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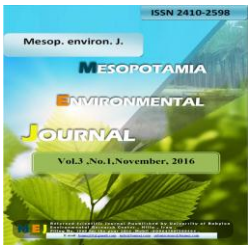

Synthesis, Characterization and Antimicrobial Studies of TransitionMetal Complexes with Azo Ligand derivative from 4-Aminoantipyrine

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Abstract

The transition metal complexes of Cu(II) and Ni(II) were synthesized from the AZo ligand derived from 4-aminoantipyrine , the ligand: 4-((2,4dihydroxyphenyl) diazenyl)- 1,5-dimethyl -2-phenyl -1-H- pyrazol- 3(2H)-one, (DDPPL1) and 4-((2-hydroxynaphthalen-1-yl) diazenyl) -1,5-dimethyl -2-phenyl -1-H- pyrazol- 3(2H)-one, (HDPPL2).The ligand and its metal complexes were characterized from their melting point, infrared and UV-visible spectroscopy. The biological activity also was investigated against eight bacterial samples including: *Streptococcus pyogenes*and *Staphylococcus aureus*(Gram Positive Bacteria)and *Escherichia coli* , *K.lebsiella pneumonia* , *proteus mirabilis*, *Salmonella typhi*, *Acinetobacterbaumannii and Vibrio cholera* (Gram Negative Bacteria). The metal complexes were showed higher antibacterial activity compared to the free ligands.

Keyword: azo group, biological activity, metal complexes.

Introduction:

In recent years, azo compounds and its complexes are a very important class of chemical compounds receiving attention in scientific research; these compounds are explored for their applications in different field [1-2-3].Antipyrine and its derivatives are well known for pharmaceutical as well as medicinal applications also evaluated as analgesic,[4] anti-inflammatory,[5] antimicrobial,[6]and anticancer activity,[7-8-9] also Coordination complexes of 4-aminoantipyrine derivatives with transition metal had been widely studies for their anti-cancer properties and antimicrobial [10]. Most of the azo compound and their complexes have a variety of biological, clinical and analytical applications [11]. It is known that chelation of metal ions with organic ligand acts synergistically to increase their biological activities [12]. antipyrine containing azo group have been investigated to have significant biological, antifungal, antibacterial activities and some industrial achiviements[13]Azo compounds are also used in the pharmaceutical industry. Azo compounds show herbicidal, anti-inflammatory, antimicrobial, or antiparasitic activity, antiulcer drug, antifungal, antibacterial, antitubercular, antibiotics [14-15]. Biological importance of azo compounds is well known for

their use as antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [16-17]. The synthesis and properties of transition metal complexes with azo-ligands have been widely investigated owing to their possible applications in a variety of fields. In this respect, an attempt has been made to synthesize and characterize azo bidentate ligand, derived from 4- aminoantipyrene as diazo component, and resorcinol or 2-naphthol as coupling agent. The structural investigation and antimicrobial activity of the synthesized compounds are discussed. In this research we tried the synthesis and characterization of Azo compounds and metal complexes derived from 4-aminoantipyrine then study the biological activity against gram positive and gram negative bacterial species including: *Streptococcus pyogenes*and *Staphylococcus aureus*(Gram Positive Bacteria)and *Escherichia coli , K.lebsiella pneumonia , proteus mirabilis, Salmonella typhi, Acinetobacterbaumannii and Vibrio cholera* (Gram Negative Bacteria).

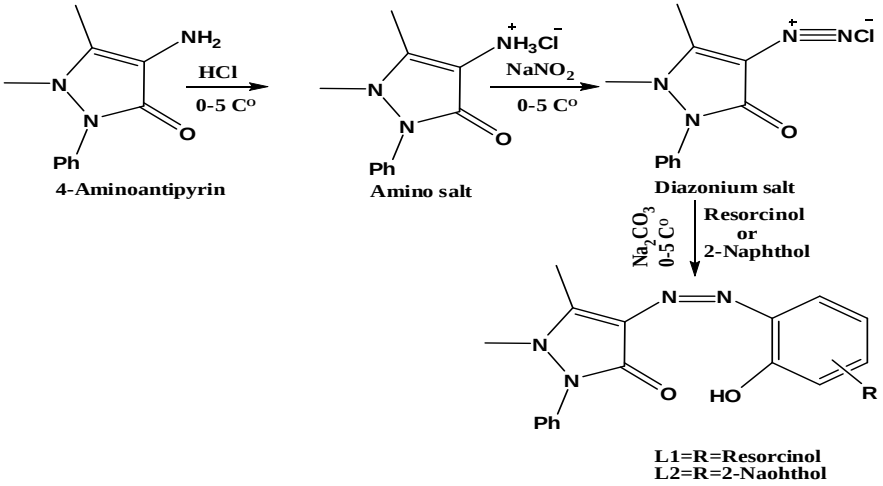
2-Experimental

General

All the reagents and solvents were of reagent-grade quality, Melting points were taken on SMP10 Melting points apparatus, Infrared spectra (in ATR) were recorded on pruker TensorIII FT-IR spectrometer. The electronic spectra of the ligands and complexes were recorded on a UV-1800 Shimadzu spectrophotometer in methanol, Magnetic susceptibility measurements were determined on a Sherwood Scientific Magnetic Susceptibility Balance (Model MK 1) at room temperature. Metal concentrations were determined with a GBC Avanta Atomic Absorption Spectrometer in solution, prepared by decomposition of the complexes with HNO_3 followed by dilution with deionized water.

Synthesis of ligands ((Z)-4-((2,4-dihydroxyphenyl)diazenyl)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one) (L1) and ((Z)-4-((3-hydroxynaphthalen-2-yl)diazenyl)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one) (L2).

The azo dyes reagents (DDPP L1) and (HDDP L2) were synthesized by dissolve of (0.11g,0.001mol) resorcinol or (0.5 g,0.001mol) β-naphtholin 100mL of 2M sodium bicarbonate and cold it (0-5C°) then the resulting solution wasadded slowly to a solution of diazonium chloride was prepared from (0.25g) $NaNO_2$ dissolved in 5 mL distilled water. added with keeping temperature between 0-5 ℃ to (0.255 g, 0.001mol in 10 mL2M hydrochloric acid) of compound (2).The mixture was stirred for 1 h at 0 ℃. Then allowed to warm slowly to room temperature and acidified with 1 mL ofconcentration HCl. It was suction filtered and recrystallized from suitable solvent, then dried in vacuum.



Schem1. Show the mechanism of reaction

Preparation of Metal Complexes (general procedure)

An ethanolic solution of the ligand (0.35g L_1 and 0.32g L_2, 2mmole) was added gradually with stirring to the 0.085g and o.118g (1mmole) of$CuCl_2.2H_2O$ and $NiCl_2.6H_2O$ respectively dissolved in the buffer solution of the required pH. The mixture was cooled until dark color precipitate was formed, filtered and washed several times with (1: 1) water: ethanol then with acetone.

Collection of specimens and bacterial identification:

An eight samples were collected from patients with different infections on May 2016 , these samples including (UTI, stool, burn infections,otitis media, skin infections). Those patients did not receive any antibiotic treatment earlier, the samples were transported immediately to the laboratory,then the samples had been inoculated on

the diagnostic culture media then identified by culture and biochemical differentiation tests . The samples were cloned three successive times nutrient agar and stored on a nutrient agar slant at 4 ̊c[18-19-20]

In Vitro Antibacterial activity testing using Agar well diffusion assay NCCLS: Loop full growths from bacterial isolates were inoculated into nutrient broth incubated at 37°C for 18 hours. The bacterial suspensions were diluted normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5 x 108 CFU/ ml. Sterilized cotton swab was dipped and streak into adjustment suspension the entire Mueller Hinton agar (for all tested bacteria) surface of plates and the plates were left for one (5-15) minutes at room temperature to dry. Media were cut into six wells (5mm diameter) by cork borer and add (20µ) of the test agent dilutions (The plates were performed in triplicates). All plates of the cultered plates were then allowed to incubate at 37°C for overnight. After (24 h) of incubation, the diameters of inhibition zones for all tested agent dilutions for each tested bacteria were measured by using measuring scale in millimeter [21]**.**

Results and Discussion

The azo ligands were prepared by reacting equimolar amounts of 4-aminoantipyrin hydrazonium salt with 2-Naphthol and Resorcinol in aqueous medium. The structures of the ligand and the complexes were established from their UV-Visible spectroscopy, IR, elemental analyses, and magnetic susceptibility measurements. The complexes are intensely colored stable solids, The results of the elemental analysis (Table1) of the Azo compounds are in good agreement with those calculated for the suggested formula and agree with a 1 : 2 metal to ligand stoichiometry for all the complexes.

Table1. Physical properties and elemental analysis of the prepared ligands and its metal complexes.

Compound	Molecular weight g.mol^{-1}	Color	m. p. (C^{o})	Yield	Molar ratio	Calculated (found)			
						C%	H%	N%	M%
DDPP L1	358.39	Red	256	67	-	70.38 (71.11)	5.06 (5.10)	15.63 (15.01)	-
HDDP L2	324.33	Brown	185	69.5	-	62.95 (61.98)	4.97 (5.01)	17.27 (17.11)	-
[Cu(DDPP)]	778.20	Black	224	70.4	1:2	64.81 (64.55)	4.40 (4.50)	14.40 (15.01)	8.16 (7.99)
[Ni(DDPP)]	773.46	Light brown	>300	73.2	1:2	73.46 (72.90)	4.43 (4.40)	14.49 (14.51)	7.59 (7.80)
[Cu(HDDP)]	710.20	Black	240	71.3	1:2	57.50 (57.66)	4.26 (4.10)	15.78 (15.20)	8.95(9.90)
[Ni(HDDP)]	705.35	Dark red	105	69.8	1:2	57.90 (57.89)	4.29 (4.49)	15.89 (15.99)	8.32 (7.99)

Infrared Spectra

The structurally significant FT-IR spectrum bands for free ligand and its complexes have been reported in Table 2. The IR spectra of the free ligands show a broad weak intensity band, due to the intra molecular hydrogen bond centered at around 3374.0 and 3417.5cm^{-1} for L_1 and L_2 respectively[22]The new absorption band appears at 1602.6, 1604.7 cm^{-1}return to –N=N– for DDPP and HDDP respectively, this demonstrated that azo compound was formed. The strong absorptions at 1675.2, 1675.4 cm^{-1}for L_1 and L_2 respectively are typical for C=O moieties respectively, which has been shifted towards lower region at around 1616-1595 cm^{-1} in the spectra of complexes, this may suggest the linkage of metal ion with nitrogen atom of the azo group and carbonyl group [23].

Table2. Show the FT-IR spectral data of the azo-dyes and their complexes(cm^{-1})

Compound	υ (O-H)	υ(C-H)Ar.	υ (N=N)	υ (C=O)	υ (C-O)
DDPP L1	3374.0	3245.5	1602.6	1675.2	1370.0
HDDP L2	3417.5	3205.3	1604.7	1675.4	1324.6
[Cu(DDPP)2]	3448.3	3170.0	1590.2	1662.0	1311.6
[Ni(DDPP)2]	3355.7	3065.5	1520.4	1599.5	1313.4
[Cu(HDDP)2].	-	3070.3	1512.0	1627.6	1285.7
[Ni(HDDP)2].	-	3068.4	1587.3	1611.6	1299.4

In the complexesthe broad band at 3448.3 and 3355.7 $cm^{-1}$for Copper and Nickel complexes with DDPP L1 respectively due to phenolic OH vibrations in para position for resorcinol remained unaltered suggesting the non-involvement of the phenolic proton in the complexes, but there is no band at this region for Copper and Nickel complexes with HDDP L_2, which confirms coordination of metal at OH group[24].
However, the infrared spectra of all the ligands exhibit bands around 1512-1590 $cm^{-1}$and 1324-1370 cm^{-1}corresponding to azo ν(-N=N-) and phenolic ν(C-O) stretching frequencies respectively. On complexation ν (-N=N-) appears at lower frequency in the range 1512–1590 cm^{-1} and this red shift supports the coordination of azo nitrogen to metal ion.
This finding may be due to bonding of the ligand with the metal ions through the enolic deprotonated oxygen. The appearance of the new bands in the ranges 660-620cm^{-1} and 597-457cm^{-1} was taken as on indication of coordination between the metal ions and the oxygen and nitrogen, respectively [25-26].

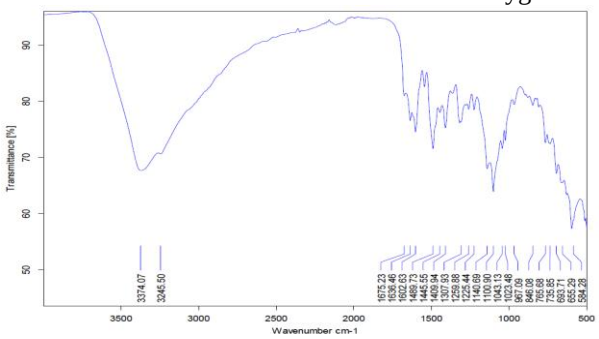


Fig1: Show the FT-IR spectrum to ligand (L_1)

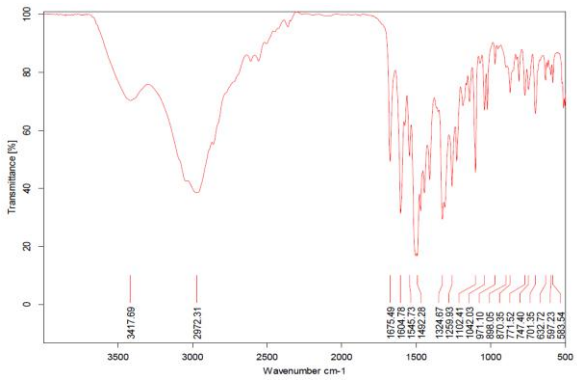


Fig2:Show the FT-IR spectrum to ligand (L)

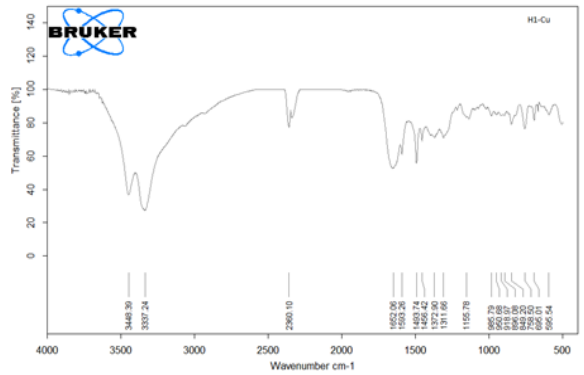


Fig3: Show the FT-IR spectrum to complex of $L_1$with Cu

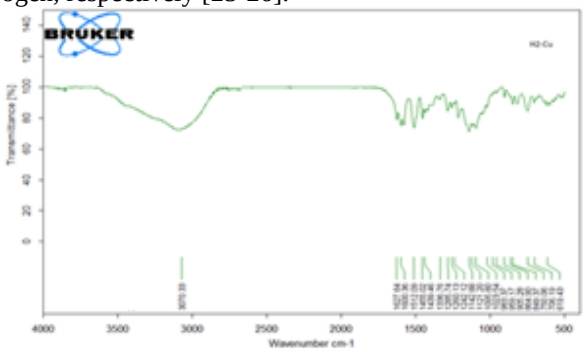


Fig4: Show the FT-IR spectrum to complex of $L_2$with Cu

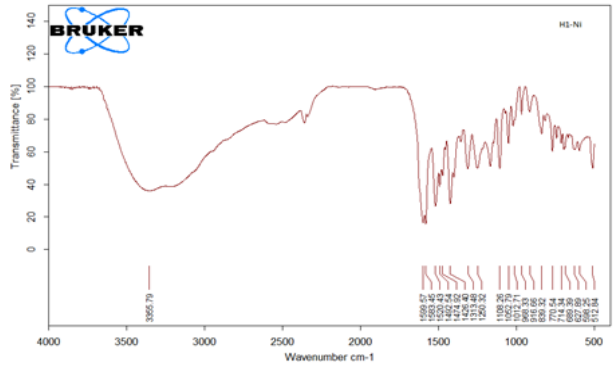


Fig5: Show the FT-IR spectrum to complex of $L_1$with Ni

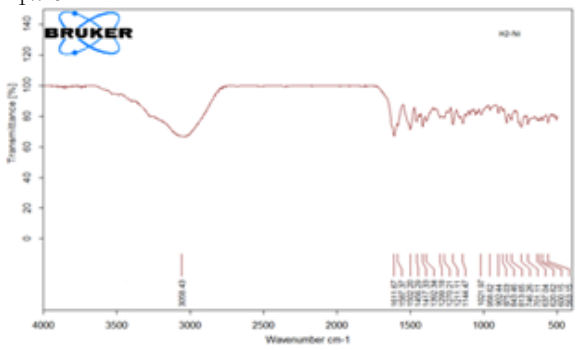


Fig6: Show the FT-IR spectrum to complex of $L_2$with Ni

Electronic Spectra and Magnetic Moments

The Uv- Vis spectra data for the free ligand and all metal complexes have been taken in ethanol . The values of band positions together with the magnetic moment values are listed in Table. The UV-Vis spectra of both two ligands showed bands at 376 **,** 413 and 410**,** 436 nm for L_1 and L_2 respectivly assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions within the molecule, these inner ligand transitions are common due to the presence of (C=O), (N=N) and (C=C) groups in the ligands' structures[27] In the metal complexes, new bands at higher wave numbers

support the formation of strong (M–O) and (M–N) bonds[28] The spectrum of Cu (II) complex, exhibit one broadband at 481 nmand Black color of complex which may be assigned to the C.T. transitions, corresponding to a in distorted octahedral geometry. The electronic spectra coupled with magnetic moment (1.82)BM. indicate octahedral geometric around Cu (II) complex [29-30]. The conductivity measurement shows that the complex is a non-ionic[31].

The Electronic spectrum of Ni(II) complex exhibited three transitions bands at the750,452and 237These bands are assigned due to $^3A_2g \rightarrow {}^3T_2g$ (F), $^3A_2g \rightarrow {}^3T_1g$ (F) and $^3A_2g \rightarrow {}^3T_1g$(P) transitions respectively . These transition suggested octahedral geometry for Ni(II) complex [32-33]. The magnetic moment measurement shows to be of high spin with (2.78 B.M.) and the conductivity measurementshows that it is a non-ionic compound [34-35].

According to the results of Copper and Nickel obtained an octahedral structure has been suggested to these complexes.

Table 3 : Electronic spectra, magnetic moments and molar conductance in DMSO of the prepared metal complexes

Compound	λmax (nm)	υ (cm^{-1})	Electronic transition	Λm($\Omega.cm^{-2}.mole^{-1}$) in DSMO 10^{-3}M	μeff (B.M)	Suggested Molecular formula
DDPP L1	376 , 413	26595 24213	π→π*, n→π*	-	-	-
HDDP L2	410 , 436	24390 26666	π→π*, n→π*	-	-	-
$[Cu(DDPP)_2]$	481 375	20790 26666	2B1g→2A1g (*ν*1) 2B1g→2B2g (*ν*2)	19.20	1.74	Octahedral
$[Ni(DDPP)_2]$	658.5 750 452	11675 13333 22123	3A2g→3T2g(ν1) 3A2g→3T1g(ν2) 3A2g→3T1g(p)(ν3)	21.20	2.78	Octahedral
$[Cu(HDDP)_2]$	510 380	19607 26315	2B1g→2A1g (*ν*1) 2B1g→2B2g (*ν*2)	20.20	1.82	Octahedral
$[Ni(HDDP)_2]$	752.5 733 485	13289 13642 20618	3A2g→3T1g(ν1) 3A2g →3T1g(ν2) 3A2g→3T1g(p)(ν3)	19.40	3.30	Octahedral

Antibacterial activity:

In this research antibacterial bio effects of the ligands and their complexes were tested against eight bacterial samples namely:*Streptococcus pyogenes*and *Staphylococcus aureus*(Gram Positive Bacteria)and *Escherichia coli , K.lebsiella pneumonia , proteus mirabilis, Salmonella typhi, Acinetobacterbaumannii and Vibrio cholera* (Gram Negative Bacteria). by agar well diffusion method using Mueller Hinton agar medium for antibacterial activity. The diameter of inhibition zones were measured and expressed in millimeters (mm).The metal complexes [Cu(DDPP)], [Ni(DDPP)], [Cu(HDPP)],[Ni(HDPP)] were showed higher antibacterial activity than free ligands (DDPPL1),(HPPL2). The biological activity of ligand and its metal complexes were showed in the Table (4). About *V.cholerae*WHO had received notification from the National IHR Focal Point of Iraq of additional laboratory-confirmed cases of cholera. As of 8 October 2015, a total of 1,263 laboratory-confirmed cases of *Vibrio cholerae* O1 Inaba were reported. These cases were reported from at least 15 governorates of the country – These are Babylon (469 cases), Baghdad (304 cases), Qadisiyyah (146 cases), Muthanna (155 cases), Basra (61 cases), Wassit (41 cases), Karbala (33 case), Najaf (32 cases), Thi-qar (6 cases), Maysan (6 cases), Diyala (2 cases), Duhok (2 cases), Erbil (2 cases), Kirkuk (2 cases), Salah al-din (1 case) and Suleimaniyah (1 case) [36]. Later, on 22 November 2015, a total of 2,810 laboratory-confirmed cases of *Vibrio cholerae*01 Inaba had been confirmed at the Central Public Health Laboratory in Baghdad, and only 2 deaths related to cholera were reported. These cases were reported from 17 Governorates of the country, namely Baghdad (940 cases), Babylon (675 cases), Qadisiyyah (442 cases), Muthanna (287 cases), Karbala (157 cases), Basra (102 cases), Wassit (68 cases), Najaf (46 cases), Thyqar (20 cases), Missan (21 cases), Dahuk (16 cases), Kirkuk (19 cases), Erbil (10 cases) Diyala (3 cases), Salaheddine (2 cases) Sulaimanneya (1 case) and Ninewa (1 case) [37]. All of tested compounds exhibited remarkable antibacterial activity against tested bacteria. A comparative study of the antibacterial activity values of the ligand and their

complexesindicate that the metal complexes exhibited higher antibacterial activity compared to the free ligand. In the best of our knowledge, there are very few studies related to investigation the biological activity of Azo compounds on pathogenic bacteria. The antimicrobial studies of the ligand 4-aminoantipyrine and its metal complexes indicate that the metal complexes showed greater antimicrobial activity than the free ligand against the microorganisms such as *S. aurous, E.coli,P. aeruginosa*and *Candida albicance*by disc diffusion method. [38]Appreciate activity was observed as anti-bacterial activities against Gram-positive and Gram-negative bacteria (*E.coli*and *S. aureus*) when azo compound tested using disc diffusion method [39]. In study done performed in the college of science/ Al-Kufa University in 2013 [40]they found that 4-aminoantipyrine(Azo) gave highest activity against *P.aeroginosa* and *E.coli* had lowest sensitivity, while *S.aureus* didn't affected by this compounds. In other research in Egypt [41] a series of copper (II) complexes of azo ligands tested to detect the antimicrobial activity of Azo complexes on some affected bacteria such as (*S. aureus , E. coli* and *K. pneumoniae*). The tested complexes have good antibacterial activity against *S.aureus* and *E.coli*. Our ratherly different from the results of the mentioned studies and that may be due to various factors such as:- Differences in chemical preparation methods of Azo compounds and using diverse metals as ligands may affect the resultsor, the possibility of using different concentrations of the ligands and its complexes in the studying the biological activity. The biological activity of these compounds has been attributed to its scavenging activity against reactive oxygen and nitrogen species (ROS and RNS), as well as to the inhibition of neutrophil's oxidative burst. Indeed, aminopyrinewas demonstrated to be a highly efficient scavenger of the ROS hydroxyl radical (HO_) [42].

Table-4-Antibacterial activity of AZO compounds on Bacterial isolates -Inhibition Zone in (mm) at concentration of ($1x10^{-5}$).

No	Bacteria	1	2	3	4	5	6
1.	*S. aureus*	15	18	18	20	23	25
2.	*Streptococcus spp.*	15	15	17	19	20	20
3.	*E. coli*	15	16	20	25	25	27
4.	*K. pneumonia*	9	9	11	17	17	19
5.	*Proteus spp.*	12	13	14	17	20	20
6.	*S. typhi*	15	18	20	20	21	26
7.	*Acinetobacter spp.*	7	8	8	11	11	12
8.	*V. cholera*	20	20	25	25	25	26

Conclusion

The coordination ability of the synthesized has been proved in complexation reaction with Ni(II)and Cu(II). IR, UV-vis spectra,and magnetic measurements confirmed the octahedral geometry for all synthesized metal complexes. through phenolic carbonyl oxygen, oxygen of OHgroup, and nitrogen of the Azo group as tridentate. The elemental analyses along with metal content were in good agreement with the Predicted structure. Also, the reflectance spectra along with magnetic measument confirm the octahedral geometry for all synthesized metal complexes.The process of chelation dominantly affects the biological activityof the complexes that are potent against pathogens.In general Azo ligands and their complexes had noticeable effect against Gram Positive Bacteria andGram Negative Bacteria . The metal complexes [Cu(DDPP)], [Ni(DDPP)], [Cu(HDPP)],[Ni(HDPP)] were showed higher antibacterial activity than free ligands (DDPPL1),(HPPL2).

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