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Synthesis, Characterization, and Antibacterial activity of Cobalt (II) and Copper (II) Complexes with Urea

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توصيف وتشخيص ودراسة النشاط البكتيري لمعقدات الكوبلت (*II*) والنحاس (*II*) مع اليوريا.

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Abstract:
This study focuses on the Synthesis and Characterization of Cobalt(II) and Copper(II) complexes with Urea using FT-IR. Their antibacterial actaivity was evaluated using both of positive Gram (Staphylococcus.aureus), and Negative Gram(Escherichia.coli). The result of the findings indicated that the cobalt (II) complex showed the high inhibitory activity compared to copper (II) complex which showed poor effective. Wile, the ligand did not show any effective. This study will be benefit to grow novel possible therapeutic agents.

Keywords: Cobalt(II), and Copper(II) Complexes, Urea, FTIR spectroscopy, Positive Gram (Staphylococcus.aureus), and Negative Gram (Escherichia.coli).

الملخص
ركزت هذه الدراسة على توصيف وتشخيص معقدات الكوبلت (II) والنحاس (II) مع اليوريا بواسطة FT-IR Spectroscopy. أجري اختبار النشاط البكتيري باستخدام نوعين من البكتيريا متضمنة البكتيريا الموجبة جرام (Staphylococcus. Aureus) و البكتيرياالسالبة جرام(Escherichia.coli). وأظهرت النتائج أن معقد الكوبلت يمتلك فعالية عالية مقارنة بمعقد النحاس والذي أظهر استجابة طفيفة. بينما لم يظهر الليجاند أي تأثير ضد نوعي البكتيريا المستخدمة في هذه الدراسة. وهذه الدراسة ستكون مفيدة جدا لتطوير المضادات البكتيرية لإجراء عقاقير جديدة لها القدرة على الحد من النشاط البكتيري.

الكلمات المفتاحية: معقدات الكوبلت (II) والنحاس (II)، اليوريا، FTIR spectroscopy، البكتيريا موجبة الجرام (Staphylococcus.aureus)، البكتيريا سالبة الجرام (Escherichia.coli).

Introduction

Transition metals consider one of the most significant group in the periodic table due to their partially filled d-orbitals, which enable them to form a wide range of coordination complexes with diverse geometries and distinctive physicochemical properties. These characteristics helps their extensive applications in industrial and biological systems, especially in chemical catalysis and metal-dependent biological. **[14]** processes

Among these elements , copper(Cu) and cobalt (Co) are the most important, due to that Copper plays an essential role in the structure and function of numerous enzymes involved in oxidation-reduction reactions and intracellular electron transfer, while the cobalt consider as a necessary component of vitamin B_{12}, which is vital for proper nervous system function.**[15]**

Most significant global health challenges at present, particularly in light of continuous increase in bacterial resistance to conventional antibiotics. This growing concern has driven researchers to explore alternative therapeutic agents with enhanced efficacy and distinct mechanisms of action Transition metals that have been study exhibit a strong tendency to form stable complexes with various ligands, including urea (**NH_2CONH_2**), which is a simple molecule capable of coordinating through its carbonyl oxygen atom. Such complexes have attracted considerable attention due to their relevance in spectroscopic investigations of electronic transition and their potential biological applications, including antimicrobial activity **[11]**.

There are several previous studies about such as this topic , it was studied by Baruah and others in 2025 **[5]**, and they studied the reaction of the Urea complexes with Copper(Cu) and Cobalt(Co) at different temperatures using viours analytical methods. However, they didn't study antibacterial activities of the complexes under studied.

Otherwise, in 2020 Mukdad et all **[25]** showed that cobalt and copper complexes that prepared using urea as a ligand can be stable either at med-temperature, but they didn't carry out the relationship between the structure of the complexes and their activity as antibacterial.

Studies showing that the metal complexes are generally more effective than free metal ions of free ligands, such as Schiff bases and amino acid derivatives, and the biological activity depends on the nature of the ligand and the coordination geometry, which justifies exploring new or simple ligand such as Urea due to their an important properties. While most recent studies have focused on complexes urea derivatives, research employing the simple urea molecule as a chelating ligand with copper and cobalt ions and evaluating their biological activity against resistant bacterial strains remains limited rare in the modern literature [27].

To the best of our knowledge, there are few researches about this topic. This work aimed to synthesized , characterized the Urea complexes with copper (II) and cobalt (II) by FT-IR , and study their effective on two kinds of bacteria, including positive Gram (**Staphylococcus.aureus**), and Negative Gram (**Escherichia.coli**).

2. Materials and Methods:

1.2- Chemicals and Instruments used:-

This study employed analytical-grade chemicals, including Copper(II) sulfate penta hydrate($CuSO_4.5H_2O$), Cobalt chloride hexa hydrate($CoCl_2.6H_2O$), Urea(($CO(NH_2)_2$), and Ethanol (C_2H_5OH). All chemicals that used in the synthesis were of reagent grad, and they were used without further purification.

The experimental work was conducted using a set of standard laboratory instruments. Since an analytical balance was utilized for precise mass measurements. While, water bath maintained controlled temperatures during reaction. and a Fourier Transform Infrared (FT-IR) Spectrometer was used for characterization of synthesized compounds and their ligand to determine thier functional groups. moreover a bacterial incubator provided optimal conditions for microbial growth, while an autoclave ensured complete sterilization of equipment and materials. Additionally, an optical microscope was used for microscopic examinations. All instruments used in this study were available in the laboratory and operated according to standard operating procedures.

2.2 Synthesis of Complexes:-

Synthesis of Copper complex $[Cu(CO(NH_2)_2(H_2O)_4)]SO_4$ (C_1)

The complex was prepared by dissolving (5g, 0.020mol) Copper(**II**) sulfate penta hydrate($CuSO_4.5H_2O$), in 50 ml of distilled water with continuous stirring until completely dissolved. Separately, 3g of urea were dissolved in an equal volume of distilled water. The urea solution was then added gradually to the copper sulfate solution. The mixture was heated at 50-60C^o for two hours, during which a color change from blue to green was observed, followed by the formation of green precipitate. The mixture was lifted to cool at room temperature, then kept in an ice bath for 30min. The precipitate was filtered, washed with distilled water and ethanol, dried, and stored for usage.

$$CuSO_4.5H_2O+4CO(NH_2)_2 \rightarrow [Cu(CO(NH_2)_2(H_2O)_4)]SO_4$$

2.2.2 Synthesis of Copper complex $[Co(CO(NH_2)_2(H_2O)_4)]Cl_2$ (C_2) :-

of The complex was prepared by dissolving (5g,0.021mol) Cobalt Chloride hydrate($CoCl_2.6H_2O$), in 50ml of distilled water with continuous stirring to unsure complete dissolution. Separately, 3g of urea were dissolved in 50ml of distilled water. The urea solution was then added slowly to the Cobalt Chloride solution. The mixture was heated at 50-60C^o for two hours, during which a color change from red to purple was observed, with formation of precipitate. The mixture was allowed to cool at room temperature, then kept in an ice bath for 30min. the precipitate was filtered, washed with distilled water and ethanol, then dried, and stored for usage.

$$CoCl_2.6H_2O+4CO(NH_2)_2 \rightarrow [Co(CO(NH_2)_2(H_2O)_4)]Cl_2$$

3.2 Qualitative Detection:

1.3.2-Detection of the Sulfate SO_4 in Cupper (II) complex (C_1):-

In a tube test, we putted a few amount of Cupper (II) complex, and dissolved in water, then heated to 50 C^O, to conform that it dissolved completely, after that drops of Barium Chloride $BaCl_2$ solution were added.

$$2Cl^-+H_2SO_4 \rightarrow 2HCI+SO_4$$

2.3.2-Detection of Chloride (Cl_2) Cobalt(II) complex(C_2):-

a few amount of Cobalt(II) complex was putted in a test tube, and the concentrated sulfuric Acid H2SO4 was added, then heated until boiled. and it was immersed in a solution of an ammonia using a glass rod, and then passed over the mouth of the tube.

$$HCl+ NH_4OH \rightarrow NH_4Cl+H2O$$

4.2 - FT-IR spectrum:-

The synthesized complexes and their ligand were characterized using Fourier-transform infrared(**FT-IR**) spectroscopy. The infrared spectra were recorded using potassium bromide (**KBr**) disc technique, covering a spectral range from **400-4000 cm^{-1}.** These analytical procedures were conducted at the Research and Consultation Center,University of Sebha.

5.2-Antibacterial activity:-

The antibacterial study of the synthesized complexes and thier ligand was tested against two types of bacteria, including a Gram-positive strain (**Staphylococcus.aureus**), and Gram- Negative strain **(Escherichia.coli).**

1.2.5- Preparation of Culture Media and Bacterial Isolation:-

Ten (10) urine samples were collected from pregnant women at the Central Medical Laboratory of Sebha, for purpose of bacterial isolation. The samples were cultured on three distinct types of nutritional media to facilitate identification:

- **Blood agar**

MacConkey agar& Eosin Methylene Blue (EMB) agar. The inoculation process was performed by applying a drop of the urine sample using sterile loops via the streaking method. This procedure was strictly carried out within a safety box following alcohol sterilization to prevent contaminations. The inoculated plates were then maintained in an incubator at 37C^o for a 24hour period. After that the bacterial growth was examined to insure the absence of external contamination and to diagnose the specific strains. The results successfully identified the presence of two types of a Gram-positive strain (**Staphylococcus.aureus**), and Gram- Negative strain **(Escherichia.coli).**

-2.2.5- Preparation of Solution Concentrations:

Five (**5**) serial dilutions were prepared for three samples used in this stud (**L, C_1, &C_2**), by dissolving **2g** of each sample in **2ml** of distilled water to obtain a 100 % stock. Subsequently, additional concentrations were prepared through progressive dilution with distilled water as following:-

- l of the solution+ 250 µl of distilled water. µ 750=% 75
- l of the solution+ 500 µl of distilled water. µ 500=% 50
- l of the solution+ 750 µl of distilled water. µ 250=% 25
- l of the solution+ 250 µl of distilled water. µ 750=% 75
- l of the solution+ 850 µl of distilled water. µ 150=%12.5

over at all, five 5 concentrations were obtained (100%,75%,50%,25%,&12.5% v/v) , and the distilled water used as the diluents. After that, the filter paper was cut into discs using a puncher, sterilized by autoclaving, impregnated with the different concentrations, and then dried before usage.

3.2.5 - Treatment Method:-

Mueller-Hinton agar was used as the culture medium. Moreover bacterial samples spread evenly on the plates using sterile swabs. Discs containing different concentrations of the tested compound were placed on the agar, with each concentration applied separately. Fifteen (15) plates were prepared to asses antibacterial activity against Gram-positive and Gram-negative bacteria. Therefore, the plates were incubated at 37C° for 24hours, and inhibition zones were measured to determine antibacterial effects.

3- Results and Discussion:-

1.3- Physical properties of Complexes:-

Table 1:- The physical data of the synthesized complexes and their ligand.

Compound	Color	M.Wt	Yild%
C_1) (Cu(II) Complex	Green	1547	%75
Co(II) Complex(C_2)	Pink	1065	%40
Ligand (L)	White	464	-----

2.3- FT-IR Spectra study:-

In FT-IR spectra of the ligand (figure1), it showed appearance of a set of inspected functional groups. Since, the bands of NH_2 was found in the range of (**3500-3400Cm^{-1}**). While, the band of (C═O) was observed at **1617.72 Cm^{-1}**.However, the band at the range of (**1457-1150Cm^{-1})** was assigned to (C–N) vibration mode.

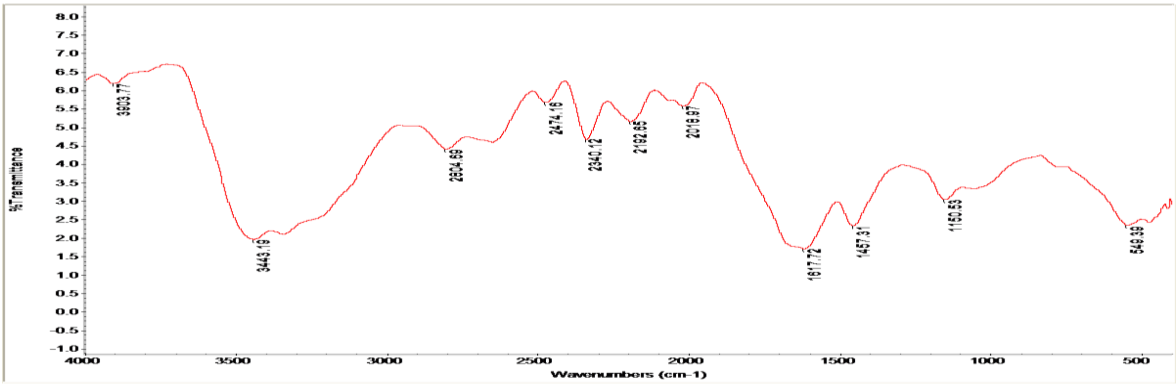

Fig.1. IR spectrum of free ligand in KBr.

Otherwise, in comparison with the spectrum of the ligand, the bands of complexes spectrums, figure(2&3) were shifted to lower frequency (table2), and that consider as the most thing that conform sharing that groups in the bands. The IR spectra also showed new bands in the low frequency at 421.32 Cm^{-1}, and 487.63Cm^{-1} which can be attributed to v(Co-O) and v(Cu-O) vibrations, respectively, and that gives confirmation of coordination of the ligand with metal ions, figure(2&3).

Strong band at 3548.23 Cm^{-1} was found, and it can be tend to (O-H) vibration. Moreover, the band at 1640.72 Cm^{-1} was assigned to (H-O-H), and this ensure that the complex containing coordinated water. In addition to shifted of this peak to 1640.72 Cm^{-1}, which was clear in Urea spectra at 1617.72Cm^{-1}which conform bonding of Urea with metal ion by (C═O) carbonyl group. The change of the spectra of the complexes conforms that, bonding through Oxygen in (C═O), while NH_2 does not share in the coordination with Copper or Cobalt ions.

Due to disappearance of (Co-Cl) vibration at the above of (400Cm^{-1}) band, and the coordination bonding was by Urea and water, Chloride ion may will be out of coordination bonding.

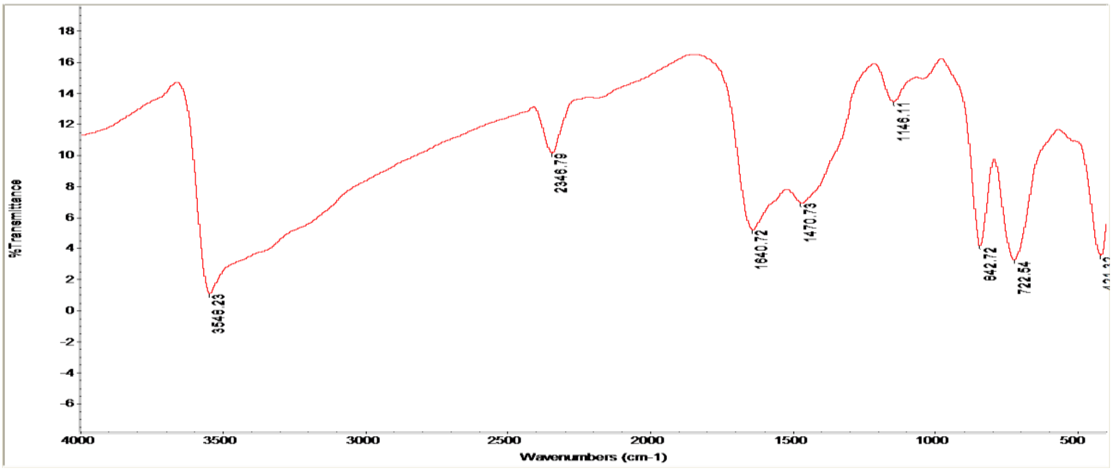

Fig. 2. IR spectrum of Co Complex in KBr

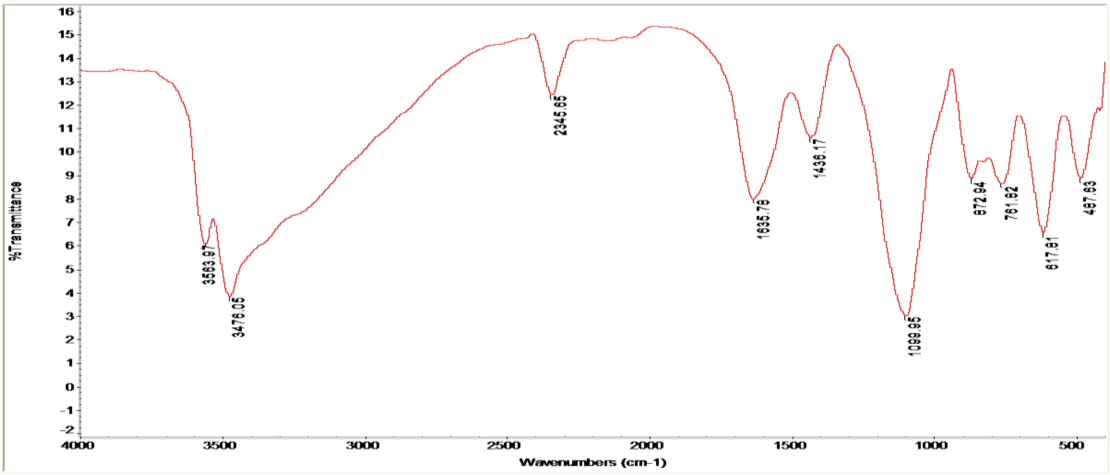

Fig. 3. IR spectrum of Cu Complex in KBr.

Table (2): IR spectrum of the ligand and its complexes.

VIPRATION	COBALT COMPLEX $^{-1}$)CM(	CUPPER COMPLEX $^{-1}$) (CM	PURE UREA $^{-1}$)CM(	FONCTIONAL GROUP
ν(N-H)	3548.23	3563.97	3903.77 3443.19	N-H
ν(C=O)	1640.72	1635.78	1617.72	C=O
δ(N-H)	1470.73	1436.17	1457.31	N-H
ν(C-N)	1146.11	1099.95	1150.53	C-N
	421.32	487.63	----	M-O

3.3-Qualitative Detection:-

The detection of sulfate SO_4 and chloride Cl_2 was evaluated, as we mentioned in the experimental part, and it was found that a white precipitate was formed which confirm a formation of SO_4 and Cl_2 out of coordination zone**.**

$$[Cu(U)_2(H_2O)_4]SO_4 + BaCL_2 \rightarrow [Cu(U)_2(H_2O)_4]Cl_2 + BaSO_4\downarrow$$

$[Co(U)_2(H2O)_4]2Cl+H_2SO_4 \rightarrow [Co(U)_2(H2O)_4]SO_4+2HCl$

4.3Proposed Structure Geometry and Molecular Formula:--

Based on FT-IR spectra study, results of Co(II) and Cu(II) complexes tend to form Octahedral complexes with coordination number of (6), and the inspected formula is **$[Co(U)_2(H_2O)_4]Cl_2$** and **$[Cu(U)_2(H_2O)_4]SO_4$** for Co(II) & Cu (II) complexes , respectively. Fig (4 & 5).

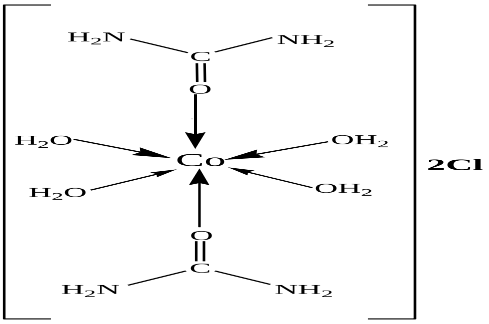

Fig. 4 : proposed structure of Co(II) complex.

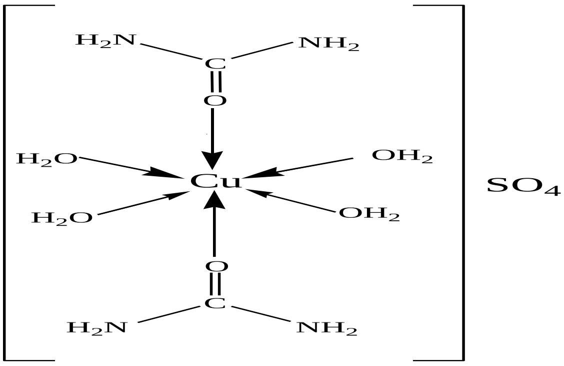

Fig. 5: proposed structure of Cu(II) complex.

-5.3- Antibacterial Activity Study:-

In this study, the ligand and its complexes were evaluated for their antibacterial activity against both of used bacterial. Figure(6) showed the result of a-Gram positive (Staphylococcus aureus) which obtained.

It was found that, the capacity of the complexes was high, especially with Co(II) complex (C_2) since showed little effect at low concentration and up to be strong effective , started from 25 mM up to 100 mM. that can be attributed to that the C_2 has a big effective at high concentration. Wile, the C_1 result finding the medium effect at 50mM up to high concentration at 100 mM. However, the ligand did not show effective at all,(Figure6).

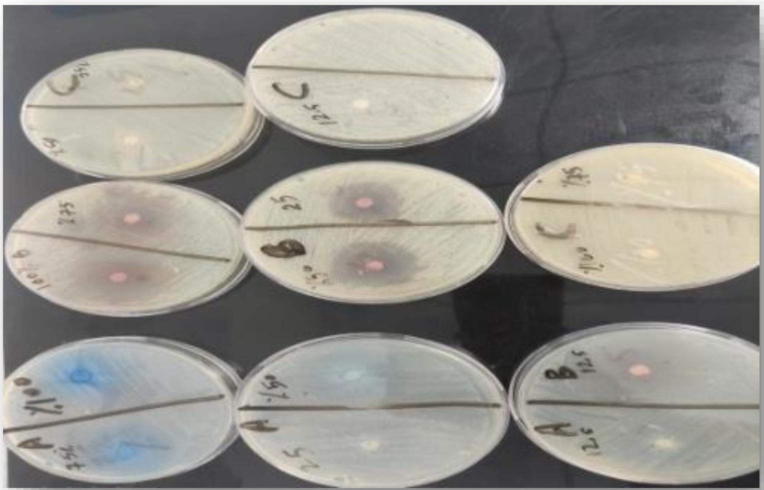

Fig. 6: a-Gram positive (Staphylococcus aureus).

(**Escherichia .coli**) in addition to that, the result of **a-Gram** Negative(figure 7), has been evaluated and it found that , little effect of C_2 at low concentration, and by the increasing of the concentrations the capacity was increased, it was started from 12.5 mM up to 100mM. However, there is no effective observed in L and C_1 among all the concentrations.

Fig.7:) a-Gram Negative **(Escherichia .coli**

Over at all, as it showed at figures(8,9,&10) , we found that the capacity of Cobalt complex C_2 against a-positive Gram was higher compare to that of a-gram negative , and that can be attributed to structural differences in the bacterial cell wall. This finding is agreed with (M. K. Agarwal,2013) [18], which stamate that the inhibitor of cobalt(II) and copper(II) complexes against positive higher the negative Gram. The antibacterial activity results (mm) of cobalt complex (C_2) **are summarized in Table 3.**

Table (3):- Antibacterial activity results (mm) of cobalt complex (C_2) .

a-Gram Negative **(Escherichia .coli)**	**a-Gram positive (Staphlococcs aureus)**	**Concentrations**
6mm6	-----	mM12.5
mm11	mm25	mM25
mm10	30mm	mM50
mm15	mm15	mM75
mm18	mm20	mM100

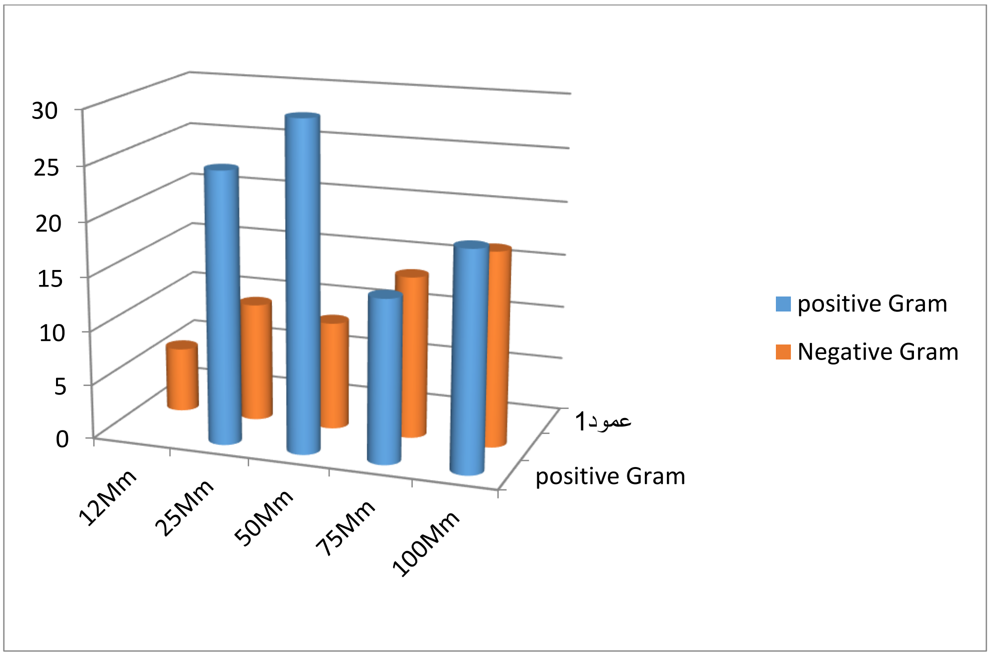

Fig 8: inhibiter of cobalt complex C_2 against used bacteria.

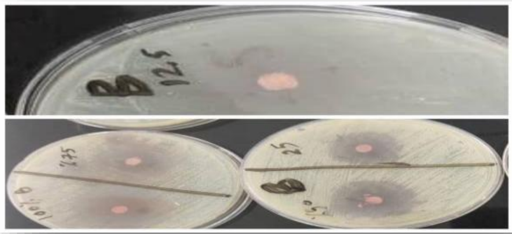

Fig 9: inhibiter of cobalt complex C_2 against a-Gram positive (Staphlococcs aureus)

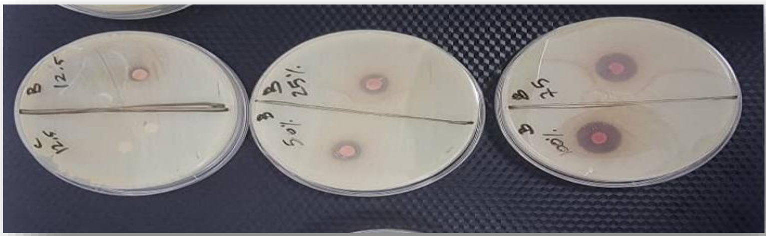

Fig 10: inhibiter of cobalt complex C_2 against a-Gram Negative (Escherichia .coli)

4 Conclusion-

In this study cobalt(II) and cupper(II) complexes with Urea as a ligand were synthesized and characterized using FTIR Spectrometry, and their antibacterial activities were studied. The ligand and its complexes were tested against both of a-Gram positive (Staphlococcs aureus) and Negative (Escherichia .coli). it was found that the complexes exhibited more effective antibacterial activity compared to the ligand, especially Co(II) complex. Lastly , the information achieved from the current study would be helpful to grow novel possible therapeutic agents.

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