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RESEARCH ARTICLE
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Synthesis, Characterization, Biological and Antioxidant estimation of Mixed Schiff Base Ligand and Heterocyclic Compound with Some Metal ions

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ABSTRACT

In this research, a group of complexes were prepared which were derived from Schiff base ligands, which is called (1E,1'E)-1,1'-(1,2-phenylene)bis(N-(2,4-dichlorophenyl) methanimine) (L) with ortho-phenanthroline (o-phen). The prepared complexes are M(II) [Co(II),Ni(II),Cu(II), Zn(II), Cd(II),and Hg(II)]. A range of spectroscopic and technical techniques have been used to characterize these materials, including: The FTIR, 1H-NMR, LC-Mass Spectrum, UV-Visbale, molar conductance, and magnatic moment, atomic absorbtion, chlorid contents. Spectral results obtained are showen that (ortho-phen) and (L) behave as neutral coordinating to the central metal ion by the donating atoms (N2) of the both compounds. The geometry shape of the mono ligand (L) complexes were tetrahedral, while the geometry shape of mixed ligands (L) as first ligand with (ortho-phen) as second ligand were octahedral geometry. The antimicrobial activities of the prepared compounds were experienced by using disc diffusion process touching different types of selected bacteria; two gram positive and two gram negative, and one type of fungi.

Keywords: *Schiff base ligand, ortho- phen, elemental analysis, antimicrobial activities, geometry shape*

INTRODUCTION

The Schiff bases are compounds have a larg role in coordination chemistry because of the precence of azomethine group (-N=CH-)as a functional group, which can coordinate with transition metal ions(1). These chemical compounds are derived from carbonyl compounds such as: (aldehyde, ketone) and amines that have coloured crystal (2). Hugo Schiff is the science who synthesized them(3). Schiff bases compounds are important in many fields such as: medicine, industry they have many of applications like dyes, artificial, and pigment(4).Schiff bases ligands, during recent years, had expected greatly interest for the reason that of their ease of manufacture, sensitivity, and selective ones concerning the transition metal ion(5). Also, metal complexes of Schiff bases with hetrocyclic compounds find applications potential drugs (6-8). ortho-phenanthroline is a hetrocyclic hydrate organic compounds. It is a white solid substance which has a white color and can be soluble in nonpolar solvents. In coordination chemistry the o-phen. can be considered as ligand, form strong compounds with most metallic ions (9). The parent of amainclass of chelating agents.

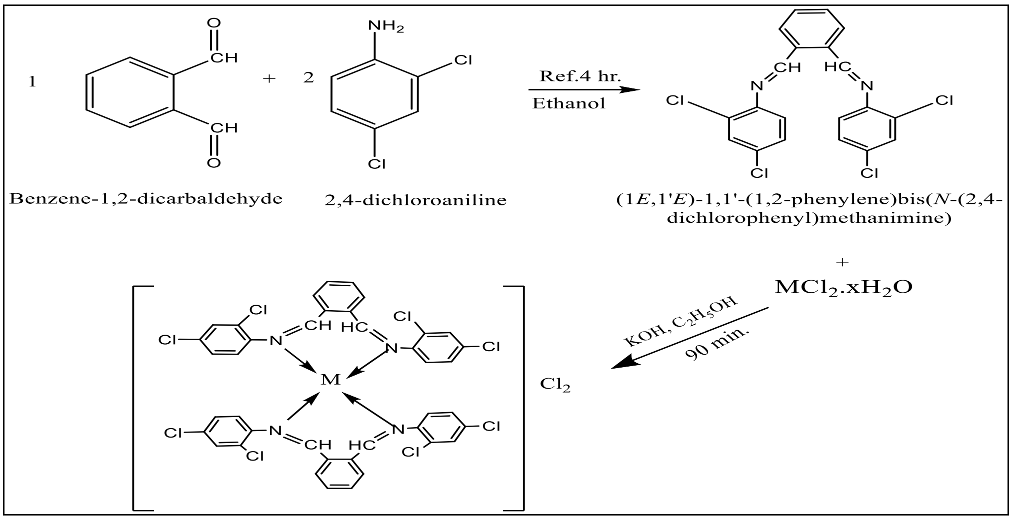
Considerable of notice has been paid in latest years to the search of the complexes forming properties of o-phen because of its excellent biological importance (10-11). Saba H. Mahdi and *et.a l*(12) synthesized a new mixed ligand Schiff base complexes of M^{2+} (cobalte, Nickle, cupper, zinck, cadmium, and Mercury) which are forming from the Schiff base ligand that derived fromortho phthaladehyde with para nitro aniline. All the compounds are characterized by spectral and elemental methods. The data are showen that all the prepared compounds are octahedral geometries. The biological activities of complexes and ligand are testedagainst different types of bacteria. Also the 1,10-phen behave as bidentate ligand because of a nitrogen atoms on the middle ring(13). Three new 1,10-phenanthroline and 2,2-bipyridine mixed ligand complexes of $[Co(bpy)(phen)_2](NO_3)_2.2H_2O$, $[Cu(bpy)(phen)(H_2O)_2]Cl_2.2H_2O$, and $[Zn(bpy)(phen)]Cl_2.6H_2O$, were synthezied by Agwara M. O. and other workers(14). All the compounds were characterized by spectroscopic analysis: IR, electronic transitions, and elemental. The resultsindicate that all ligands have octahedral shapes through coordination with metal ion. The antimicrobial activities of the complexes and ligands revealed that the Cu complexes have the highest activity.

Equipment's and Devices

Every substance has been purchased from reputable sources and is of the utmost quality; so, no further purification is necessary. The Stuart Melting Point Kit is utilized to determine the melting points, while the Euro (EA 3000) instrument is employed for elemental microanalysis of the ligand. The Brucker DRX system (400 MHz) is used to acquire the ^1H NMR spectra, and the Shimadzu UV- 160A Ultraviolet-Visible Spectrophotometer is utilized to conduct UV-Vis spectra on KBr discs within the (4000-400) cm^{-1}range. The IR-spectra are established by FTIR- 8400S Spectrophotometer. To calculate the metal content (A.A.S) of the complexes, the atomic absorption method is employed using the AA 620G Shimadzu spectrophotometer. Lastly, the Chloride compounds of the substances are particular. The process involved testing all the complexes, breaking them down using Nitric acid, and then diluting them with water. The magnetic properties of the resulting solution were determined using the Faraday's way and Bruker BM6 device at room temperature.

preparation of the Ligand Complexes

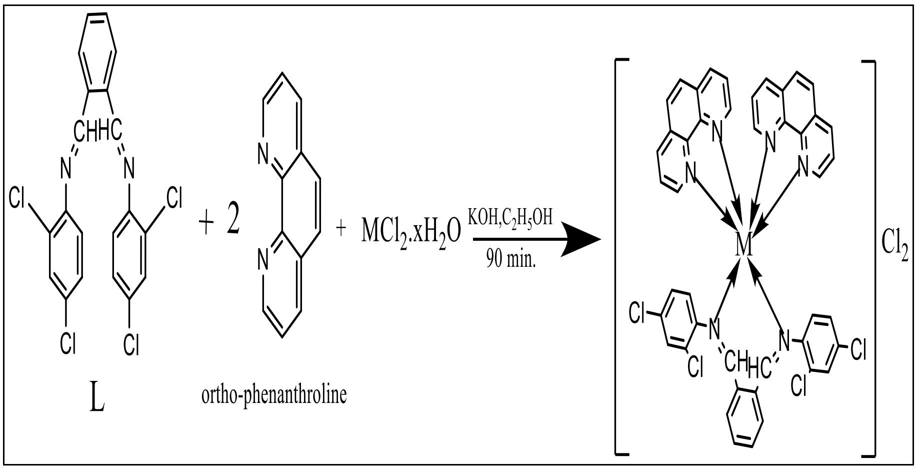
To stirred one millimole of metal chloride in 10 milliliter ethanol;0.237g (Co^{+2}-chlorid.$6H_2O$,0.237g, Ni^{+2}-chloride.$6H_2O$, 0.17g Cu^{+2}-chlorid.$2H_2O$, 0.201g Cd^{+2} -chlorid.H_2O, and 0.272 g Hg^{+2}-chloride, the ligand solution (L) (0.422g, 2millimole) in five milliliter absolute ethanol is added. These symbols have been given for complexes and respectively; (LS_1, LS_2, LS_3, LS_4, LS_5). The resulting mixture is stirred for one houre and 30minutes, filtered and dried using anhydrous $CaCl_2$. The physical properties of the schiff base ligand and newly formed compounds are displayed in table-1, schem-1where



SCHEM1: preparation of ligand and complexes

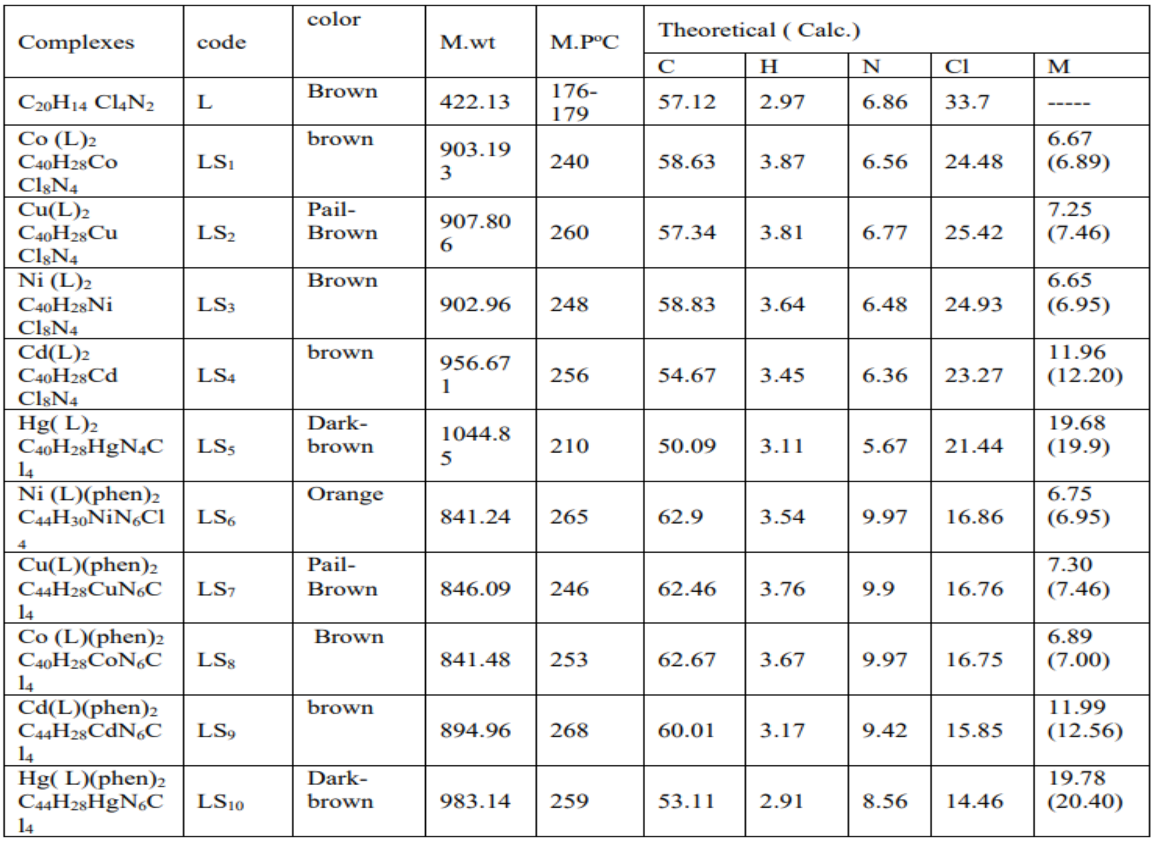
Preparation of the Mixed Ligand Complexes

A stirred solution consisting of 1 millimole of metal chloride in ten millileter ethanol (composed of 0.126g Mn(+2) chloride, 0.237g Co(+2) chloride.6H_2O,0.237g Ni(+2) chloride.6H_2O, 0.17Cu(+2) chlorid.2H_2O,0.201g Cd(+2) chlorid.H_2O, and 0.272 g Hg(+2) chloride) is mixed with a solution of schiff base ligand (0.422g, millimole) and orthophenanthroline (0.360g, millimole) in 5milliliter absolute (C_2H_5OH) . The mixture is stirred for 120 minutes, filtered and dried using anhydrous $CaCl_2$.The physical characteristics of schiff base ligand and newly formed compounds are revealed in table-1 according to the following symbols; (LS_6, LS_7, LS_8, LS_9, LS_{10}), schem-2.



SCHEME 2: preparation of mixed complexes

TABLE 1: all the physical properties of the prepared materials



Complexes	code	color	M.wt	M.P°C	Theoretical (Calc.)				
					C	H	N	Cl	M
$C_{20}H_{14}Cl_4N_2$	L	Brown	422.13	176-179	57.12	2.97	6.86	33.7	-----
Co $(L)_2$ $C_{40}H_{28}CoCl_8N_4$	LS_1	brown	903.193	240	58.63	3.87	6.56	24.48	6.67 (6.89)
Cu$(L)_2$ $C_{40}H_{28}CuCl_8N_4$	LS_2	Pail-Brown	907.806	260	57.34	3.81	6.77	25.42	7.25 (7.46)
Ni $(L)_2$ $C_{40}H_{28}NiCl_8N_4$	LS_3	Brown	902.96	248	58.83	3.64	6.48	24.93	6.65 (6.95)
Cd$(L)_2$ $C_{40}H_{28}CdCl_8N_4$	LS_4	brown	956.671	256	54.67	3.45	6.36	23.27	11.96 (12.20)
Hg(L$)_2$ $C_{40}H_{28}HgN_4Cl_4$	LS_5	Dark-brown	1044.85	210	50.09	3.11	5.67	21.44	19.68 (19.9)
Ni (L)$(phen)_2$ $C_{44}H_{30}NiN_6Cl_4$	LS_6	Orange	841.24	265	62.9	3.54	9.97	16.86	6.75 (6.95)
Cu(L)$(phen)_2$ $C_{44}H_{28}CuN_6Cl_4$	LS_7	Pail-Brown	846.09	246	62.46	3.76	9.9	16.76	7.30 (7.46)
Co (L)$(phen)_2$ $C_{40}H_{28}CoN_6Cl_4$	LS_8	Brown	841.48	253	62.67	3.67	9.97	16.75	6.89 (7.00)
Cd(L)$(phen)_2$ $C_{44}H_{28}CdN_6Cl_4$	LS_9	brown	894.96	268	60.01	3.17	9.42	15.85	11.99 (12.56)
Hg(L)$(phen)_2$ $C_{44}H_{28}HgN_6Cl_4$	LS_{10}	Dark-brown	983.14	259	53.11	2.91	8.56	14.46	19.78 (20.40)

Biological & Antioxidant studies

The ready-made substances underwent a test via the disc diffusion process next to a single type of fungus, specifically *candida albicans* as well as various strains of bacteria including *Escharia coli*, *psuedomonas aruginosa* and (*Staphylococcus aureus*, *Streptococcus pyogenes*).

The solution for the solution is prepared using DMSO as the solvent with a concentration of 0.001M. The dishes are then kept at room temperature for 24 hours, during which the diameter of the inhibition is calculated to determine the increase of bacteria and fungi [10-12]. To evaluate the antioxidant activity, the radical scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is assessed, which is a widely-used method in antioxidant studies. The DPPH radical scavenging effects of different concentrations (25, 50 and 75) µg/ml of all compounds and associations are measured. The solution is incubated in the dark for thirty min., and the absorbance (A) is considered at 517 nm using UV-VIS Shimadzu spectrophotometer against a blank. The IC inhibition of DPPH color values is then calculated, and experiments were repeated. Antioxidant Activity percentage (DPPH inhibition percentage) was determined using the following formula [13-15]: Antioxidant % = A (blank) – A (sample) / A (blank)× 100, where: A (blank) represents the absorbance of the blank and A (sample) represents the absorbance of sample].

RESULTS AND DISCUSSION

The outcomes demonstrate that in general, the complexes have low solubility in water, but they are dissolvable in in both DMSO and DMF. Table – 1 shows the compounds' noteworthy characteristics and CHN. When referring to the complexes with metal (II) ions and Schiff base ligand , they are denoted as [M(L)$_2$] and [M(L)(phen)$_2$], respectively.

FT-Infrared Spectra

The FT-Infrared spectra of the ligand(L) and its complexes, which are embedded in KBr disc within range from 4000 to 200 cm^{-1}, display a faint absorption band at 3117 cm^{-1} indicating the stretching vibration of the aromatic bond (C-H) whereas the regions (3053-3142) cm^{-1} indicate the aromatic bond (C-H) of complexes (15). The azomethine group (HC=N) stretching vibration of the ligand revealed at 1625 cm^{-1} which is considered as a medium band, whereas the bands at (1610-1664) cm^{-1} corresponds to (HC=N) of complexes(16). The stretching vibration of the (C=C) bond is located at 1567 cm^{-1} for the ligand, whereas the bands at (1545-1569)cm^{-1} are allocated to the (C=C) stretching vibration of complexes (15 . The peaks displaying a weak absorption in range (268-297)cm^{-1} refer to (C-Cl) stretching vibration(17). In all complex FT-Infrared spectrum, new bands emerge within frequency (544-558) cm^{-1} indicating frequency of (M-N) mode while the peaks (256 - 295) cm^{-1} indicate (M-Cl) stretching vibration(18). The FT-IR information of ligand and complexes are shown in Table-2. IR of [M-L-Ph] spectrum the bands are shifted to lower frequencies. The strong bands at (1633-1640)cm^{-1} are due to azomethine group, whereas the weak bands at (3098-3144) cm^{-1} are allocated to frequency of aromatic ring. In the spectrum of complexes with ortho- phen. There are stretching bands in the region (534-558) cm^{-1}and (248-235) cm^{-1} are refered to frequency of (M-N) and (M-Cl) in that order.

TABLE 2: FTIR details of cm^{-1} free ligands and their complexes

Compound	**υ(C-H) aromatic**	**υ(C=N)schiff**	**υ(C=N)1,10**	**υ(C=C)**	**υ(C-Cl)**	**υ(M-N)**	**υ(M-Cl)**
L	3117	1625	_	1567	278	----	----
LS$_1$	3133 3062	1652	_	1557	254	553 546	256
LS$_2$	3131 3076	1644	_	1545	255	553	287
LS$_3$	3130	1664	_	1553	289	561	284

	3075						
LS$_4$	3142 3053	1654	_	1583	273	584	283
LS$_5$	3118 3058	1644	_	1569	268	558	295
LS$_6$	3102 3110	1633	1623	1540	236	538	235
LS$_7$	3115 3131	1639	1629	1543	232	547	268
LS$_8$	3144 3098	1640	1615	1523	267	543	246
LS$_9$	3152 3114	1638	1633	1529	259	569	279
LS$_{10}$	3157 3123	1634	1617	1530	257	528	284

LC-Mass Spectrum and 1H-NMR Spectral Data of L

The primary Precision-resolution mass spectrometer of the synthesized ligand (L) is depicted in illustration (3- 9). The Observant peak at m/z = 421.4 is due to molecular ion for ($C_{20}H_{14}Cl_4N_2$) that exhibited a remarkable degree of conformity with the theoretical value documented in table (3), figure (1) is listed the fragmentation data for L. The ^{1}H-Neoclear Magnetic Resonance spectrum of the (L) is determined by using Dimethylsulphoxide-d6 as solvent at room temperature (19-21), as exposed in table (4) figure (2). The singlet signal, which is appeared at (2.48 ppm), can be assigned to the solvent DMSO. The signal at (3.34 ppm) refers to the residual of the water. The signals of the aromatic ring protons, shown in the region (6.00-8.06 ppm), were due to the aromatic rings' (protons H$^+$) of the free ligand. The signal within range at (8.43 ppm) is due the azomethine group' (HC=N) (protons H$^+$) in the ligand.

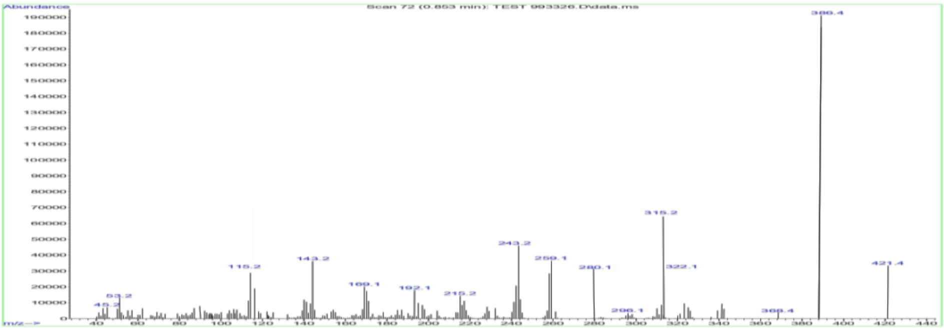
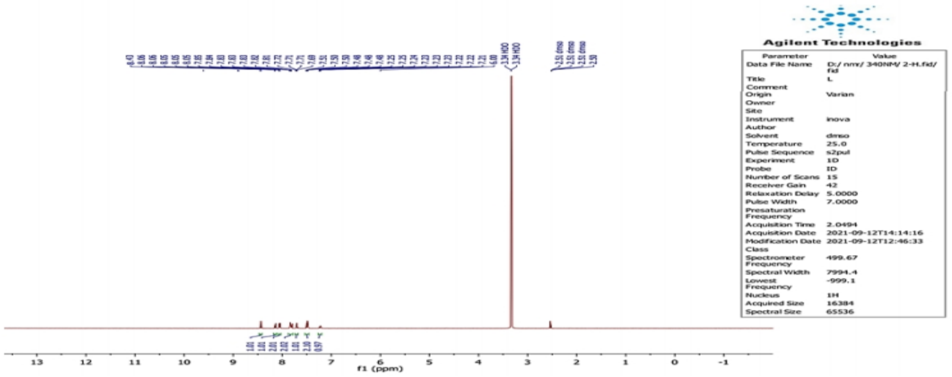


FIGURE 1: LC-Mass Spectrum of (L)



Parameter	Value
Data File Name	D:/ nmr/ 340NM/ 2-H.fid/ fid
Title	L
Comment	
Origin	Varian
Owner	
Site	
Instrument	inova
Author	
Solvent	dmso
Temperature	25.0
Pulse Sequence	s2pul
Experiment	1D
Probe	ID
Number of Scans	15
Receiver Gain	42
Relaxation Delay	5.0000
Pulse Width	7.0000
Presaturation Frequency	
Acquisition Time	2.0494
Acquisition Date	2021-09-12T14:14:16
Modification Date	2021-09-12T12:46:33
Class	
Spectrometer Frequency	499.67
Spectral Width	7994.4
Lowest Frequency	-999.1
Nucleus	1H
Acquired Size	16384
Spectral Size	65536

FIGURE 2: ^{1}HNMR spectrum of (L)

TABLE 3: The δ(ppm)of L

Functional group	**δ(ppm)**
DMSO-d6	2.51
D-OH	3.34
Ar-H	6.00 – 8.06 ($12H^+$, m)
N=C-H	8.43($2H^+$, s)

TABLE 4:^{1}H-NMR of L

Assignments	**Peak m/z**
$[M^{+\cdot}]= (C_{20}H_{14}N_4O_4)$	421.4
$[M^{+\cdot}]$ - Cl = $[M_1^+]$	386.4
$[M_1^+]$ – C_3HCl = $[M_2^+]$	315.2
$[M_2^+]$ – C_7H_3N = $[M_3^+]$	215.1
$[M_3^+]$ – C_7H_4N= $[M_4^+]$	115.2
$[M_4^+]$ – C_2HCl = $[M_5^+]$	45.2

Electronic Spectra, Molar Conductivity, and Magnetic Moments

Mono ligand complexes

Table (5) shows the Ultraviolet-Vis. spectrum for ligand and its complexes. The ligand's spectrum has peaks (251 nm) indicating an electronic shift from π to π*, and at (334,367 nm) indicating a shift from n to π*. The [LS_1] complex has four absorption peaks in its UV- Vis. Spectrum. The peak at (290 nm) is due to intra ligand, while the band at (434 nm) is recognized to charge transfer transition. The remaining 2 peaks at (659, 696 nm) are back to $^4A_2$ (F) →^{4} T $_1$(P) and ^{4} A $_2$(F) →^{4} T $_1$(F). UV-Vis. spectrum for ligand and its complexes were listed in table (5). The peaks for the ligand's spectrum at (251 nm) refers to (π→ π*) electronic shift and the two peaks in (334,367 nm) refer to (n → π*). The UV- Visible spectrum of [LS_2] exhibits (4) absorption peaks, the peak at (290 nm) due to intra ligand while the peak at (434 nm) is attributed to (C.T.) transition and the two peaks at (659, 696 nm) are back to $^4A_2$(F) →^{4} T $_1$(P) and ^{4} A $_2$(F) →^{4} T $_1$(F). The absorption peaks of electronic spectrum of Ni complex shown at (257, 392, 787, 836 nm) is assigned to intra ligand, C.T. and (d-d) transition respectively, the shape was suggested of complex is tetrahedral (22). In contrast with the complex of [LS_3] the spectrum shown four absorption bands one is could be assigned to I.T, and two peaks to C.T. and the last for (d-d) electronic transition at (281, 400, 445, 830 nm) respectively Characterizing those peaks is an indication of the fact that the compound has tetra-hedral shape(23).. The data in the table (5) shown the peaks at (262, 423 nm) of the [LS_4] complex and (273, 497 nm) of [LS_5] complex which are refered to (in. ligand) and C.T. in that order. Tetrahedral geometry of Cd and Hg complex is suggested by their peaks (24-25).

TABLE 5: Spectral data and magnetic moments of mono ligandcomplexes

Compound	**λ max. nm**	**ῡ cm^{-1}**	**ϵ max. $mol^{-1}.L.cm^{-1}$**	**Assignments**	**μ_{eff} (BM)**
L_1	251 334 367	39840 29940 27247	896 2017 1139	π → π* n → π* n → π*	---
LS_1	679 691	24213 14727	40 33	$^4A_2(F) \rightarrow {}^4T_1(F)$ $^4T_2(F) \rightarrow {}^4T_1(F)$	4.66

LS$_2$	797 824	12547 12135	23 16	$^3T_1(F) \rightarrow {}^3A_2(F)$ $^3T_2(F) \rightarrow {}^3A_2(F)$	4.41
LS$_3$	850	11764	46	$^2T_2 \rightarrow {}^2E$	1.64
LS$_4$	428	23364	245	C.T.	Dia.
LS$_5$	488	20491	238	C.T.	Dia.

Ortho phenanthroline and ligand complexes

The UV-Vis. for ligand was listed in table (5). The five absorption peaks in the UV- Vis. spectrum of [LS$_6$] at (289,363,347 nm) due to (in. ligand), while the peak at (464 nm) due to (charge transfer transition). and the peaks of low intensity at (759,796 nm) point to ^4T_1g (F) →^4A_2g (F) and ^4T_1g (F) →^4T_2g (F) respectively . In [LS$_7$] complex, the spectrum is shown six peaks, the first three peaks are attributed to intra ligand at (257,342,367 nm) , while the peaks at (392 nm) is due to C.T., and the two peaks at (787,836 nm) is refer to (d-d electronic transition) type ^3A_2g (F) →^3T_1g (F) and ^3A_2g (F) →^3T_2g (F) respectively. The peaks at (260,355nm) in the UV-Vis spectrum of [LS$_8$] refer to intra ligand, whilst the peak at (445 nm) is due to charge transfer whereas the other weak peaks at (569, 820 nm) are indicate to (d-d) electronic transition type^{2}Eg →^2T_2g. The [LS$_9$] complex has four peaks as shown in the UV-Vis spectrum, two are for intra ligand at (262,352 nm) and two in the region (385,423 nm) is assigned to charge transfer. As the same thing with the spectrum of [LS$_{10}$], there are four peaks, (258,375 nm) which are due to intra ligand and (419,486 nm) are attributed to charge transfer (C.T.). The entire spectrum are improved that there are octahedral shape around the metal ions in the complexes (26-29). Table (6) contains a list of all the μe$_{ff}$ (BM) values for the synthesized complexes, which indicate that the metal ions have an octahedral structure (30). Based on the molar conductivity values in the same table, it can be concluded that all the complexes are non-conductive.

TABLE 6: Spectral data and magnetic properties of mixed ligand complexes

Compound	**λ max. nm**	**ῡ cm^{-1}**	**ϵ max. mol^{-1}.L.cm^{-1}**	**Assignments**	**μe$_{ff}$ (BM)**
L	251 334 387	39840 29940 25839	896 2017 1139	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $n \rightarrow \pi^*$	---
LS$_6$	759 796	13175 12562	35 38	$^4T_1g(F) \rightarrow {}^4A_2g(F)$ $^4T_1g(F) \rightarrow {}^4T_2g(F)$	4.76
LS$_7$	787 836	12706 11961	18 11	$^3A_2g(F) \rightarrow {}^3T_1g(F)$ $^3A_2g(F) \rightarrow {}^3T_2g(F)$	4.51
LS$_8$	569 820	17574 12195	24 48	$^2Eg \rightarrow {}^2T_2g$	1.79
LS$_9$	385 423	25194 23640	1160 256	C.T. C.T.	Dia.
LS$_{10}$	419 486	23866 20576	415 228	C.T. C.T.	Dia.

Biological Activity&Antioxidant Activity by DPPH Radical Scavenging Method

The disc diffusion technique is utilized to experiment the synthesized ligand and complexes with various types of bacteria; *psuedomonas aruginosa*, *Escharia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*, one type of fungi such as candida albicans (19-20). The solvent of dimethyl sulfoxide (DMSO) was used to prepare the chemical solutions of the biological study, and they are provided as the same concentration of 1×10^{-3}M and all the plates are placed at room temperature for twenty four hours. The standard method employed to evaluate the impact of ligand and chemical complexes on the growth of specific bacterial strains involves measuring the inhibition zones(IZ) in millimeters, which are produced after 24hours. The synthesized ligand and mono & mixed ligand complexes exhibit a strong activity next to all kinds of bacteria gram(+), gram (-) and fungi. The data are listed in table (8). The increased (IZ) of the metal complexes can be attributed to bigger lipophilic environment of the complexes compared to the ligand alone, as per Tweedy's complexes theory (31), The DPPH assays method, the ability of the newly synthesized Schiff bases ligands and their some complexes to search free of charge radicals may be assessed, and the findings are shown in Table 5 and Figure.4. Because of its stability and simplicity, the DPPH radical is frequently used to evaluate antioxidant activity quickly. The creation of stable DPPH resulted in a decrease in the intensity of the DPPH band, demonstrating the ability of ligand and metal (II) complexes and Vitamin C to scavenge free radicals [29]. The IC50 values of the test ligand and some complexes were observed has a stronger scavenging activity [30].

TABLE 8: Biological activity and IC50 inhibation of Mono & mixed ligand complexe

Compound	***Staphylococcus aureus***	***Streptococcus pyogenes***	***psuedomonas aruginosa***	***Eschari a coli***	***candida albicans***	***IC50***
Vit. C	---	---	---	----	----	14
L	12	14	22	14	12	320.8
LS1	15	18	13	16	15	620
LS2	19	12	14	24	17	560
LS3	26	17	20	28	12	446
LS4	28	26	16	21	17	216
LS5	30	29	18	25	19	580
LS6	15	16	18	15	19	587
LS7	15	13	16	15	20	663
LS8	17	16	15	18	16	612
LS9	25	26	31	22	26	477
LS10	33	29	30	28	27	528

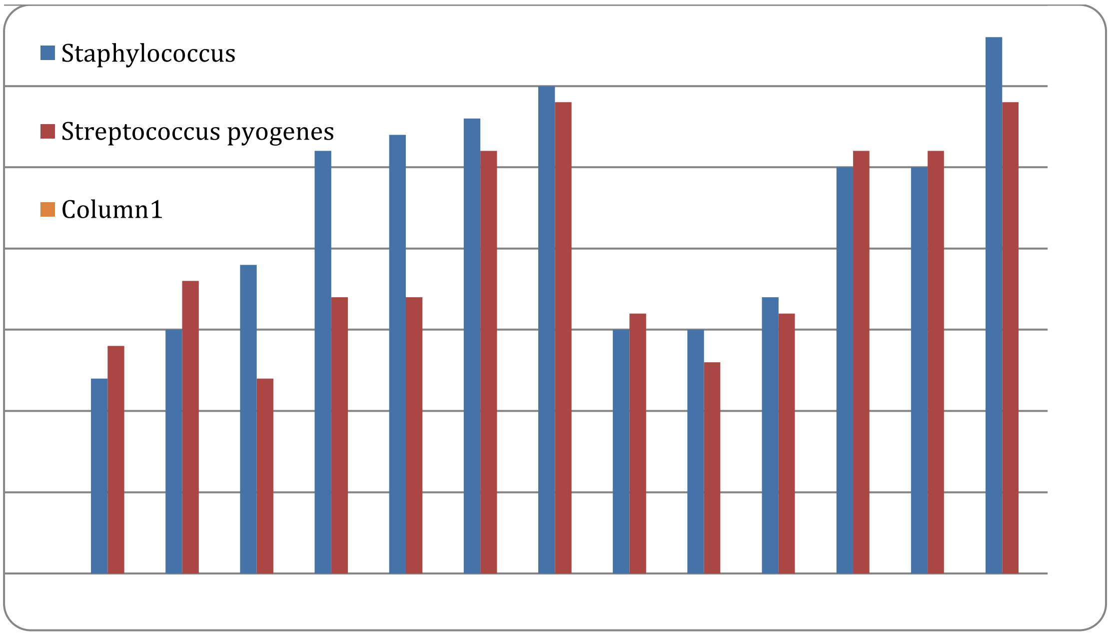


FIGURE 3: The values of Zone inhibition for Antimicrobial Activity

CONCLUSION

The synthesis and characterizations of the new Schiff base involves the coordination of metal ions with Schiff basefree ligand and phenanthroline via the N donor atom. Results of the analysis of the spectra electronic and the magnetic suncitivity data revealed that all compounds had either octahedral shape or tetrahedral shape. The biological activity of the preparation complexes exposed good activity toward types of bacteria.

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