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Abstract. This study demonstrates the synthesis of new ligands derived from trimethoprim with their Iron(III) and copper (ll) complexes. At the beginning, preparing new ligands , the first namly N,N'-(5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diyl)bis(3,4,5-trihydroxy benza mide)and the Ligand second namly N,N'-(((5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diyl) bis(azanediyl))bis(carbonthioyl) bis(3,4,5-trihydroxybenzamide) which prepared by nucleophilic addition of trimethoprim to Gallic chloride and prepared also by nucleophilic addition of trimethoprim to the Solution of Ammonium thiocyanate and Gallic chloride . Ferric Ion (III) and Copperic Ion (ll) complexes have been prepared with molar ratio [1:2]. The synthesis ligands have been characterized by Uv-Visible, FT-IR, ^{1}HNMR, ^{13}CNMR and EI-mass, while the complexes have been characterized by elemental analysis, Uv-Visible, FT-IR, Conductivity measurements and thermogravemtric (TgA) analysis. The ligands acts as multiple sites coordinating with ferric ion and copper ion , Via lone pair of nitrogen atom of NHC=O and phenolic oxygen. In Vitro, the ligands and complexes have been tested for their growth Inhibitory activity against Gram negative bacteria *Salmonella Spp* and Gram Positive *Staphylococcus Spp*. The results of the test indicate that the synthesized compounds possessed a high inhibition effectiveness comparative with trimethoprim.

1. Introduction

Antibiotics are among the medicines that are widely used in the field of health, as there are many about these antibiotic compounds and their derivatives, which have succeeded in eliminating a group of bacteria and fungi that infect living organisms[1].

It is known that most of these compounds are susceptible to being interfered with by hemoglobin in the blood by nitrogen or oxygen and sulfur atoms present within the formulations of these drugs [4].

The binding property leads to a reduction in the proportion of iron it is found in the body of people who take large amounts of these antibiotics, and it is noticed that the diuretic changes of these people during the course of treatment with these medicines.

Therefore, thinking and searching for ways to reduce this effect of these drugs began while preserving or increasing their therapeutic efficacy, as the combinations of these antagonists have been used and contain groups through which to add groups that have the ability to supply the body with iron instead of reducing its percentage in the body and increasing its therapeutic effectiveness. the research dealt with one of these types of antibiotics that are widely used to treat ear infections Central, urinary tract infections and diseases that affect the respiratory system[2], which is Trimethoprim[3] and its abbreviation TMP Trimethoprim[5] as in the Figure1 and Gallic acid[6] derivatives Figure 2 and

Figure 3 have the ability to be persistent with many ions of transitional elements, and among these ions that the body of the organism needs are the iron ions[7], and some of its derivatives are able to bind with the antibiotic containing the amine groups that have been used.

In this research, by increasing the efficacy of trimethoprim, preventing its association with hemoglobin, and increasing the second derivative of the new antibiotic and its complexes in water and increasing its biological effectiveness[8].

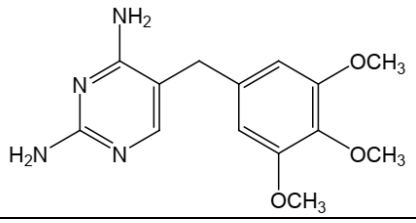

Figure 1. The structure of TMP.

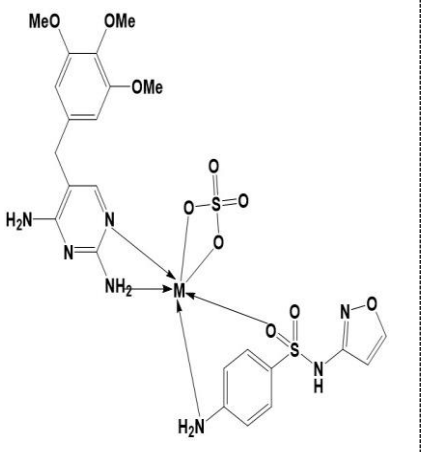

Figure 2. Trimethoprim derivative , their Iron (III) Complex.

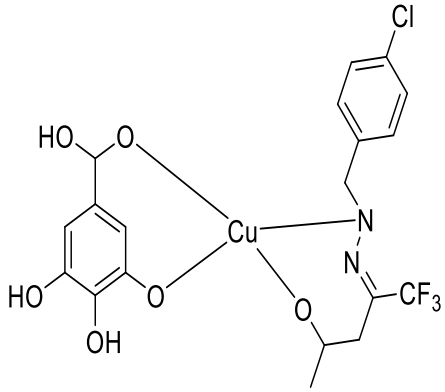

Figure 3. Gallic Acid derivative , their Copper (ll) Complex .

2. Experimental

2.1. Materials

Trimethoprim was purchased from Aldrich . Gallic acid , Thionyl chloride , Ammonium thiocyanate, Copper nitrate of dehydrate ($Cu(NO_3)_2.3H_2O$) and Ferrie chloride ($FeCl_3$) were obtained from Fluka, all solvent used were of analytical grade and used without further purification.

2.2. Instrumentation

Infrared (IR) were recorded for KBr pellets using Shimadzu FTIR model 8400s in the range 4000-400 cm^{-1}. Electronic spectra for the synthesized compounds were recorded by using scan 80 D (England) at range 200 – 800 nm using H_2O as solvent and 1 cm pathway quartz cells.The ^{1}H-NMR ,^{13}CNMR were recoded on a Bruker (400 MHz for H-NMR and 500 MHz, for CNMR) using (DMSO-d6) as a solvent, and tetra methyl silane (TMS) as an internal standard.Thermal analyses measurements (Tg and DTG) were recoded and a Rheometric Scientific Inc. 1998. Nitrogen flow rate 10 cm^3/min and heating rate 10 ºC min^{-1}. Mass spectrum scanned by EI-technique at 70 ev using Agilent Technologies 5975 C spectrometer.

2.3. Synthesis of Gallic chloride

Gallic Acid (1.7 g, 10 mmol) has been dissolved in anhydrous DCM (20 mL), in thionyl chloride (1.6 mL, 10 mmol) added to the solution with stirring with reflux at 60 °C for 90 min, Then the mixture has been washed with dried toluene (20 mL) to afford Gallic chloride as a pale yellow product.[9]

2.4.Synthesis of N,N'-(5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diyl)bis(3,4,5-trihydr oxybenzamide) (L1)

Gallic chloride (1.508g, 4mmol) was suspended in 20 ml of acetone. (0.58g , 2 mmol) of Trimethoprim was added dropwise ,with constant stirring .The reaction mixture was reflux for 2 hrs. The solid product which obtain , filtrated off , washed with ethanol and dried . yield 72% , Rf = 0.87, M.p.188-190 ºC .

2.5. Synthesis of N,N-(((5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diyl)bis(azanediyl))bis(carbono thioyl)) bis(3,4,5-trihydroxybenzamide)(L2)

The solution of thiocyanate (0.761 g .10 mmol) in 20 ml acetone was added to (1.88 g . 10 mmol) of Gallic chloride ,with constant stirring, the reaction mixture refluxed for 2 hrs.The mixture was filtered .The filtrate solution added dropwise to solution of Trimethoprim (1.6 g, 5 mmol) with continuous stirring. The resulting solution refluxed for 3 hrs. The solid white product which obtain was filtrated off, washed with ethanol and dried. yield 66%, Rf = 0.56, M.p. 190-192 ºC .

2.6. Synthesis of Copper (ll) Comlex.$Cu_2L1.4NO_3.H2O$ (C1)

(L1) (10 mmol) dissolved in ethanol and (20 mmol) of aqueous solution of Copper nitrate of dehydrate has been added dropwise with constant stirring at room temperature for 2 hrs with molar ratio 2:1 (M:L).Green precipitate formed which was filtered , washed several time with water and dried ,yield 63% , m.p. 220-222 ºC. The obsered physical properties are given in Table 1**.**

2.7. Synthesis of Iron(lll) Comlex. $Fe_2L1.(Cl)_6.2H_2O$ (C2)

Trimethoprim derivative (L1) (10 mmol) dissolved in ethanol and (20 mmol) of aqueous solution of ferric chloride has been added dropwise with constant stirring at room temperature for 2 hrs with molar ratio 2:1 (M:L). Black precipitate formed which was filtered , washed several time with water and dried ,yield 86% , m.p. 195-197 ºC. The obsered physical properties are given in Table 1.

2.8. Synthesis of Copper (ll) Comlex.$Cu_2L1.4NO_3.H2O$ (C4)

(L2) (10 mmol) dissolved in ethanol and (20 mmol) of aqueous solution of Copper nitrate of dehydrate has been added dropwise with constant stirring at room temperature for 2 hrs with molar ratio 2:1 (M:L).Green precipitate formed which was filtered , washed several time with water and dried ,yield 78% , m.p. 176-178 oC. The obsered physical properties are given in Table 1.

2.9. Synthesis of Iron(lll) Comlex. $Fe_2L1.(Cl)_6.2H_2O$ (C5)

(L2) (10 mmol) dissolved in ethanol and (20 mmol) of aqueous solution of ferric chloride has been added dropwise with constant stirring at room temperature for 2 hrs with molar ratio 2:1 (M:L). Black precipitate formed which was filtered , washed several time with water and dried ,yield 81% , m.p. 245-244 ºC. The obsered physical properties are given in Table 1.

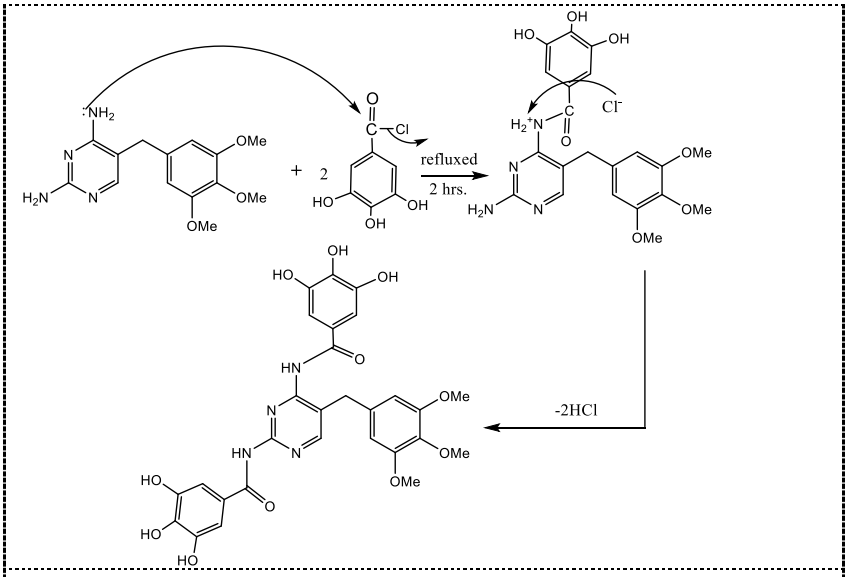

Figure 4. The reaction mechanism of L1.

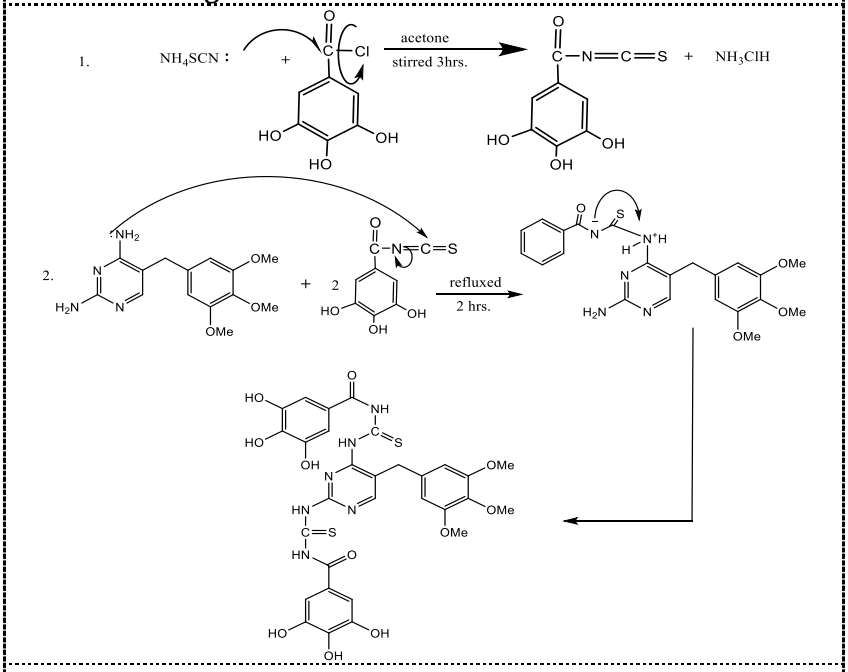

Figure 5. The reaction mechanism of L2.

$$L + Cu(NO_3)_2.3H_2O \xrightarrow[\text{Stir .2h}]{EtOH / H_2O} Cu_2L.nH_2O \quad (1)$$

$$L + 2FeCl_3.5H_2O \xrightarrow[\text{Stir .2h}]{EtOH / H_2O} Fe_2L.nH_2O \quad (2)$$

Table 1. Analytical and physical data of ligands and complexes

	Colour	M.p. ºC	Ohm^{-1} Cm^2.mol^{-1}	Yield %
L1	White	190-188	-	72
L2	White	190-192	-	66
C1	Green	222-220	180.5	86
C2	Black	197-195	182.7	79
C4	Green	178-176	137.1	81
C5	Black	245-244	202.3	69

Table 2. CHN results for the prepared complexes

		Elemental of analysis found (calc.)			
Compound	Formula	% C	% H	%N	% S
C1	$C_{28}H_{30}Cu_2N_6O_{19}$	42.20(42.37)	4.89(4.32)	7.50(7.06)	-
C2	$C_{28}H_{22}Fe_2N_4O_{11}$	39.84(39.66)	3.67(3.09)	6.79(6.61)	-
C4	$C_{30}H_{32}Cu_2N_6O_{13}S_2$	41.79(41.14)	3.11(3.68)	9.85(9.60)	7.68(7.32)
C5	$C_{30}H_{36}Fe_2N_6O_{15}S_2$	34.23(34.71)	3.46(350)	8.55(8.09)	6.62(6.91)

3. In vitro antimicrobial activity

The ligands and complexes were Screend for antibacterial activity in vitro against two kinds of organisms species . Gram(-ve) *Salmonella spp* and gram(+ve) *Staphylococcus spp* , by agar well diffusion method .

These compounds were dissolve in DMSO to prepared solution of different weights (5µg ,4µg, 3µg) in (0.1ml) of DMSO . Sterile discs dipped in this solutions , dried it and place on nutrient agar plate spreaded with the bacteria .

The plate were further incubated for 24 to 48 hours at 37 ºC and the diameters of inhibition zone measured in millimeter[10] .

4.Result and Discussion

New oregano Ferric (lll) and Copperic (ll) were synthesized by the reaction of Trimethoprim derivative in molar ratio 2:1 [M:Ligand] . The Ferric (lll) and Copper(ll) complexes are subjected to elemental analysis. The result obtained is in good agreement with those calculated for suggested formula of the complexes. The high value of molar conductance of aqueous solution of complexes confirm the electrolytic nature and indicate that the chlorine ion and nitrate ion out of coordination sphere. Table 1.

The IR spectroscopy is important to diagnose the Prepared complexes, as through it is possible to infer and determine the coordination sites between the ligand and the metal ion. It's can be the deflection the Values of IR due to coordination between metal and ligand as shown in the table 3.The IR spectra of the ligands (L1 and L2) indicate broad bands at (3367, 3437) cm^{-1} respectively which attributed to stretching of OH group . While the bands within ranges (3290, 3163) cm^{-1} characteristic of stretching vibration of NH group[11]. The ligand(L1) showed two strong bands at the rang 1701 and 1666 cm^{-1} that attributed to C=O moiety . Also two strong bands appeared within rang 1539 cm^{-1} which attributed to asymmetrical and symmetrical stretching of aromatic C=C . The Ligand(L2) showed one strong band at the rang 1670 cm^{-1} that attributed to C=O and showed one strong band at 2080 cm^{-1} that attributed to C=S. The IR spectra of the ligands showed weak bands at the range (3073, 3090) cm^{-1} respectively which attributed to aromatic C-H stretching , the aliphatic C-H stretching bands appeared at rang (2847, 2939) cm^{-1} respectively further more the rang at (1246 , 1238) cm^{-1} can

be attributed to stretching vibration of C-N group . In other hand the L1 showed aband at 1030 cm^{-1} that attributed to stretching vibration of C-O while the L2 showed the band of C-O at 1130 cm^{-1} [12] [13] .

Table 3. IR data spectrum of ligands and complexes.

	υ(O-H) cm^{-1}	υ(N-H) cm^{-1}	υ(C=O) cm^{-1}	υ(C=S) cm^{-1}	υ(N-H bond) cm^{-1}	υ(C-H Arom.) cm^{-1}	υ(C-H al ph.) cm^{-1}	υ(C-O) cm^{-1}	υ(C-N) cm^{-1}	υ(C-Hbend.) cm^{-1}
L1	3367(br)	3290(br)	1701(s)	-	1620(m)	3070(w)	2847(w)	1450(m) 1342(m)	1246(s)	1030(m)
C1	3452(br)	3390(br)	1705(s)	-	1620(m)	3080(w)	2850(w)	1446(m) 1338(m)	1242(s)	1118(s)
C2	3452(S)	3190(m)	1666(s)	-	1593(m)	3075(w)	2974(w)	1458(w) 1334(w)	1238(s)	1118(s)
L2	3437(m)	3163(m)	1670(s)	2080(s)	1531(m)	3090(w)	2939(w)	1458(w) 1346(w)	1238(s)	1130(m)
C4	3452(S)	3136(br)	1666(s)	2180(m)	1593(m)	3070(w)	2978(w)	1462(w) 1334(w)	1234(s)	1118(s)
C5	3406(br)	3171(br)	1732(m)	2066(m)	1589(m)	3080(w)	2850(w)	1404(w) 1303(w)	1238(s)	1126(s)

s: strong, m: medium, w: weak, br : broad

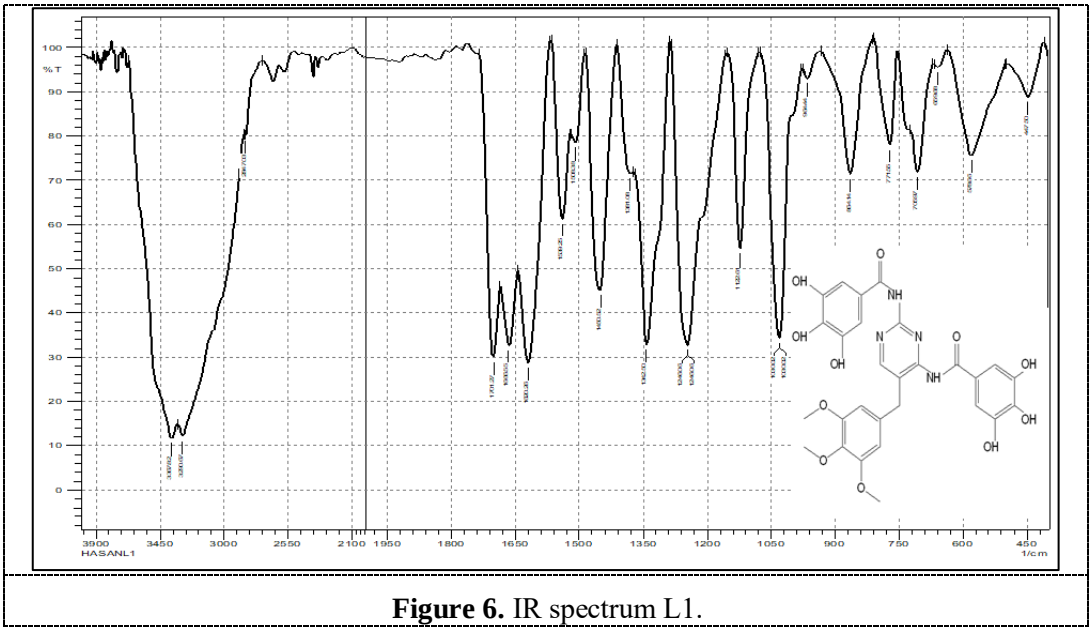

Figure 6. IR spectrum L1.

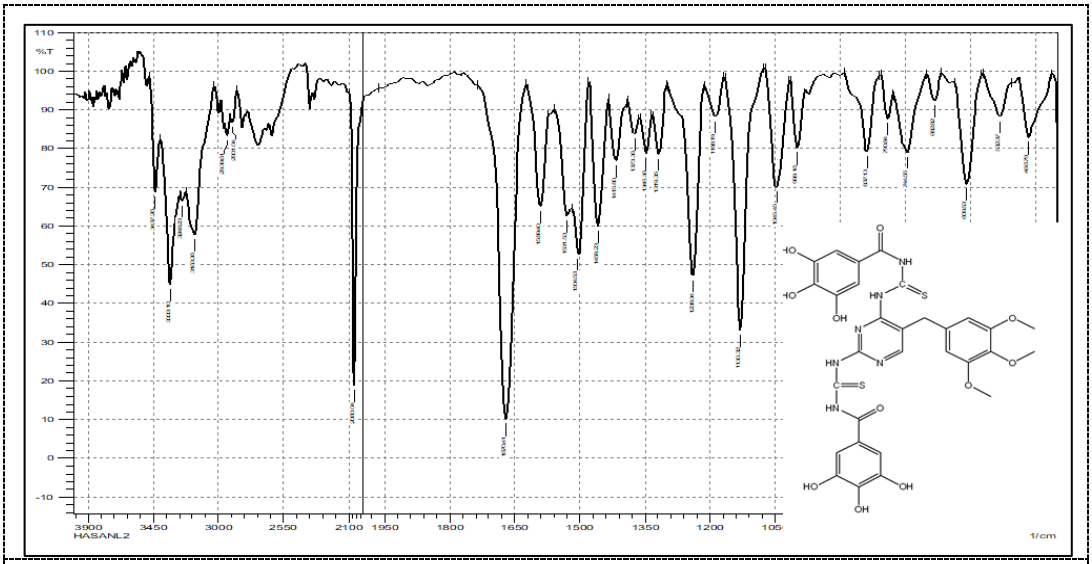

Figure 7. IR spectrum L2.

The UV-visible spectra of the ligands and their complexes have been studied (figure 8) at 1x10-3 M aqueous solution at range 200-800 nm by using quartz cell. The UV-vis. Spectrum of the ligand L1 showed four absorption region at 245 nm, 290 nm, 360 nm and 405 nm while the ligand L2 showed two absorption at the region at 210 nm and 270 nm which may be all these attributed to n→π* transition of the aromatic heterocyclic group of the Trimethoprim molecule .

The Electronic transitions for UV-visible spectra of the Ferric(lll) and Copper(ll) complexes have the spectrum of the electronic range (200-400)nm which attributed to n→π * while the spectrum of the electronic at range (400-800)nm in the Ferric(lll) which attributed to transition type $^{2}T_{2}g \rightarrow {}^{2}Eg$ and the Copper(ll) complexes which attributed to transition type $T_{2} \rightarrow E$ [14] [15].

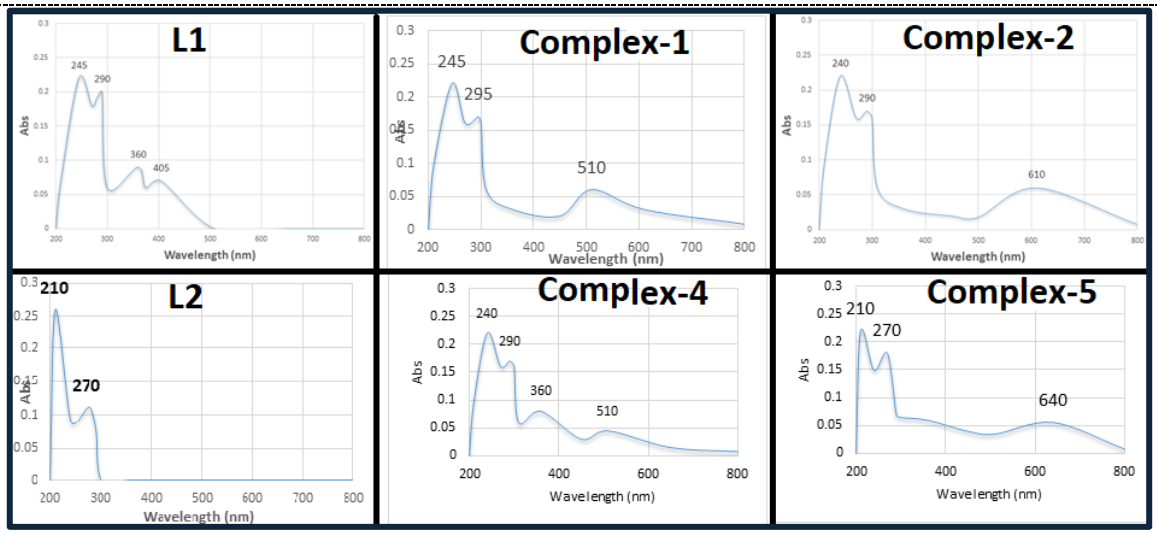

Figure 8. UV-vis. Spectrum of Prepared Compounds.

The ^{1}H-NMR ,^{13}CNMR of the prepared Ligands showed in figure(9,10,11) DMSO-d6 used as solvent . The spectrum of ^{1}H,^{13}C-NMR of the Ligand (L1) the signals showed as expected and can be given as follows:-

The ^{1}H-NMR spectra of L2 shows a singlet signal at δ 3.58 ppm which attributed to two protons of methylene group (CH_2). Also appear a singlet signal at δ 3.64-3.75 ppm which attributed to OCH_3 while the signal appeared at δ 6.60 ppm assigned to NH group . Also the Ligand L1 showed a multiple signal which assigned to aromatic range at δ 6.76 – 6.94 ppm. The signal that appeared at the rangs δ 7.21 – 7.53 ppm can be attributed to phenolic group (OH) .The ^{1}H-NMR spectra of L2 shows of a single signal at δ 3.56 ppm which attributed to two protons of methylene group (CH_2).Also the spectrum showed a single signal at the range δ 3.63 – 3.74 ppm that assigned to nine protons of methoxy group (OCH_3) . The compounds is characterized by showing abroad signal at δ 4.93 ppm which can be assigned to amino group (NH). Furthermore the multiple signals that appear at δ 6.94-7.52 ppm can be attributed to aromatic rings of this Ligand. In addition the phenolic groups OH were observed as single signals that appeared at the range δ 6.23 – 6.58 ppm .[11][12][16].

Similarly, the 13C-NMR spectra of the ligands L1 and L2 the Ligand first L1shows the single signal at δ 33.42 ppm which attributed to methylene group and the signal at the range δ 56.28 – 60.44 ppm is due to the methoxy groups (OCH3) and signals at the range δ 138.34,145.88,153.18 and 162.81 ppm which attributed to C-OCH3 , C-OH , C-N and C=N respectively . Also the signal of aromatic carbon of these Ligands observed at the range δ 106.32 -136.22 ppm . Additionally the signal at carbonyl group C=O of these compounds observed at δ168.30 ppm . while the Ligand second L2 which confirms the composition of these Ligands shows a singlet signal at δ 32.60 ppm which attributed of methylene group (CH2) and the signal at the range δ 56.38 – 60.45 ppm is due to the methoxy groups (OCH3) and signals at the range δ 130.09,133.43,136.65 and 140.28 ppm which attributed to C-OH , C-OCH3 , C-N and C=N respectively . Also the signal of aromatic carbon of these Ligands observed at the range δ 106 -136 ppm . Additionally the signal at carbonyl group C=O of these compounds observed at δ153.39 ppm While this ligand differed from the ligand L1shows the single signal at δ 164.52 ppm which attributed to (C=S) group .[17][18][12].

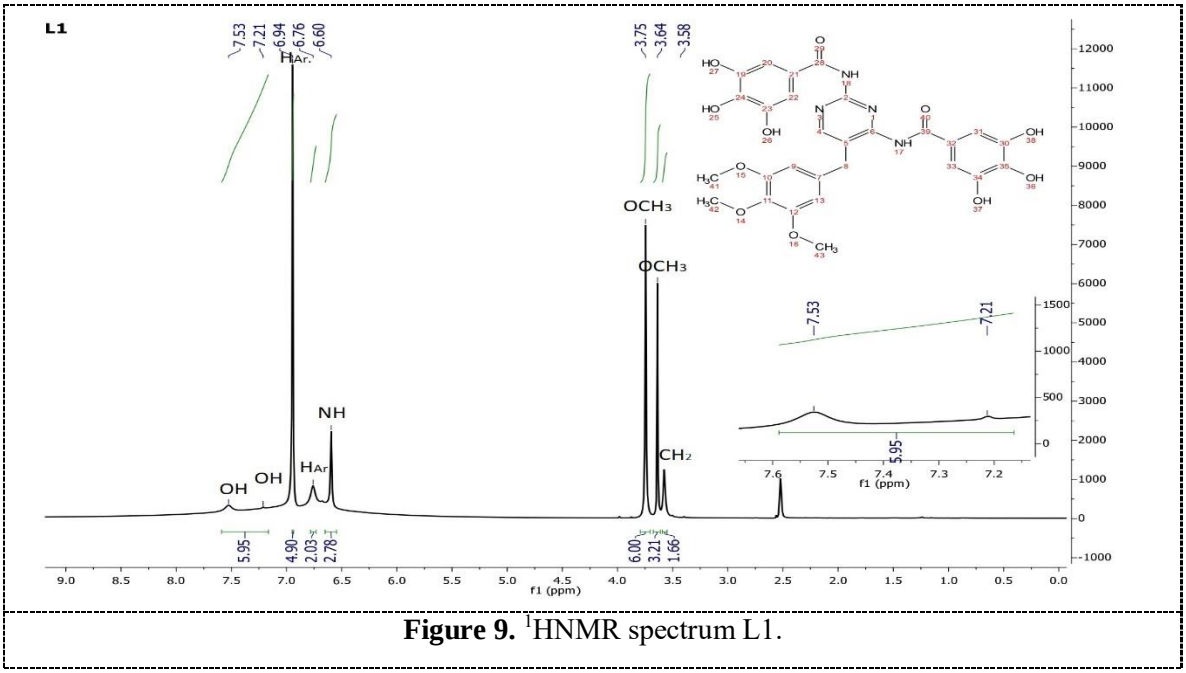

Figure 9. ^{1}HNMR spectrum L1.

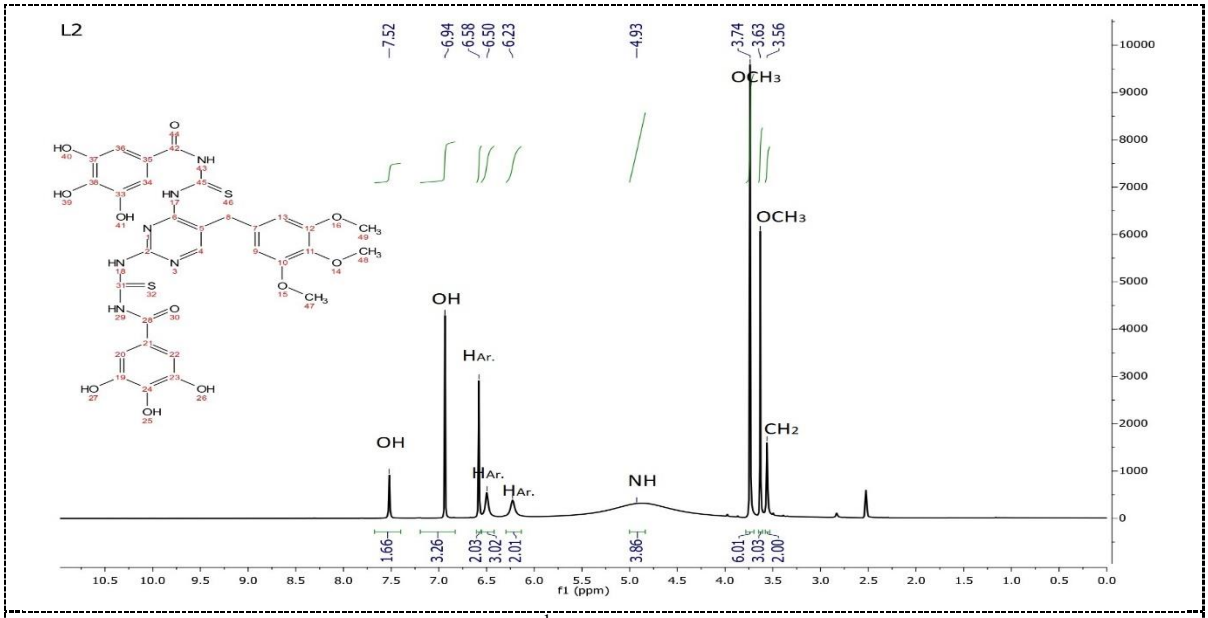

Figure 10. ^{1}HNMR spectrum L2.

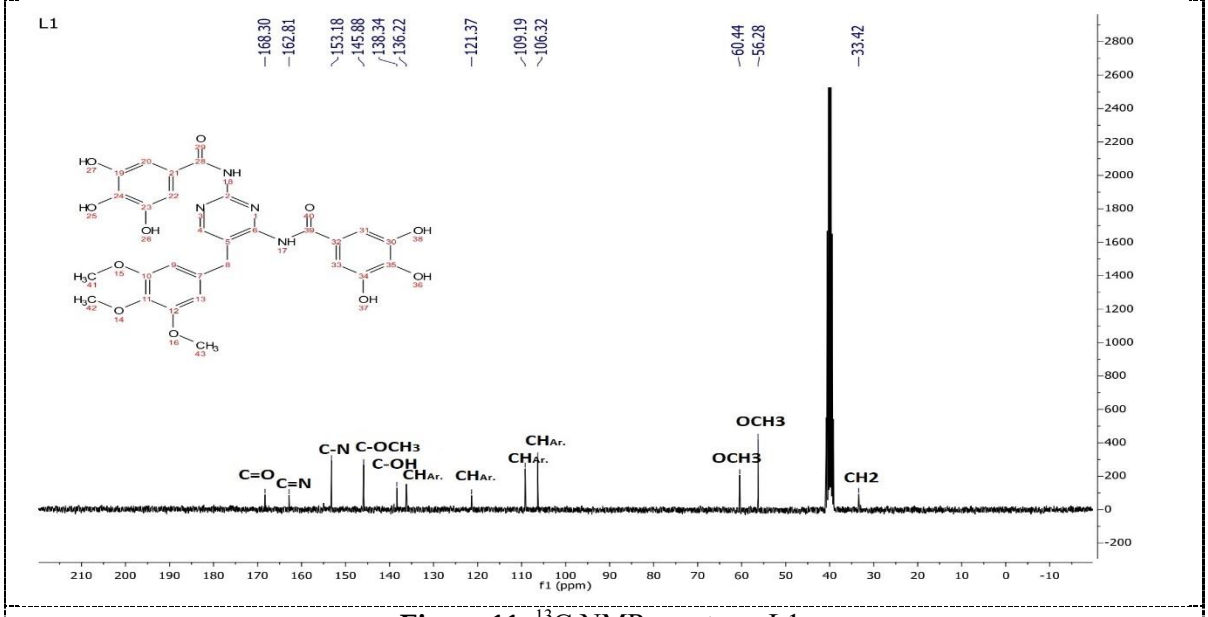

Figure 11. ^{13}C NMR spectrum L1.

El-Mass: The mass spectrum of ligand(L1) Figure 12 . show the exact molecular ion at m/z 594 with relative low abundance which indicate the condensation between Trimethoprim and Gallic acid has taken place resulting into the formation of this derivative with the rate (2:1) which agreement with the formula that suggested. Also the spectrum show peak at m/z 288 attributed to base peak [$C_{14}H_{16}N_4O_3$] $^{+}$, while the peaks which appear at m/z 275, m/z 259 and m/z 243 with relative abundance 76% , 89% and 51% can be attributed to the ion $[C_{13}H_{15}N_4O_3]^{+}$, $[C_{12}H_{11}N_4O_3]^{+}$ and $[C_{11}H_7N_4O_3]^{+}$ respectively ,while The mass spectrum of ligand(L2) figure (13) shows the exact molecular ion at m/z 712 with relative low abundance which indicate the reaction between Trimethoprim and the product who would be from interacting of gallic chlorid with ammonium thiocyanate resulting into the formation of this derivative with the ratio (2:1) agreement with the formula that suggested. Also the spectrum show peak at m/z 288 that attributed to base peak

[$C_{14}H_{16}N_4O_3$] $^+$, while the peaks which appear at m/z 58, m/z 259 and m/z 275 with relative abundance 20% , 54% and 41% can be attributed to the ion $[CHNS]^+$, $[C_{13}H_{15}N_4O]^+$ and $[C_{13}H_{15}N_4O_3]^+$ respectively .

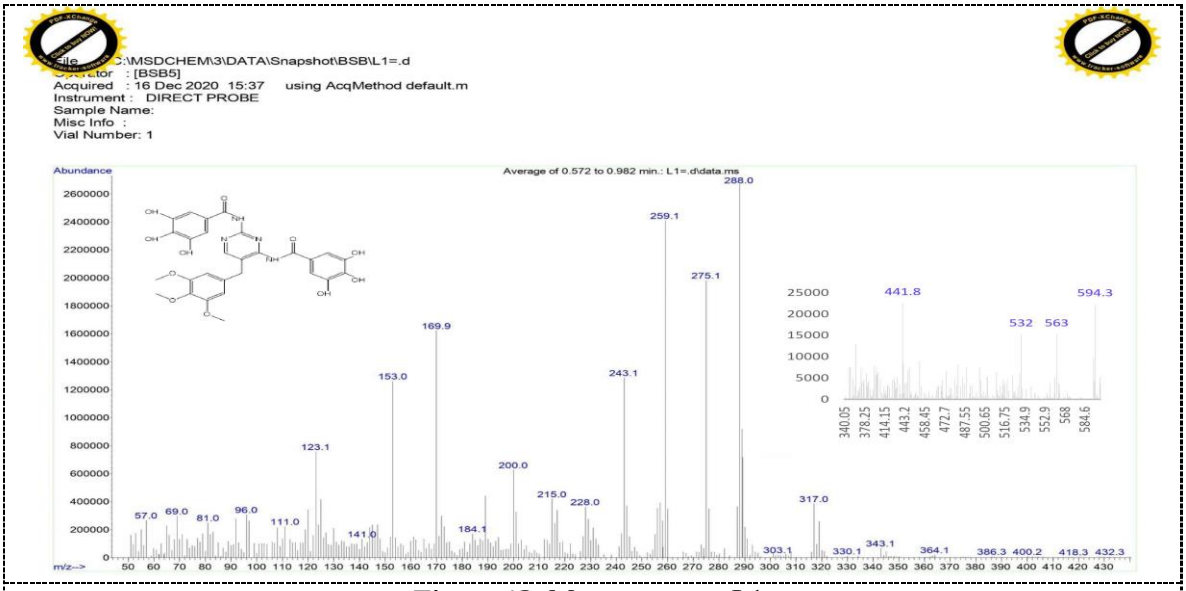

Figure 12. Mass spectrum L1.

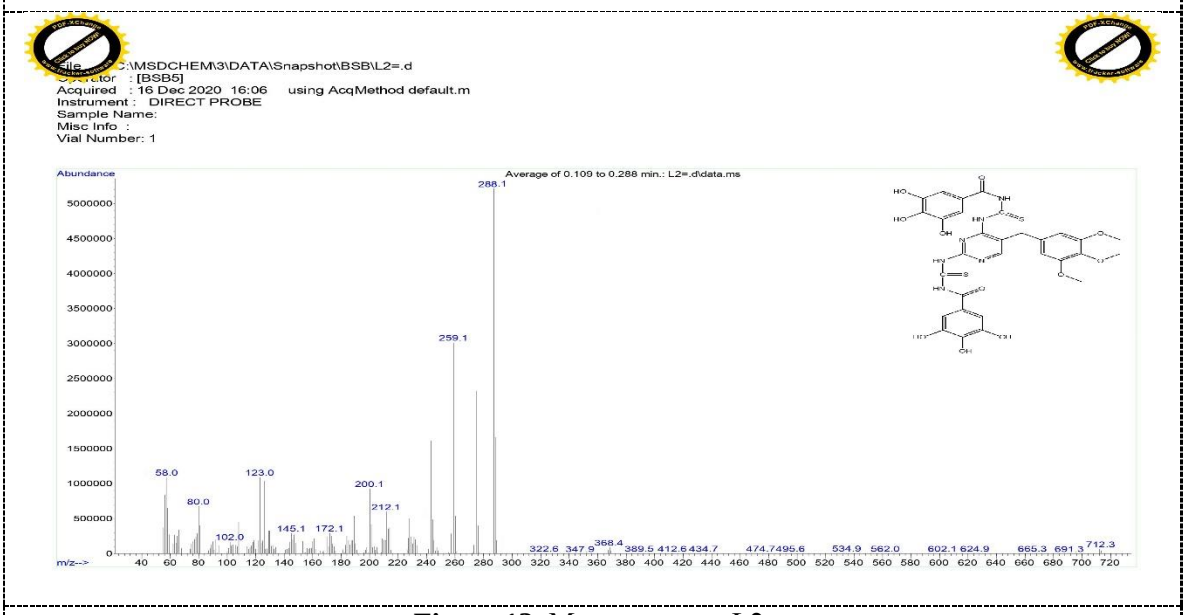

Figure 13. Mass spectrum L2.

Thermal analysis : The thermal investigation was carried out from 80 – 600 ºC under nitrogen atmosphere (20 ml/min) with heating rate 10 ºC /min . The thermos gram of the Ferric lll complex and Cu (ll) complex[19],[20]]. The TGA curves for complex C1 (figure 14) indicates three stages of weight loss from a temperature of 100 – 360 ºC with a loss of approximately 67.19%, the first stage at a temperature of 80 - 150 ºC with a loss of 6.068% in practice (theoretically 7.10%) Which is attributed to the loss of four water molecules that are symmetrically bound with the metal [21], the second phase at a temperature of 270- 150 ºC with a loss rate of 41.2% in practice (theoretically 41.09%) attributable to the loss of $C_{18}H_{13}N_4O_3$ while the third phase is at a degree Heat 350-270 ºC with a loss rate of

19.93% practically (18.50%) which is attributed to the loss 6 molecules of NO_3 [22]. While the TGA curves for complex C2 (figure 15) indicates three stages of weight loss from a temperature of 80-600 ^oC with a loss of approximately 60.84, the first stage at a temperature of 80-100 ^oC with a loss of 5.68% in practice (theoretical%) (4.27), which is attributed to the loss of two crystallized water molecules, the second stage at a temperature of 250 - 350 ^oC with a loss of 34.40% in practice (theoretically 34.19%) that is attributed to the loss of $C_{14}H_{18}N_4O_3$, while the third stage at a temperature of 550-370 ^oC , a loss of 20.76% in practice (%) 21.34) which is attributed to the loss of $C_{10}H_{13}O_3$.

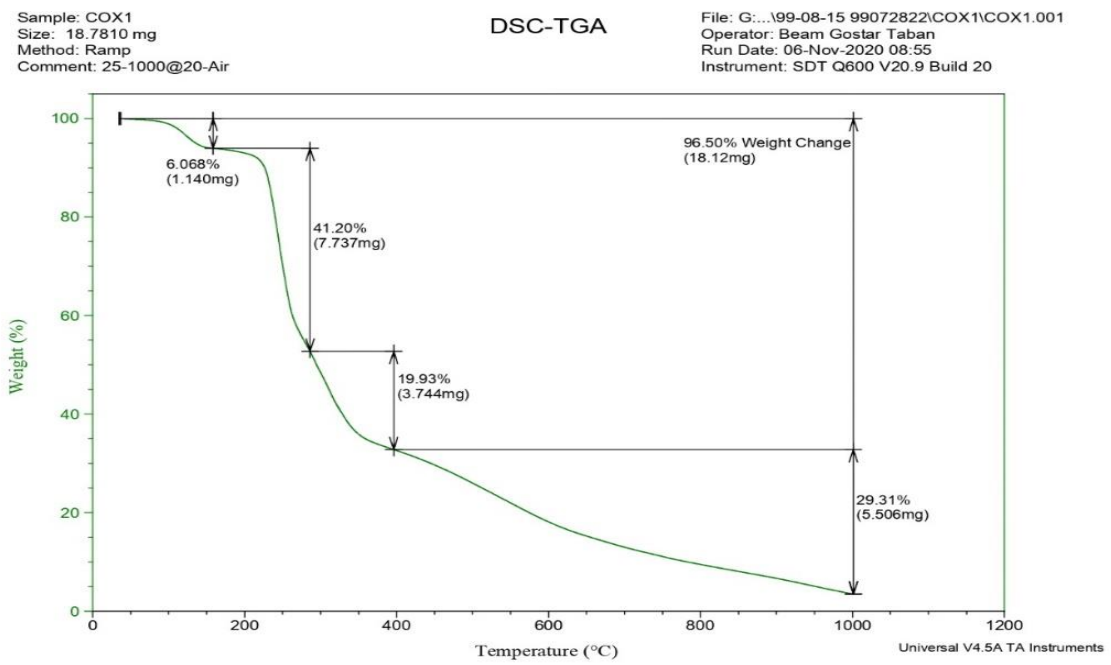

Figure 14. DSC-TGA spectrum C1.

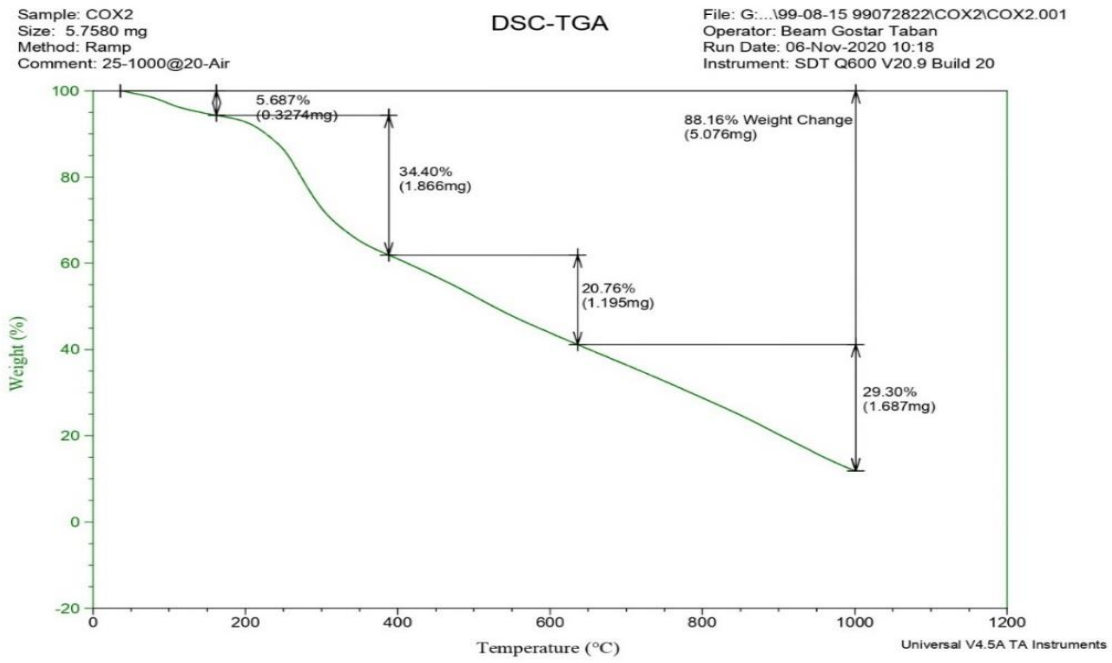

Figure 15. DSC-TGA spectrum C2.

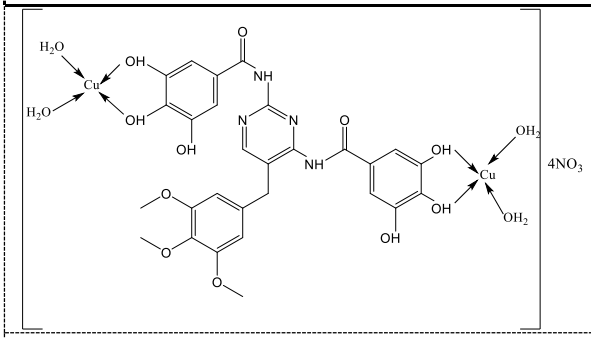

Figure 16. Suggested structure of (C1) $[Cu_2L1(H_2O)_4](NO_3)_4$.

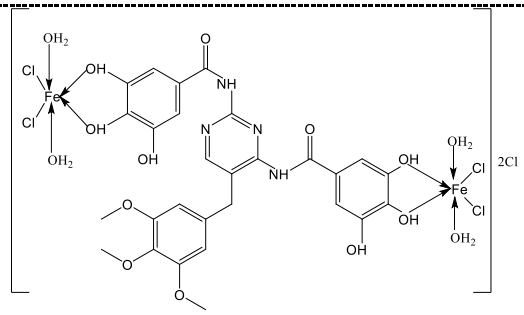

Figure 17. Suggested structure [5] of (C2) $[Fe_2L1(Cl)_4](Cl)_2.2H_2O$.

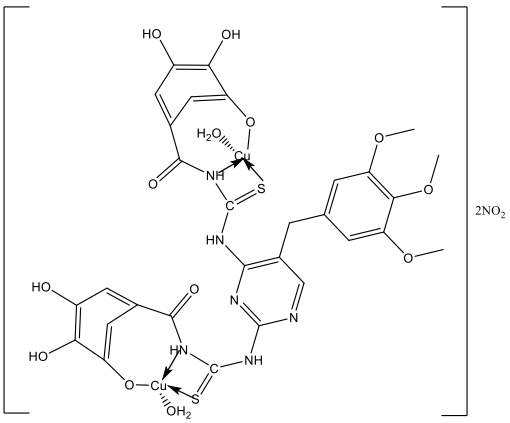

Figure 18 . Suggested structure [6] of (C4) $[Cu_2L2(H_2O)_2](NO_3)_2$.

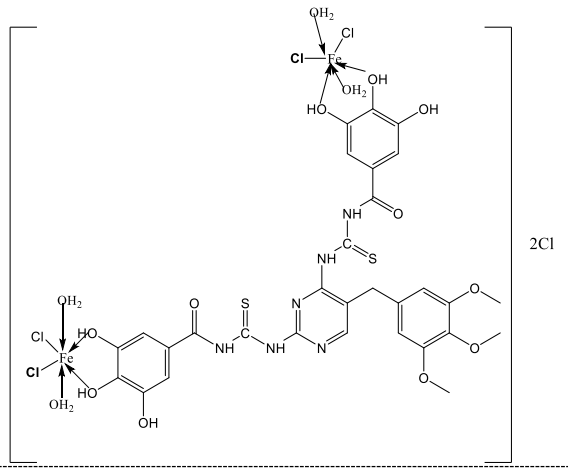

Figure 19. Suggested structure of (C5) $[Fe_2L2(Cl)_4(H_2O)_4](Cl)_2$.

The study also showed the biological effectiveness of the prepared compounds compared to the starting material against two types of bacteria As shown in the Table 4 and Figure20.The results indicate that the solvent has no inhibitory activity towards the studies of bacteria[23],[24], which confirms that the measured inhibition diameters are due to the activity of the prepared compounds. All compounds have biological efficiency for both sexes of bacteria. It is noted from the results that the compounds (L1,L2,C1,C2,C4 and C5) have a greater inhibitory activity compared to the compound trimethrim on both sexes of bacteria because the presence of a double membrane surrounding each bacterial cell. Although all bacteria have a membrane The inner cell, and Gram-negative bacteria, have a unique outer membrane. This outer membrane excludes some Cell-penetrating medicines and antibiotics [25] but the C1 haven't inhibitory activity towards bacteria *Staphylococcus app* for all weights . A table (4) shows the results of the biological effectiveness of the prepared compounds and their comparison with the anti-trimethprim (Contr.) [26],[27].

Table 4. In vitro antimicrobial activity of prepared compounds

	The diameter of the inhibition zone for negative bacteria is in mm *Salmonella*			Contr.In millimeters	The diameter of the inhibition zone for cationic bacteria is in mm *Staphylococcus*			Contr.In millimeters
	Weight				Weight			
	5µg	4µg	3µg		5µg	4µg	3µg	
DMSO	0	0	0	0	0	0	0	0
L1	35	26	25	23	30	28	27	25
L2	23	23	21	26	20	16	16	20
C1	25	20	20	26	0	0	0	20
C2	31	30	29	26	28	20	20	20
C4	26	24	22	26	20	20	18	20

C5	20	20	22	26	21	20	20	20

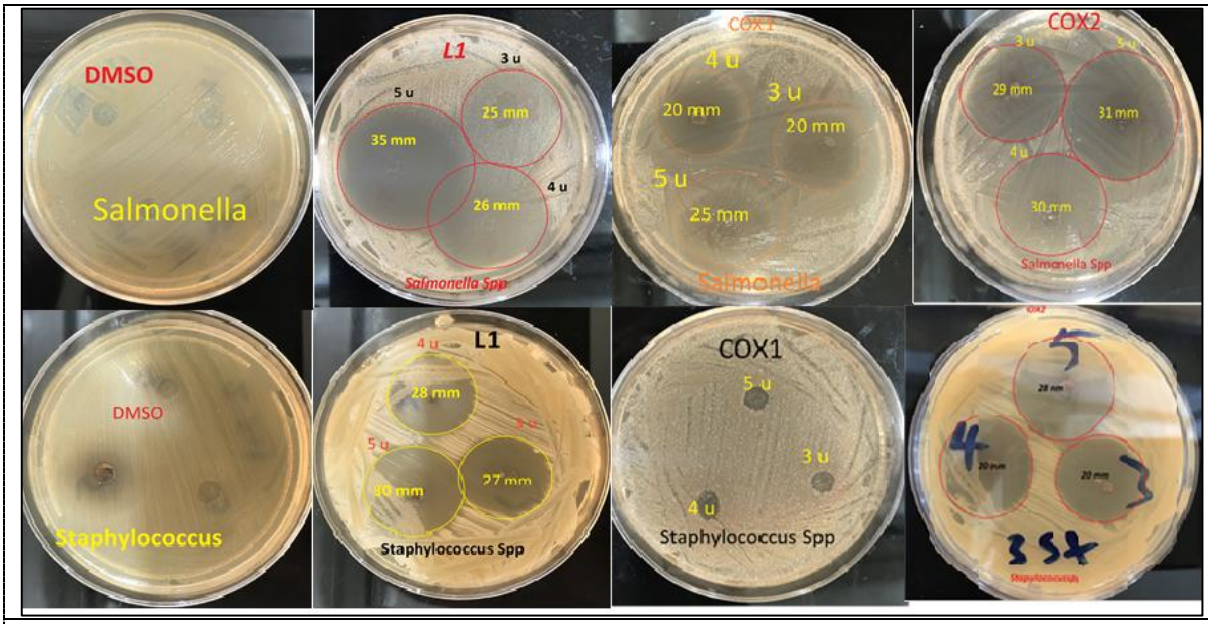

Figure 20. Biological activity of the prepared compounds.

The cytotoxicity of all compounds and complexes prepared using human blood were studied according. Different concentrations (5, 4, 3) µg / ml were prepared in DMSO as a solvent for all the prepared compounds . The blood solution was prepared by mixing (1 ml) of human blood with (20 ml) of normal saline and their cytotoxicity was measured for periods of time (60, 30, 15 minutes), and observing what happens in terms of turbidity or the appearance of sediment, it is evidence of toxicity to the prepared compounds. while no observing any chang of all compounds and concentrations .It isn't toxic compounds .[28]

5.Conclusions

Many measurements were made on the compounds prepared in this research, and through the analysis of the results, this study proved the expected formula for it, as the results of the analyzes of the infrared spectrum, the nuclear magnetic resonance spectrum and the mass spectrum of the prepared compounds were made and compared with the chemistry literature in conformity with what was planned in preparing these Compounds . which are part of a series of compounds prepared in a lengthy study of a group of compounds prepared and will be published later.

The study of thermal analysis of TGA of the prepared complex, which is part of an integrated study of the complexes of the prepared complexes of TMP derivatives, also proved that it is identical with the structural formula of the complex prepared in the study.

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