	S	S	Ŕ	S	Ŕ	Ŕ	S
Salmonella typhi	S	S	Ŕ	Ŕ	S	S	Ŕ	S	Ŕ	Ŕ	Ŕ	S

R=resistance
S=sensitive

Table 3. Inhibition zones (mm) of Pre-ligand and Zn- complex against bacteria. (- = No Inhibition)

Chemical Comps. / Bacteria species	Schiff base					Zn- complex				
	(200) mg/ mL	(100) mg/ mL	(50) mg/ mL	(25)mg/ mL	OFX mg/ mL	(200) mg/ mL	(100) mg/ mL	(50) mg/ mL	(25)mg/ mL	OFX mg/ mL
Staphyococcus aureus	15	12	10	8	32	20	15	12	-	32
Escherichia coli	16	10	10	-	22	23	19	12	10	22
Lacto bacillus	-	-	-	-	-	22	15	-	-	-
Klebsiella pneumonia	19	13	13	11	25	25	15	10	-	25
Salmonella typhi	20	15	12	-	30	18	12	10	-	30

(-)= No growth

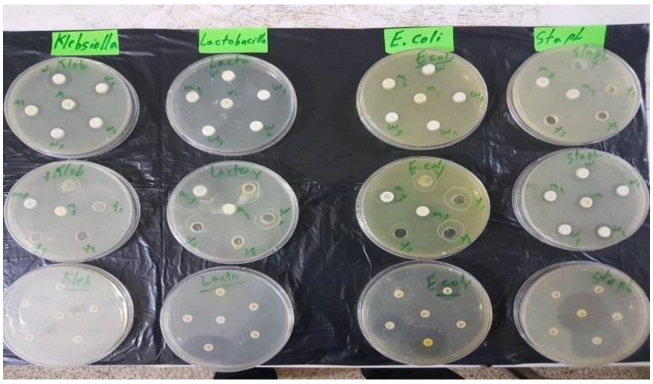


Fig. 5. Effect of Zn- complex and Schiff bases for selected bacteria

The results of antimicrobial. screening indicate that Schiff bases can provide significant activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Salmonella typhi while Zn- complex was proved to be more active against all tested bacterial strains due to Gram-negative bacteria species. In addition, Zinc ion can be bind to the proteins that were inserted into the layer of lipopolysaccharides and could act as channels (porins) filled with water. In Gram-positive bacteria, which contain structurally thicker cell walls compared to Gram-negative bacteria, the effect of zinc on Gram-negative bacteria can be less than the effect [35]. Antibacterial activity of these compounds can show ascending order when the concentration is increased, the area of inhibited growth increased too. The MIC of both compounds presented in table (4). It found that Zn- complex was effective against both tested bacteria with the MIC ranged between (60-50 mg/mL) .and the more effective against two bacteria. Moreover, Schiff bases were the MIC ranged between (100- 80 mg/mL). On the other hand, Medeiros and his group [36], studies many potential mechanisms methods where the Many pathogenetic events in the bacterium, such as the reduction of biofilm formation, adherence to epithelium, or expression of the virulence factor, can be changed by physiological zinc levels. Moreover, the special bacterium's effect on the epithelium (cytokine response to exposure of EAEC) can modify the EAEC pathogenesis in both in vivo and in vitro. These effects can be useful to explain the benefits of zinc in childhood diarrhea or malnutrition. The increasing of complexes activity can be demonstrating on the basis of chelation theory and Overtone's model theory [37]. The complex formation could, according to the chelation theory, lead to the crossing of a microorganism cell membrane. This is because chelation greatly decreases the polarity of the metal ion and allows the positive charge of metal to be partially shared with the donor groups, thus eventually improving the lipophilic existence of the chelated metal system, which performs its penetration ability through the lipid layer of microorganism cell membranes [38]. Furthermore, dithiocarbamate (dtc) and its complexes exhibit huge importance applications effect in biological system [39]. Nowadays the materials and industrial application of dithiocarbamate (dtc) species in environmental chemistry are considered promising candidates that could show multi-biological activity such as antibacterial, anticancer and antifungal [40].

The in vivo assay revealed that the treatment of infected rats skin with MIC for each compound and (OFX) antibiotic during 15 days and it was found to have marked effects (Significant at $P \leq 0.001$) on the number of Staphylococcus aureus and Salmonella typhi especially group four and five which showed more effectiveness than other groups and the number of bacteria was reduced significantly (when compared with antibiotic at $P \leq 0.001$) from130 to 19 ×106 cell/mL and 120 to 2×106 cell/mL during the treatment days and these results are attributed to Zn-complex which has antibacterial activities. After the treatment for 15 days using the antibiotic ofloxacin and both compound at 50 g / kg contrition, the results showed that the skin of the infected rat could be cured by the tumor's disappearance, redness and disappearance of the pus from the affected area compared to control where the area of the infection remained wet and continuously scrubbed. Therefore, the results showed that Zn- complex is effective and preferable to use in the treatment of pathological injuries and against skin infections as shown in table (5) and figure (6). These findings agreed with literature which establish the fact that the existence of zinc ion in the prostatic secretion under this high concentration which play a role as antibacterial polypeptides, can be so effective against (Gram-negative) and (Gram-positive) bacteria [37]. This effect of zinc is related to the capsule of (Gram-negative) and (Gram-positive) bacteria which have been tested by capsular stain and demonstrated the loss of capsule.

Conclusions

The preparation and characterization of zinc macrocyclic complex are descried. Attempts to isolate the free ligand (dithiocarbamate) from the reaction were unsuccessful. The complex's bonding mode and overall structure effect were founded upon previously remarkable physico-chemical and spectroscopic techniques. These results revealed the synthesis of four coordinate complex (tetrahedral). The development of several antibiotic-resistant Gram-positive and Gram-negative bacteria in hospitals and community settings calls for novel treatment approaches. The present study was carried out to evaluate antibacterial activity of zinc macrocyclic complex and dithiocarbamate against the growth of some bacteria isolated from hospital. Antibiotic Sensitivity was performed for all isolated bacteria which were used in this study. Results showed a range of variation in antibacterial activity of both compounds against all tested bacteria and measuring the diameter of inhibition zone. Zinc macrocyclic complex had the greatest inhibitory effect followed by dithiocarbamate. The antimicrobial activity of each compound was examined practically (*in vivo*) by treating burns rats infected with tested bacteria (*Staphylococcus aureus and Salmonella typhi*), the results have shown good recovery at short time compared to that of antibiotic (OFX) which were used simultaneously.

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Conflicts of Interest

"The authors declare no conflict of interest."

Table 4. Minimum inhibitory concentration (MIC) of Pre-ligand and Zn- complex On bacterial species

Microorganism	MIC	
	Zn- complex	Schiff bases
Staph. Aureus	60	100
Salmonella typhi	50	80

Table 5. Numbers of Staphylococcus aureus and Salmonella typhi between the rats groups

Duration of treatment per day	Group(1) Control	Group(2) OFX Staph aureus	Group(3) OFX S.typhi	Group(4) Staph aureus Zn- complex. 50mg/ml	Group(5) S.typhi Zn- complex 50mg/ml	Group (6) Staph aureus Schiff bases 50mg/ml	Group(7) S.typhi Schiff bases 50mg/ml
3	0	140	160	130	120	180	170
6	0	120	110	72*	68*	70*	110
9	0	65	40	19*	12*	30*	50
12	0	30	0	0	2	20	35*
15	0	0	0	0	0	5	10

* Significant at P≤0.001.

Fig. 6. Rats appear to be infected with burns and a bacterial infection after treatment by Zn-complex.

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تحضير ودراسة ((N, N` –(ethane–1,2–diyl)bis(1–phenyl methanimine ومشتقاتها المعقدة مع دراسة بيولوجية بكتيرية في الجسم الحي وفي المختبر

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الخلاصة

في هذا البحث تم تحضير ودراسة الفعالية البيولوجية لمركب قاعدة شف والذي تم تحضير منه ليكاند من نوع ثنائي (ثاني الكاربميت) والذي أعطى معقدا عند تفاعله مع أيون الزنك ثنائي التكافؤ . ومن خلال القياسات الفيزيائية –الكيميائية مثل درجة الانصهار وتقنيه الأشعة فوق البنفسجية والمرئية وطيف الأشعة تحت الحمراء وتقنيات الرنين النووي المغناطيسي وطيف الكتلة وبعض التقنيات التحليلية الأخرى اقترح للمعقد شكلا (رباعي السطوح).علاوة على ذلك ، أجريت الدراسة الحالية لتقييم الفعالية المضادة للبكتيريا لمركب لمركب قاعدة شيف ومعقد الزنك ضد خمس سلالات من البكتيريا (*Staphylococcus aureus*، *Escherichia coli*، *Lacto bacillus*، *Klebsiella Pneumonae* ، و *Salmonella typhi).* تشير نتائج فحص مضادات الميكروبات إلى أن قواعد شيف يمكن أن تُظهر نشاطًا كبيرًا ضد *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* بينما تبين أن مركب Zn أكثر نشاطًا ضد جميع السلالات البكتيرية المستخدمة في هذا الاختبار. تم فحص النشاط المضاد للبكتيريا لكل مركب عمليًا (في الجسم الحي) عن طريق علاج حروق الفئران المصابة بالبكتيريا المختبرية *(Staphylococcus aureus* و *(Salmonella typhi*، وقد أظهرت النتائج علامات الشفاء الجيد في وقت قصير إذا ما قورنت بالمضاد الحيوي Ofloxacin (OFX) المستخدم في نفس الوقت. وانخفض عدد البكتيريا بشكل ملحوظ ($P \geq 0.001$) خلال أيام العلاج. علاوة على ذلك، أظهرت النتائج أن معقد Zn فعال في علاج الأمراض الجلدية المختلفة. يفضل استخدامه في علاج الإصابات المرضية وضد الالتهابات الجلدية الأخرى.