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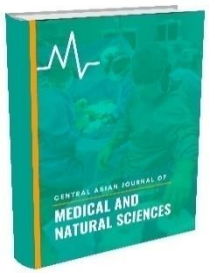

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SPECTROSCOPY, THERMAL ANALYSIS, BIOAVAILABILITY AND ANTICANCER ACTIVITY OF COPPER (II) COMPLEX WITH HETEROCYCLIC AZO DYE LIGAND

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Key words: Imidazolylazo dye, thermal analysis, Thyroid cancer (MDA-T120), spectrum studies.

Abstract: In this study, we investigate the binding of copper ion to a unique compound called (E)-2-((4, 5-diphenyl-1H-imidazol-2-yl) diazenyl) benzoic acid (IABA). Through various tests, including spectroscopy, physical analysis, and thermal measurements, we were able to confirm the structures of the synthesized compounds. Our results showed that the copper (II) complexes had a 1:2 ratio of metal ions to IABA ligand.

The ligand made up of azo dye dazzles the copper ion with its three distinct arms, featuring the mighty nitrogen atom of the azo component, the nitrogen atom of the azo group in the heterocyclic imidazole ring, and finally the oxygen atom of the carboxyl group. Thermal analysis techniques such as thermogravimetry and differential thermogravimetry were utilized to demonstrate the thermal properties of the copper complexes, revealing their higher degree of stability compared to the ligands.

The ligand and copper (II) complex that was prepared displayed noteworthy effects against cancer, with an IC50 value indicating its capacity to hinder the growth of malignant cells. The IC50 values for cytotoxicity against WRI-68 were recorded at 185.7 µg/ml for the ligand and 200.7 µg/ml for the copper (II) complex, while the specific cytotoxicity of the copper (II) complex did not align with the targeted disease cell lines, with IC50 values of 100.1 µg/ml and 330.1 µg/ml for WRI-68. The Cu (II) complexes showed strong effectiveness in inhibiting the growth of both Streptococcus (gram-positive bacteria) and Salmonella enterica serovar Typhi (gram-negative bacteria).

Introduction

"Medicinal inorganic chemistry offers important prospects for the application of metal complexes in the development of anticancer drugs. There is great potential for their application, and numerous therapeutics and other bioactive compounds have been produced and demonstrated to be effective[1]. The platinum complex cisplatin has been shown to be a very effective chemotherapeutic agent in the treatment of a variety of cancers .Since high copper levels are a common feature of many human tumors, targeting copper in tumor cells with copper chelators has emerged as an exciting new approach in cancer therapy. Copper complexes have been shown to be effective anti-proliferative agents in cervical cancer cells. Ruthenium complexes with antitumor effects have also been reported [2].

Imidazolyl azo compounds and some of their metal compounds exhibit significant anticancer activity ,as well as antiviral [3], antifungal [4],and antibacterial [5] microbial activity. In this study, we describe the preparation ,characterization ,and bio antimicrobial activity of copper(II)complex with Imidazolyl azo (IABA) ligands. The in vitro anticancer activity of Imidazolyl azo ligands and copper(II) complex against human thyroid cancer (MDA-T120) was evaluated using the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

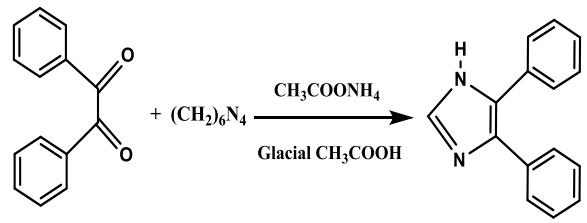
Experimental

Materials and Measurements

We sourced all of the chemicals and solvents necessary for thorough cleaning from Sigma Aldrich and Fluka, B.D.H. The melting points of both the ligand and the Cu(II) complex were determined utilizing a high-tech electrothermal melting point apparatus with a staggering output of 9300. The absorption spectrum was measured using a Shimadzu UV-Vis 1700 spectrophotometer in the (200–1000) nm range with absolute ethanol as the solvent (10^{-3} M) using room temperature, and the FT-IR spectrum was measured using a Shimadzu FT-IR-4800S infrared spectrophotometer. The KBr disk was measured with a meter, and the 1H NMR spectrum of the ligand was determined by a (BRUKER 400 MHz) spectrophotometer at the University of Isfahan, Iran (Germany) using DMSO-d6 as the solvent and TMS as the internal standard. Mass spectra were examined using a Shimadzu Ufms Gcms–TQ 8030, and conductivity was measured using a digital conductivity meter - WT-720–inolab (Germany). SEM images of both ligand and Cu(II) complex were recorded using a KYKY 3200 microscope imager.. Thermo gravimetric analyses of both ligand and Cu(II) complex have shown PL-TG by means of Perkin Elmer TGA-4000 . C.H.N has used based on a micro analytical unit of Euroverctor, EA300A, Italy. Magnetic susceptibility Measurements of the prepared Cu(II) complex have been recorded via Balance Magnetic (MSB-MKI) equipment".

Preparation of 4,5-diphenyl-1H-imidazole:

For the formation of imidazole derivatives,α-dipolar carbonyl condensation[6] with ammonia and aldehydes was employed from the reaction of benzyl with hexamine in the presence of glacial CH_3COOH. Added glacial acetic acid(50 ml) to a mixture of ammonium acetate (17.728 g, 0.23 mole) ,benzyl (2.70 g, 0.01 mole)and hexamine (0.7009 g , 0.005 mole) in a round flask (250 mL) . By using reflected condenser ,The solution was heated at reflux for 90 minutes.. then transferred solution to a beaker (1L) containing (500 ml) of distilled water and after cooling to a temperature of (5^oC) by using ice cream, add NaOH solution to equliaze the solution and obtain imidazole derivative .After addition complete , the solution was filtrate and collected the white precipitate , Wash with distilled water several times, recrystallize with hot ethanol to obtain white crystals, and dry at room temperature. Yield 86% white crystal, decomposed at 184-186^oC. The structural of the imidazole derivative as shown below":

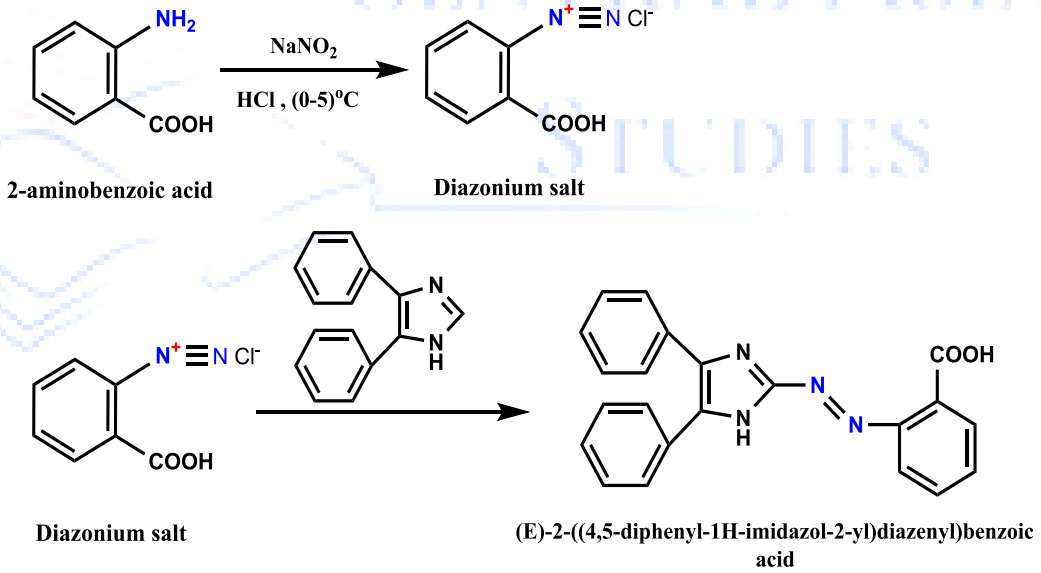


benzil 4,5-diphenyl-1*H*-imidazole

Scheme (1) :- preparation of the imidazole derivative

Synthesis of Imidazolyl azo (IABA) ligand

Using the usual technique, the diazotization coupling process was used to prepare the imidazolyl azo dye ligand [7].To a (30 ml) D.W solution of 2-aminobenzoic acid (1.3714 g, 0.01 mol) in 5 ml HCl, (0.69) g, 0.01 mol sodium nitrite ($NaNO_2$) was (dropwise) in (15 ml) D.W at (0-5)°C was added. The mixture is stirred at (0-5)°C to (15 min.) and the resulting diazonium chloride solution is added . Dropwise Imidazole derivative (2.2033 g, 0.01 mol) dissolved in alkaline medium (100 ml) ethanol and (40 ml) of NaOH (10%) in a 500 ml beaker at (0-5)°C, stirred continuously while cooling ,the mixture was stirred in an ice bath and left over night and acidified to pH=6 with dilute hydrochloric acid. The resulting precipitate was filtered, washed with distilled water, purified by recrystallization from hot ethanol, and dried at room temperature. The yield was 85 % reddish-orange crystals that decompose at 185° C. The structural structure of Imidazolyl azo (IABA) is as shown below:



Scheme (2) :- preparation of the Ligand (IABA)

General Method for the Preparation Complex $[Cu\ (IABA)_2]H_2O$:

The complex was prepared in M: L ratio 1:2 by dissolving (0. 36840 g) of L in (5ml) of ethanol. The ligand solution was added gradually with stirring to the ($CuCl_2$. $2H_2O$) solution (0.0852 g) in buffer solution (pH=5.5).The mixture was stirred until dark violate precipitate appears. The dark violate solid was collected by filtration ,and then washed several times by DDW then allowed the color solid to dry at room temperature . The % yield of solid complex formed (%79) and m.p. (210 ^{0}C).

The physical and analytical properties of both ligand and copper complex are listed in Table (1) and Figure (1) shows the results of preliminary tests for the interaction of the ligand with Mn^{2+} ,Co^{3+} ,Ni^{2+} ,Cu^{2+} , Hg^{2+} ions.

Table (1):- Physical and analytical characteristics of the Cu(II) complex and the ligand (IABA)

Compounds	Colour	M.P C°	Yield %	M. F (M.wt)	Found (Calculate) %			
					C	H	N	M
Ligand = (IABA)	yellow	185	85	$C_{22}H_{16}N_4O_2$ 368.40	(71.72) 71.00	(4.38) 4.68	(15.21) 14.32	——
[Cu $(IABA)_2]H_2O$	violet	210	79	$C_{44}H_{30}CuN_8O_4$ 798.32	(66.19) 66.97	(3.76) 4.15	(14.04) 13.54	(7.96) 7.00

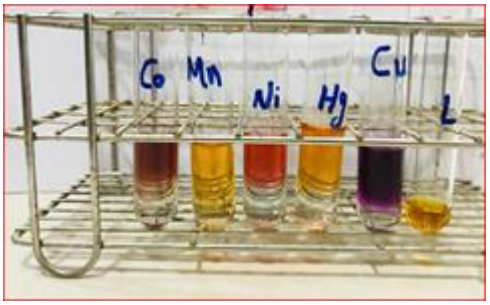


Fig. (1) Show the results of the initial tests of the Ligand (IABA) reaction with Mn^{2+} ,Co^{3+} ,Ni^{2+} ,Cu^{2+} , Hg^{2+} ions

Results and Debate

Description of azo ligand (IABA) and Cupper complex

"The imidazolylazo ligand is a reddish crystals but the prepared Cu(II) complex was crystalline and violet color .The **IABA** and the Cu(II) complex are insoluble in water and stable toward air at room temperature. It is soluble in methanol, ethanol, acetone , ,dimethyl formamide, dimethyl sulfoxide, alkaline aqueous solutions and strongly acidic solutions. The structures of the acquired ligand and the individual Cu(II) complex were elucidated by Infrared , UV– visible, mass spectra ,^{1}H-NMR, elemental analysis and several techniques" .

^{1}H-NMR Spectra

"The ^{1}H-NMR spectra of both ligand (IABA) and its Cupper (II) complex were recorded by using DMSO-d_6 as solvent and TMS as internal reference .Fig. (2 and 3). A signal at δ =2.52 ppm is attributed to solvent proton[8] ,The ^{1}H-NMR spectra of ligand (IABA) show multiplets signal around δ= 6.63-8.25 ppm are assigned to aromatic protons (Ar-H) [9] , a signal at δ=12.41 ppm is attributed to (O-H) of the carboxyl group and signal at δ=13.00 ppm is attributed to (N-H) of the imidazole ring"[10] .

In the ^{1}H-NMR spectrum of Cu(II)-complex , a signal corresponding to H_2O protons at δ=3.38 ppm [11] , multiple signals around δ= 6.75 -8.59 ppm corresponding to aromatic protons (Ar-H) , signal at δ=13.31 ppm corresponding to (N-H) in the imidazole ring , and a signal corresponding to the solvent proton at δ =2.52 ppm [8] .

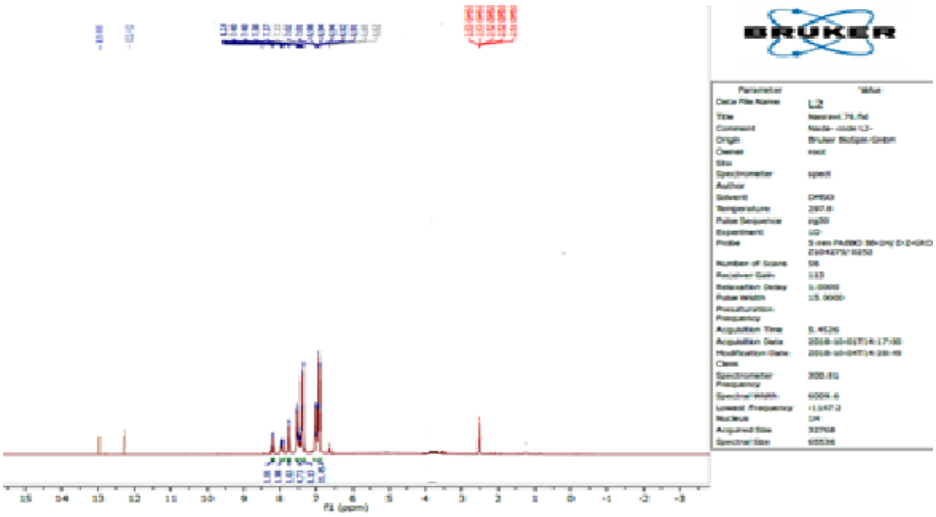


Fig. (2):- ^{1}H-NMR spectrum of ligand(IABA)

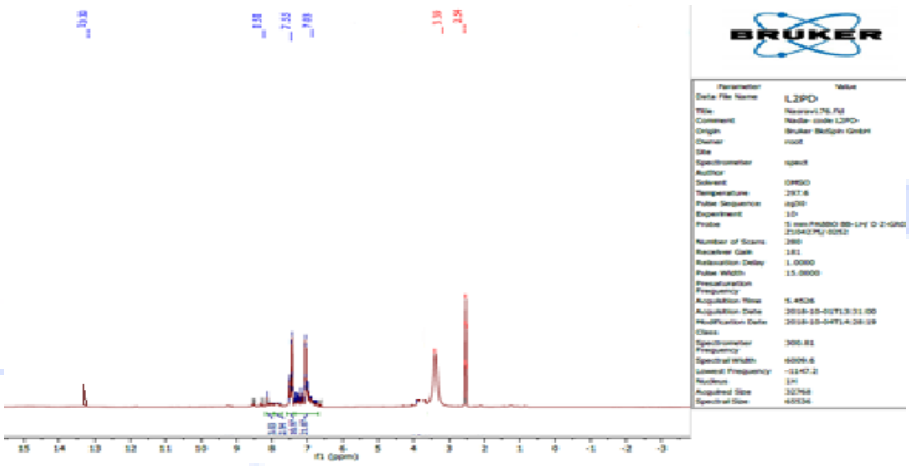


Fig. (3):- ^{1}H-NMR spectrum of Cu(II)- complex

I-R spectra of (IABA) ligand and Cupper(II) complex

"Infrared radiation submitted valuable details about the kind of the functional collection attached to the metal atom. The ulmost important IABA spectral bands of the ligand and its copper complex submit decisive structural proof for symmetry of the binding with the central metal. FT-IR spectra of the ligand are included in two bands They are located in the regions (2835.45, 2999.41 and 2958.97) cm^{-1} which were characteristic of aromatic and aliphatic (CH-H) stretching vibrations, respectively[12]. The infrared spectrum shows a band at the region (3076-3340) cm^{-1} due to The presence of (N–H) imidazole [13] while the post-coordination bands were observed at (3114-3145) cm^{-1}. This shift is related to the damage of the internal hydrogen bonds of N3 to the heterocyclic ring [14], the band at (1649.19) cm^{-1}. Due to ν(C═N) of imidazole nitrogen N3[15], while the bands were observed at (1442.80) cm^{-1} and (1309.71) cm^{-1} assigned to (N = N) and (C – N = N – C). [15] respectively. The scan band (N = N) in the IR spectra of the free ligand shows the change in position (frequency range, shape and intensity) in the IR spectra of the complexes. These confirm their participation in coordination with metal ions. The complex spectra showed new weak bands in the frequency range (419.55 cm-1) related to the bond stretching frequency (M-N) [16], and the FT-IR spectra of the ligand and its copper complex are shown in the figure (4,5)

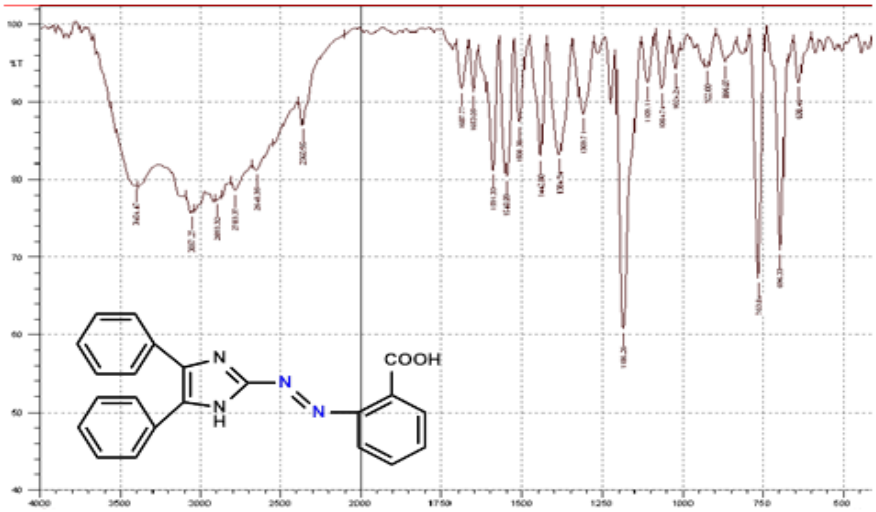


Fig.(4):- FT-IR of imidazolylazo dye ligand (IABA)

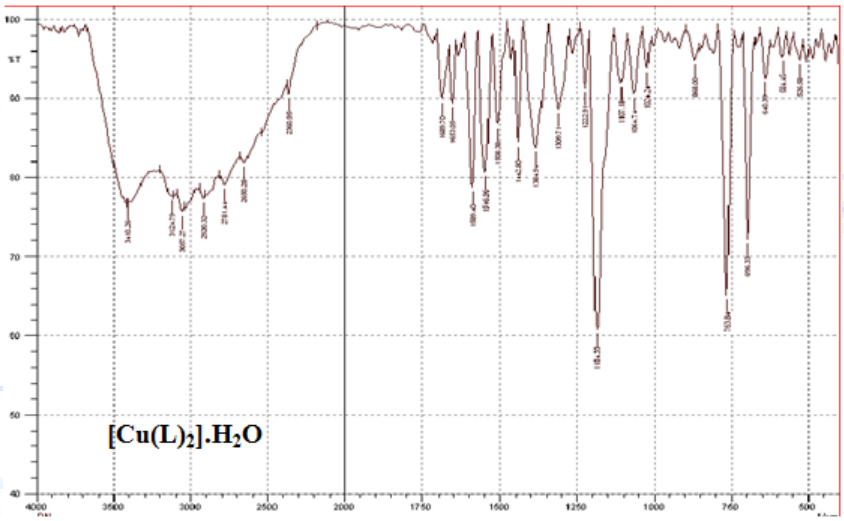


Fig. (5):- FT-IR [Cu] complex

Table (2):- Selected infrared absorption bands (4000-400) cm^{-1} for ligand(IABA) and its Cu complex (KBr disc)

Compounds	ν(O-H)	ν(N-H)	ν (N=N)	ν(C=C)	ν(C-N)	ν(C-O)	υH_2O (outer-sphere coordination)	υH_2O (inner – sphere coordination)	ν(M–N)
Ligand = IABA	3404.47 width band	3184.31	1442.80	1309.71	1222.91	1183.84	-----	-----	-----
$[Cu(L)_2].H_2O$	--------	3190.24	1442.80	1309.71	1222.91	1184.33	3410.28	-----	526.58

UV-Vis Spectra and Magnetic Moments:

"Electronic absorption spectra of (IABA) and its copper complex were recorded at 300 K, 200 - 1000 nm and absolute ethanol as solvent. Absorption bands, band assignments and proposed shapes of the copper complex are listed in Table 3. The UV-Vis spectra are shown in Figures (6,7). UV-Vis of the ligand (IABA) and its copper complex.

Electronic spectrum of the ligand is described by three absorption bands. The bands show at 219.07 nm, 281.42 nm and 427.61 nm. The first band is attributed to the n → π* transition of the azo group (-N = N-), while the second band is attributed to the n → π* transition of the vinyl π electron [17]. The third band can be attributed to the π → π* transition of the hetero imidazole ring via the azo group (-N=N-). [17] This band shows redshifts when coordinated with copper ions. It also shows electronic spectra of the Cu(II) complex bands at 214.16 nm (466.94.06 cm^{-1}), 278.54 nm (35901.48 cm^{-1}) and 537.77 nm (18595.31 cm^{-1}) , assigned to charge transfer and 2Eg → 2T2g transition respectively[18]. The magnetic moment of the complex is (1.89 B.M). An octahedral stereochemistry was proposed."

Table (3):- Electronic spectra (nm and cm^{-1}), electronic transitions, magnetic moments, proposed geometries and hybridization of the ligand (IABA) and its Cu complexes

Compounds	λ_{max} (nm)	Absorption bands (cm^{-1})	Transitions	μ_{eff} (B.M)	Geometry	Hybridization
Ligand = IABA	219.07	45647.50	n →π*	----------	_____	_____
	281.42	35537.07	n →π*			
	427.61	23385.79	π →π*			
$[Cu(L)_2].H_2O$	214.16	466.94.06	Ligand field	1.89	Octahedral (distorted)	Sp^3d^2
	278.54	35901.48	Ligand field			
	537.77	18595.31	$^2E_g \rightarrow {}^2T_{2g}$			

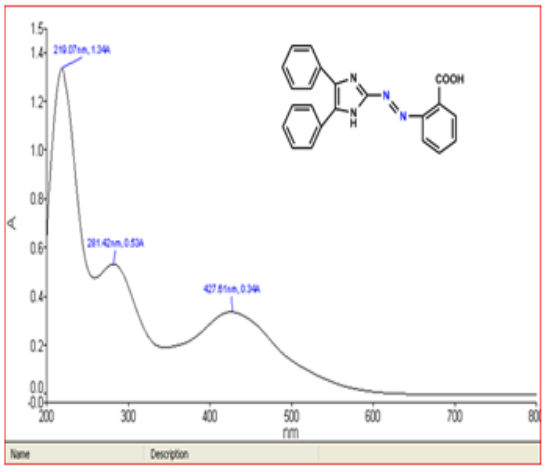


Fig. (5):- UV-Visb. spectra of ligand (IABA)

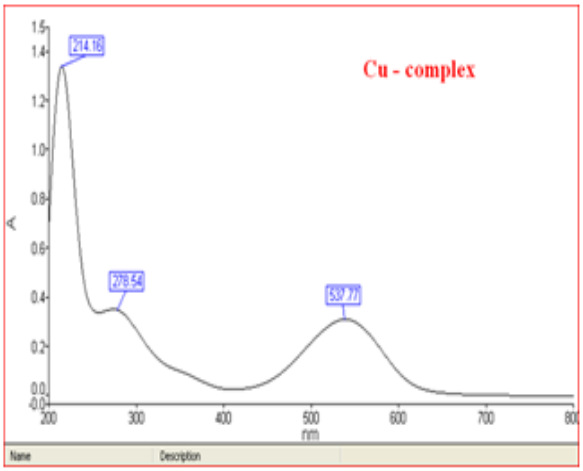


Fig. (6):- UV-Visb. spectra of Cu complex

Molar Conductance measurements

Cu(II) complex prepared in the work showed conductivity value 13.98 S. mol^{-1}. C^{-1} [19] in DMSO at room temperature this value indicating that don't conductive species exist. Based on these results, the structural formula of this ligand and complex is shown in Fig. 7.

Table (4):- Maximum wavelength (λ_{max}),Molar conductivity, molar absorptivity (ϵ), and Optimal concentration of ligand(IABA) and Cu complex.

HL=(IABA) λ_{max} =427.61nm Conc.=1x 10^{-4}M	Metal ion	Optimal Conc.×10^{-4} M	λ_{max} (nm)	Abs	(ϵ) L.mol^{-1}.cm^{-1}	Molar cond. S.cm^{-1} mol^{-1}
	Cu (II)	1.5	537.7	0.310	2066	13.98

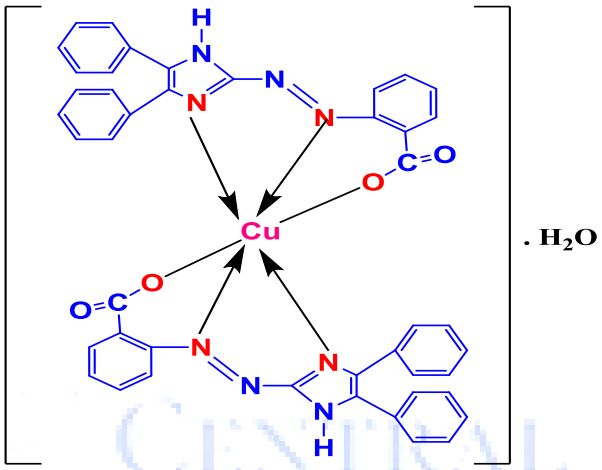


Fig. (7): The suggested structural formula of Cu-complex $[Cu(IABA)_2].H_2O$

Thermal analysis

"Thermogravimetric analysis (TGA) and differential thermogravimetric (DTG) for the ligand (IABA) and its Cu complex were done from room temperature to 900°C. Typical TG and DTG diagrams are shown in Figures (8 , 9). The calculated and confirmed mass losses are shown in Table(5) [20].

Table (5). Thermal analysis data for (TG , DTG) of ligand (IABA) and Cupper complex

Compound	Dissociation stages	TG Range (˚C)	DTG peak (˚C)	Estimated(calculated) % Mass loss %	Decomposition assignment	Residue
Ligand=IABA	Stage I	29.99-183.22	110.25	2.168(2.155)	The loss of oxygen	CH_7N_3
	Stage II	183.22 - 383.88	332..80	48.63(48.92)	$C_{14}H_{12}O$	
	Stage III	383.88 - 542.58	414.89	23.47(23.93)	$C_5H_{12}O$	
	Stage iIII	542.58 -900.1	------	12.6(12.23)	C_2H_7N	
$[Cu(L)_2].H_2O$	Stage I	29.59-150.49	116.05	2.56(2.207)	The loss of the H_2O molecule	$C_{16}H_{14}CuN_2$

					outer -sphere coordination	
	Stage II	150.49 - 359.58	295.62	27.835(26.983)	$C_{15}H_{12}N_2$	
	Stage III	359.58 - 573.60	376.80	20.801(20.351)	$C_7H_6N_3O_3$	
	Stage IIII	573.60 -900.8	-------	15.562(15.210)	$C_7H_9N_2O$	

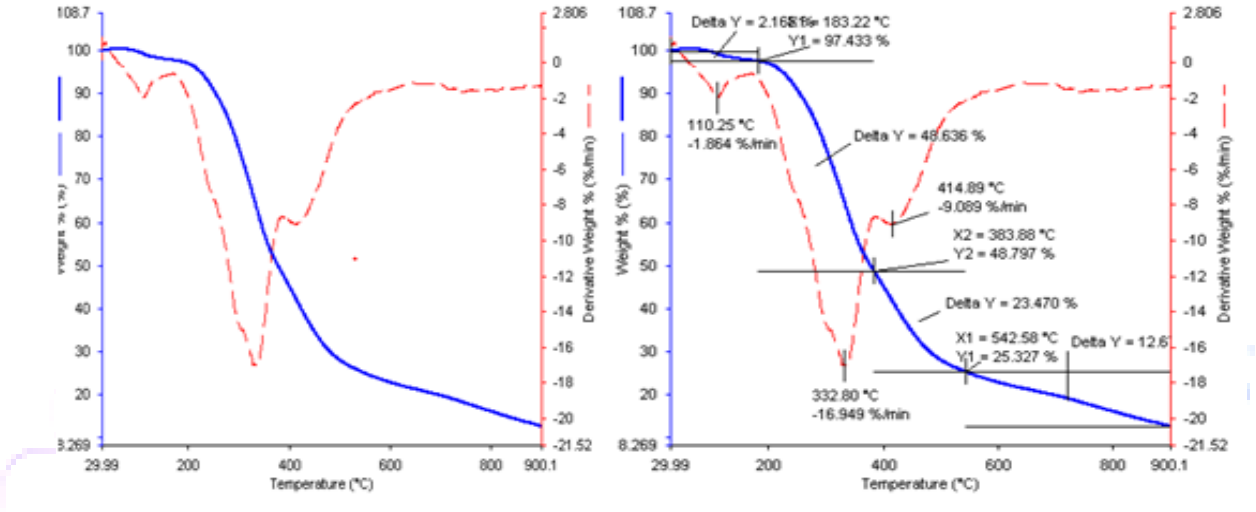


Fig. (8):- Thermal analysis (TG and DTG) of ligand (IABA)

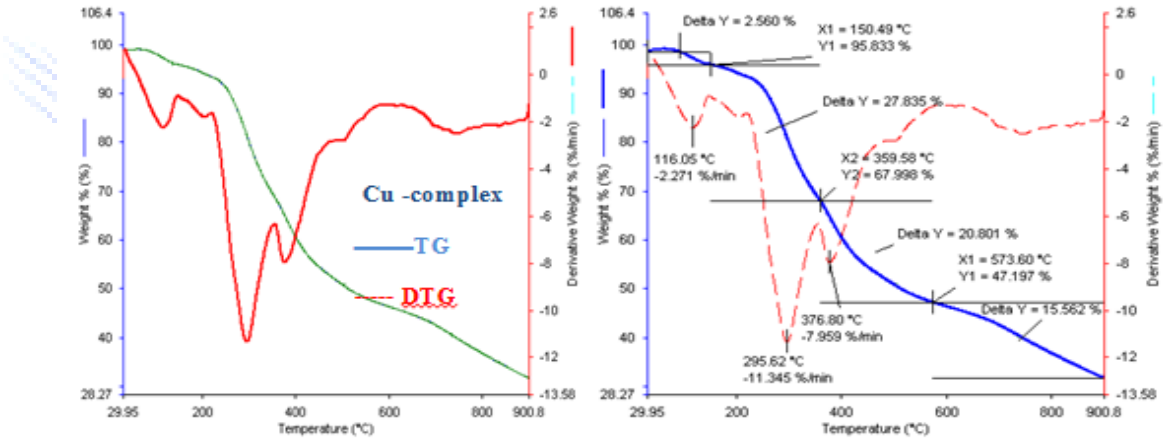


Fig. (9):- Thermal analysis (TG and DTG) of Cu Complex

Anti-Microbial studies

"Five harmful bacterial strains were used to test the ligand and Cupper complex's antibacterial effectiveness., three gram negative (Escherichia Coli , Salmonella Typhi and Pseudomonas) and two gram positive (staphylococcus Aureus and streptococcus) bacteria [Fig. 10,11,12,13,14,15] .Antibacterial and capability of the ligand and its complex were estimate regarding of zone of inhibition of bacterial growth. The consequences of the antibacterial activities are displayed in table (6). Growth of bacterial pathogens on all concentration was checked to decide the minimum concentration that prevent the growth of the organism. The ligand and its complex were effective

against all bacterial strains tested. The compounds seem to be antimicrobial based on the antibacterial screening data, and it's important to note that the metal complex exhibits more inhibitory effects than the free ligand; in other words, it's clear that the metal complex's zone of inhibition is larger than the free ligand's against the selected bacterial strains [21].

Table(6):- Antibacterial activity of ligand (IABA) and Cupper complex

Comp. No.	Anti - bacterial Activity								
	E. coli\ ppm			Salmonella Typhi\ ppm			Pseudomonas\ ppm		
	1000	500	100	1000	500	100	1000	500	100
Ligand (IABA)	+	+	-	+++	++	+	+++	++	+
$[Cu(L)_2].H_2O$	+	+	-	+++	++	+	++	++	+

Comp. No.	Anti - bacterial Activity								
	Streptococcus\ ppm			Staphylococcus\ ppm			Streptococcus Pyogenses \ ppm		
	1000	500	100	1000	500	100	1000	500	100
Ligand (IABA)	++	+	-	+++	++	+	+++	++	_
$[Cu(L)_2].H_2O$	++	+	+	+	-	-	+++	++	+

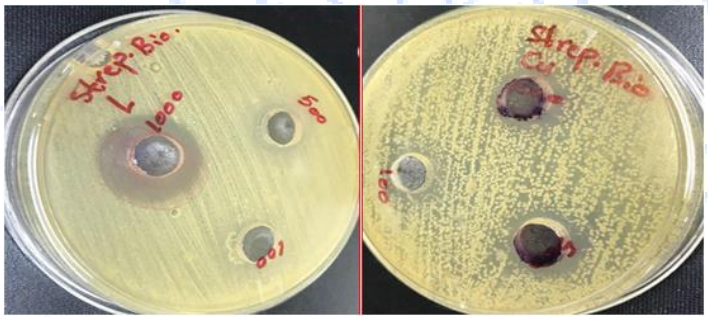


Fig. (10):- Effect of ligand (IABA) and Cupper complex on Streptococcus Pyogenses bacteria growth

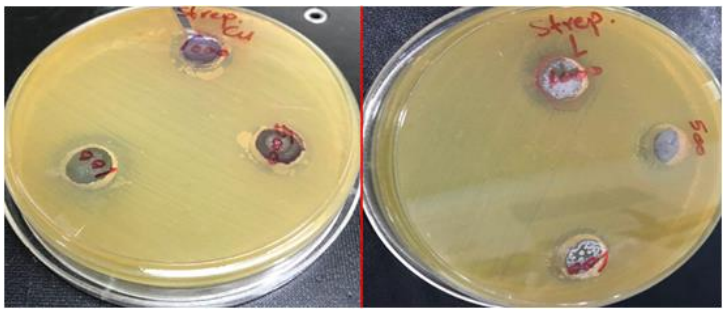


Fig. (11):- Effect of ligand (IABA) and Cupper complex on Streptococcus bacteria growth

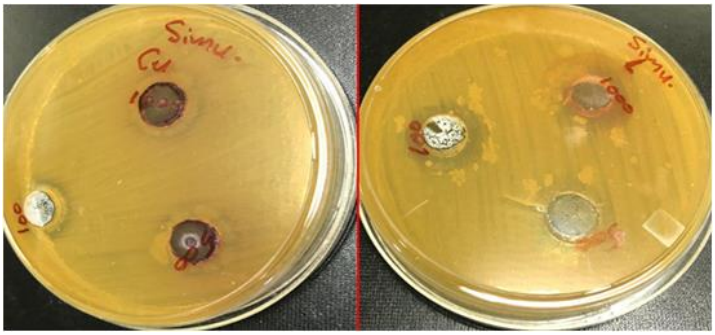


Fig. (12):- Effect of ligand (IABA) and Cupper complex on Salmonella Typhi bacteria growth

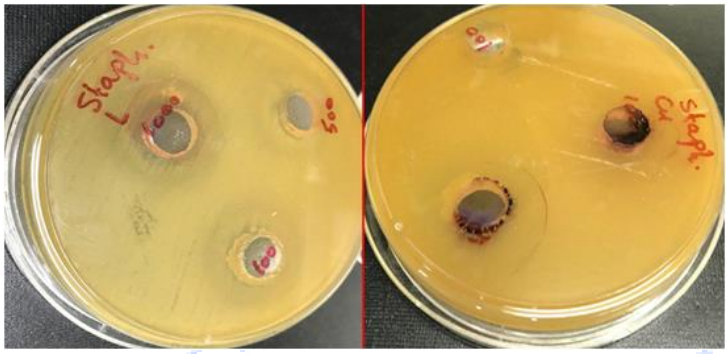


Fig. (13):- Effect of ligand (IABA) and Cupper complex on Staphylococcus bacteria growth

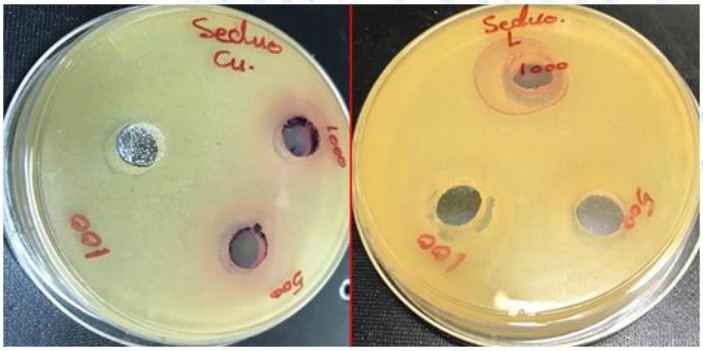


Fig. (14):- Effect of ligand (IABA) and Cupper complex on Pseudomonas bacteria growth

Figure (15):- Effect of ligand (IABA) and Cupper complex on E. coli bacteria growth

Pharmacology Results

Cell viability and cytotoxicity assay

Cytotoxicity assay: Various concentrations of [Cu((IABA)$_2$] H_2O was used to inhibit cell growth of thyroid cancer cells (MDA-T120) (6.25-400) µg/ml as well as healthy cells (WRL-68). Impact of

[Cu((IABA)2] H2O and ligand (IABA) on the proliferation of MDA-T120 thyroid cancer cells and healthy cells (WRL). Table (7) displays the concentrations utilized to limit growth against MDA-T120 thyroid cancer cells and healthy cells (WRL).

The cytotoxicity of the ligand and copper complex was expressed as the average inhibitory concentration of growth (IC50) [22], This is the concentration that would be expected to produce a (50%) cytotoxic effect on cancer cells after a (24) hours exposure to the test compound. The ligand IABA's in vitro cytotoxicity against the human cell line MDA-T120. The copper complex was specifically cytotoxic to cancer cell lines, with an IC50 of 100.1 µg/ml and an IC50 of 135.7 µg/ml for the ligand and 330.1 µg/ml and 200.76 µg/ml for WRI-68, respectively. According to the test results, a compound's type has a significant impact on how quickly cancer cells and cells of normal line age grow inhibited.Results of the screening are displayed in Figures (16, 17).

Table(7):- Effect of $[Cu((IABA)_2]\ H_2O$ **on human thyroid cellular cancerous cell line (MDA-T120) Viability compared with healthy cells (WRL-68) mat the same con. Using 24 hours MTT test at 37^0C**

Con. (µg.mL^{-1})	Mean Percentage (%) for each cell line							
	LH=(IABA)				$[Cu((IABA)_2]\ H_2O$			
	Cancerous line cells of breast MDA-T120		Normal line cells of breast WRI-68		Cancerous line cells of breast MDA-T120		Normal line cells of breast WRI-68	
	Cell Viability	Cell Inhibtion	Cell Viability	Cell Inhibtion	Cell Viability	Cell Inhibtion	Cell Viability	Cell Inhibtion
6.25	96.41	3.59	95.95	4.02	96.34	3.66	95.95	4.05
12.5	96.88	3.12	95.95	4.02	96.80	3.2	95.95	4.05
25	94.25	5.75	95.22	4.78	95.95	4.05	95.22	4.78
50	91.51	8.49	95.33	4.67	91.09	8.91	95.33	4.67
100	81.02	18.98	93.60	6.4	66.17	33.83	93.60	6.4
200	73.23	26.77	84.80	15.2	46.03	53.97	88.27	11.73
400	61.21	38.79	76.00	24.00	37.50	62.5	78.63	21.37

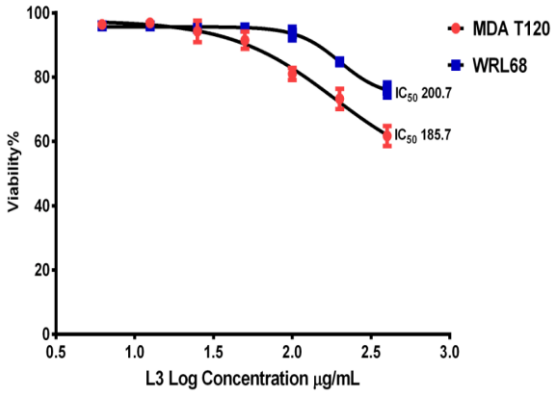


Fig. (16):- Showing anticancer activity data ligand (IABA) against human cancer cell lines

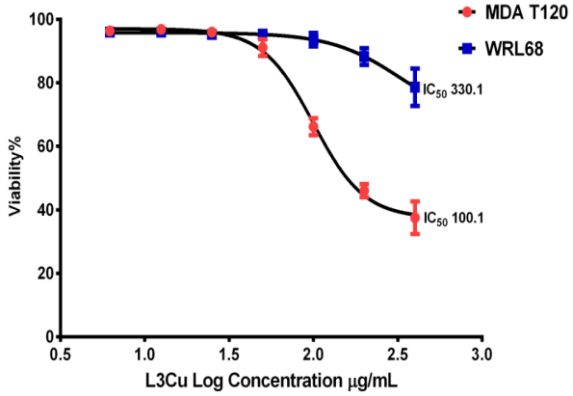


Fig. (17):-Showing anticancer activity data of copper Complex against human cancer cell lines

Conclusions

Based on the results of the spectral and analytical diagnosis of the heterogeneous azo ligand and its metal complex under study, we conclude The proposed structural formula of the complex shows the behavior of Ligand (IABA) as tridentate ligand when it is coordinated with Cu ion .The metal : ligand ratio is 1:2 for the Cu complex under study and have non- electrolytic nature and non ionic.Thermal analysis TGA proved the thermal stability of the ligand and its prepared metal complex and is not affected by the surrounding conditions.Ligand and Cu complex prepared showed a biological effect when treated with Five strains of bacteria that are highly sensitive to antibiotics".

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