

Synthesis, Characterization, and In-Vitro Anti-Microbial and Anti-Oxidant Activities of Co^{+2} , Ni^{+2} , Cu^{+2} and Zn^{+2} Complexes of 5-chloro-2-hydroxybenzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazone

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ABSTRACT

New transition metal complexes of a Schiff base (5-chloro-2-hydroxybenzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazone) have been synthesized, characterized and evaluated in-vitro for their anti-microbial and anti-oxidant activities. Modern characterization techniques including CHN, IR, UV-Vis, AAS and SEM have been employed for characterization. The bis Schiff base found to be a tridentate ligand and coordinated to the metal centers through its phenolic oxygen, azomethine nitrogen and carbonyl group. On the basis of spectral evidences and magnetic susceptibility data of complexes, square planar geometry is proposed for Cu^{+2} complex, distorted tetrahedral geometry is proposed for Zn^{+2} while octahedral geometries are proposed for Co^{+2} and Ni^{+2} complexes. Results of antimicrobial studies revealed that the metal complexes are more potent bactericide and fungicide agents. This boost in activity can be explained on the basis of chelation theory suggesting that chelation reduces the polarity of metal ions which increase the lipophilic nature of the complex and hence is able to cross the lipid barrier layer of cell membrane to interfere normal cell functions leading to cell death. On the other hand however, Schiff base ligand showed more DPPH radical scavenging activity compared to the complexes which is attributed to the presence of OH group in the Schiff base.

Keyword: Schiff bases, azomethine derivatives, anti-microbial, anti-oxidant

1. INTRODUCTION

Schiff bases constitute incredible organic compounds and reported to have a range of biological activities which is mainly attributed to the presence of the electron rich azomethine group in their structures¹⁻⁴. The effect of coordination on the activity of Schiff bases has been studied extensively and it is reported that coordination with the metal centers alters the electron density around the azomethine moiety which in turn vary the biological response, many workers have reported such metal complexes with bi-dentate, tri-dentate and, tetra-dentate Schiff base ligands²⁻⁶ to study the coordination behavior of these compounds that is found to be dependent on pH of medium, types and nature of substituents and the position of hydrazone group in their structures⁷.

Literature review on Schiff bases revealed that metal complexes of Schiff bases have been well studied but metal complexes of bis Schiff bases have been studied noticeably less so far and this work is a continuation of our previous research² to study $M+2$ complexes of these Schiff bases. Schiff bases and their derivatives are extensively reported for antimicrobial and antioxidant potential besides other bioactivities. M. Saranagapani et al. have reported a series of “ β -isatin aldehyde-N,N – thiocarbohydrazone” derivatives with highly significant antimicrobial activities against gram positive and gram negative bacteria including *B. Subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. These compounds were also reported to be active against fungi including *C. albicans*, *P. notatum* and *A. niger*⁸. Studies on antioxidant potentials of Schiff bases and their derivative compounds revealed a number of examples available in literature, [(3-bromobenzylidene)-amino] phenol, (E)-4-(3,4-dihydroxybenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one and (E)-N'-(4-nitrobenzylidene)-4-chlorobenzohydrazide have been reported by various workers as potent antioxidant agents⁹⁻¹¹.

In current study an attempt is being made to synthesize Co^{+2} , Ni^{+2} , Cu^{+2} and Zn^{+2} with 5-chloro-2-hydroxybenzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazine. Newly synthesized complexes were evaluated in vitro for their anti-microbial and anti-oxidant activities by reported methods.

2. MATERIAL & METHODS

Synthesis, characterization of all compounds were performed according to the reported methods². The bis Schiff base ligands and their metal complexes were screen for their anti-bacterial and anti-fungal activities using agar and potato dextrose agar diffusion methods respectively. All compounds were taken from 100, 200 and 400 $\mu\text{g}/\text{mL}$ in DMSO and tested against three bacteria (*B. subtilis*, *E. coli*, and *S. aureus*) and two fungi (*A. niger* and *T. viride*) by MIC method¹². Bacterial strains were incubated for 24 hours at 37°C and fungi strains were incubated for 48 hours at 37°C. “Streptomycin” was used as standard for anti-bacterial assay while “Nystatin” was used as standard for anti-fungal assay under similar conditions. Activity was determined by measuring the diameter of zone of inhibition in millimeter.

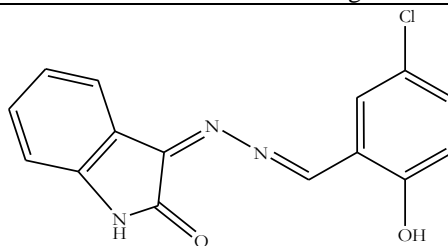
3. RESULTS & DISCUSSION

3.1 Characterization of Schiff Base Ligand

Analytical and spectroscopic characterization provide sufficient evidence about the Schiff base to be a tri-dentate ligand with three potential electron rich binding sites at phenolic oxygen, azomethine nitrogen and carbonyl groups. The ligand compound was stable at room temperature, non-hygroscopic, insoluble in water but soluble in DMSO. **Table-3.1.1** summarizes the physical characterization of the ligand.

Table-3.1.1: Physical & Analytical Data of Schiff Base Ligand

Structure of Schiff Base Ligand

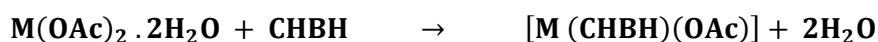


5-chloro-2-hydroxybenzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazine

Yield	85%
Color	Orange
EI MS: m/z (rel. abund.%)	299 (M ⁺ , 44)
Molecular Formula	C ₁₅ H ₁₀ ClN ₃ O ₂
Elemental Analysis	C = 60.11 (59.61), H = 03.36 (03.64), N = 14.02 (13.83)

3.2 Characterization of Complexes

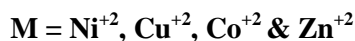
Elemental analysis of complexes indicated the 1:1 metal-ligand ratios for Cu⁺² and Zn⁺² complexes while 1:2 metal-ligand ratios for Co⁺² and Ni⁺² complexes. General equation of formation of the Cu⁺² and Zn⁺² complexes can be written as:



While the formation of the complexes of Co⁺² and Ni⁺² can be presented by the following general equation.



Where:



CHBH = 5-chloro-2-hydroxybenzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazine

The attempts to grow larger crystals for complexes did not work thus SEM analysis were performed. It was revealed that all the complexes have micro-crystalline structures that are very clear and have sharp edges. **Table-3.2.1** to **Table-3.2.4** illustrates the physical characterization data of complexes.

Table-3.2.1: Physical & Analytical Data of Ni⁺² Complex

Structure	SEM Image
Yield	75%
Color	Green
Molecular Formula	NiC ₃₀ H ₂₂ N ₆ O ₄ Cl ₂
Formula Mass	660.13 g.mol ⁻¹
Elemental Analysis	Ni = 08.99 (08.81), C = 54.58 (53.43), H = 03.36 (03.21) N = 12.73 (12.78)
Molar Conductance	4.5 ohm ⁻¹ .cm ² .mol ⁻¹
Magnetic Moment	3.88 BM

Table-3.2.2: Physical & Analytical Data of Co⁺² Complex

Structure	SEM Image
Yield	69%
Color	Pink
Molecular Formula	CoC ₃₀ H ₂₂ N ₆ O ₄ Cl ₂
Formula Mass	660.37 g.mol ⁻¹
Elemental Analysis	Co = 08.92 (08.90), C = 54.56 (54.48), H = 03.36 (03.30) N = 12.73 (12.67)
Molar Conductance	7.4 ohm ⁻¹ .cm ² .mol ⁻¹
Magnetic Moment	4.22 BM

Table-3.2.3: Physical & Analytical Data of Cu⁺² Complex

Structure	SEM Image
Yield	76%
Color	Dark Brown
Molecular Formula	CuC ₁₇ H ₁₄ N ₃ O ₄ Cl
Formula Mass	423.31 g.mol ⁻¹
Elemental Analysis	Cu = 15.01 (14.87), C = 48.23 (48.06), H = 03.33 (03.10) N = 09.93 (09.75)
Molar Conductance	5.3 ohm ⁻¹ .cm ² .mol ⁻¹
Magnetic Moment	2.00 BM

Table-3.2.4 – Physical & Analytical Data of Zn⁺² Complex

Structure	SEM Image
Yield	65%
Color	Red
Molecular Formula	ZnC ₁₇ H ₁₄ N ₃ O ₄ Cl
Formula Mass	425.15 g.mol ⁻¹
Elemental Analysis	Zn = 15.38 (15.29), C = 48.03 (47.91), H = 03.32 (03.18) N = 09.88 (09.47)
Molar Conductance	6.6 ohm ⁻¹ .cm ² .mol ⁻¹
Magnetic Moment	D

3.3 Electronic & Magnetic Data of Complexes

UV-Vis. spectrum of Co^{+2} complex showed absorptions at 391nm, 518nm, and 848 nm which are assignable to ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}$, ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}$ and ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{1g}$ transitions respectively. Similarly the UV-Vis. spectrum of Ni^{+2} complex showed absorption at 389nm, 645nm and 871nm which can be attributed to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$, ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}$ and ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}$ transitions respectively. UV-Vis. spectrum of Cu^{+2} complex showed absorptions at 377nm and 729nm and can be assigned to ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ and ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_{1g}$ transitions, while Zn^{+2} only showed one absorption at 355 nm. Zn^{+2} complex was found to be diamagnetic in nature. Figures 1 – 4 shows the UV-Vis spectra of all four complexes.

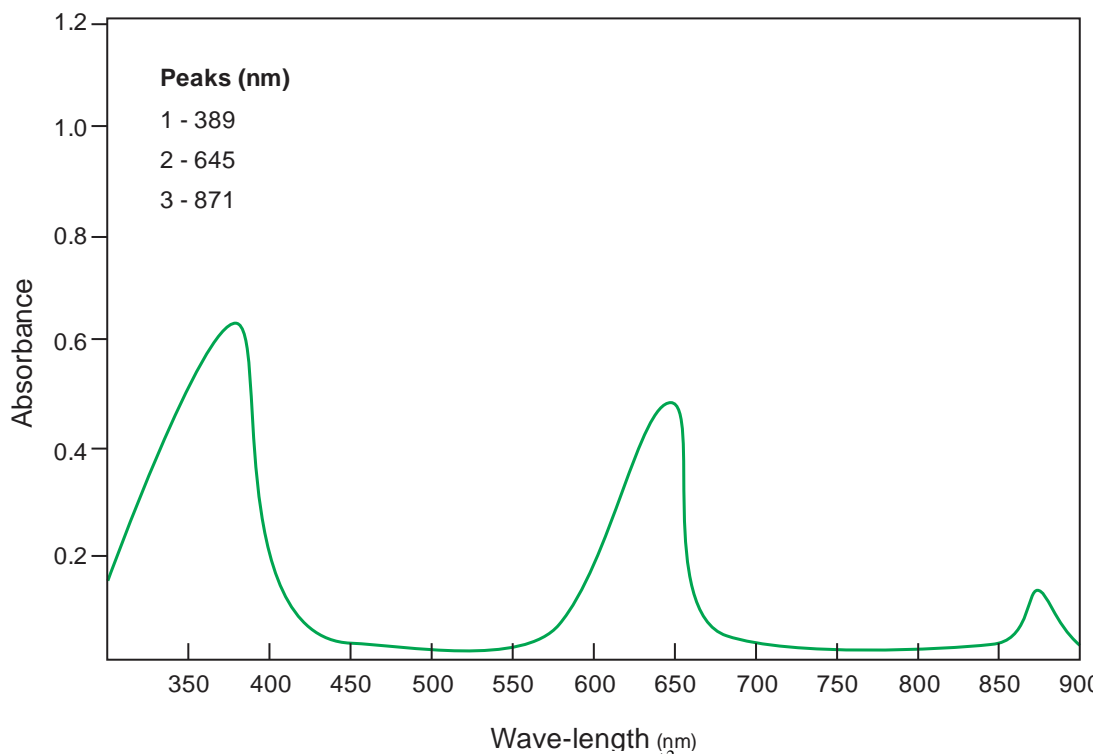


Fig.1: UV-Vis. Spectrum of Ni^{+2} Complex

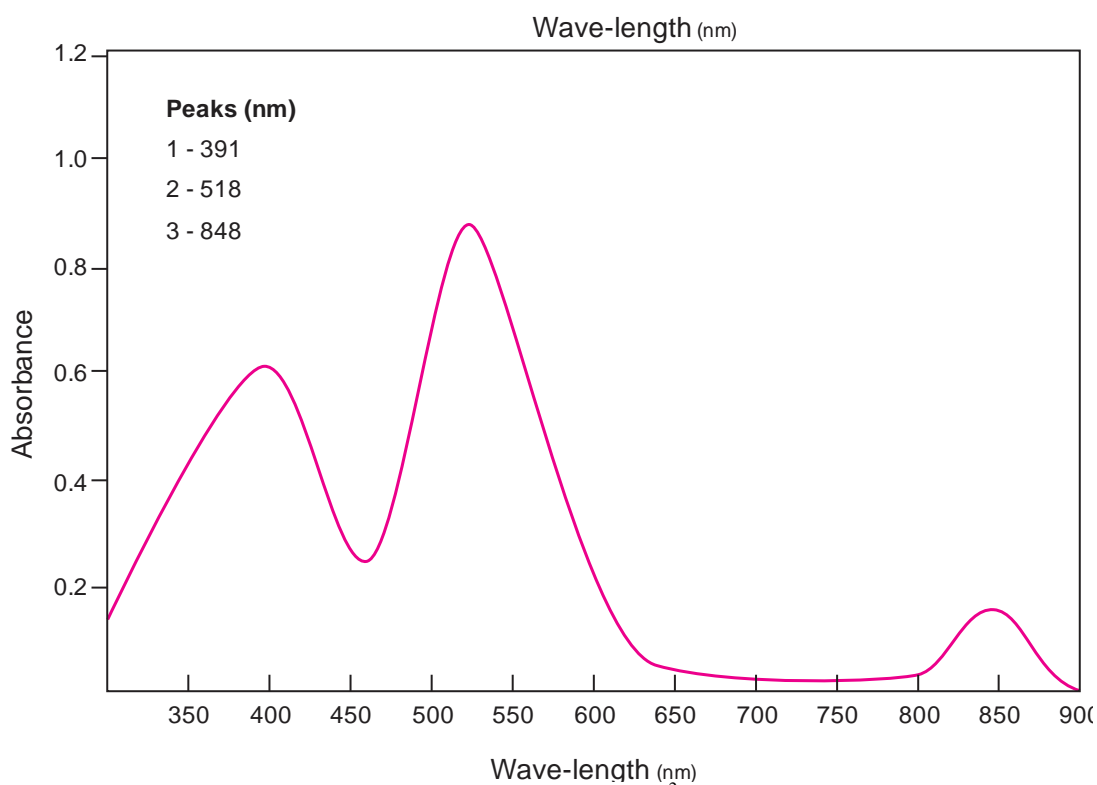
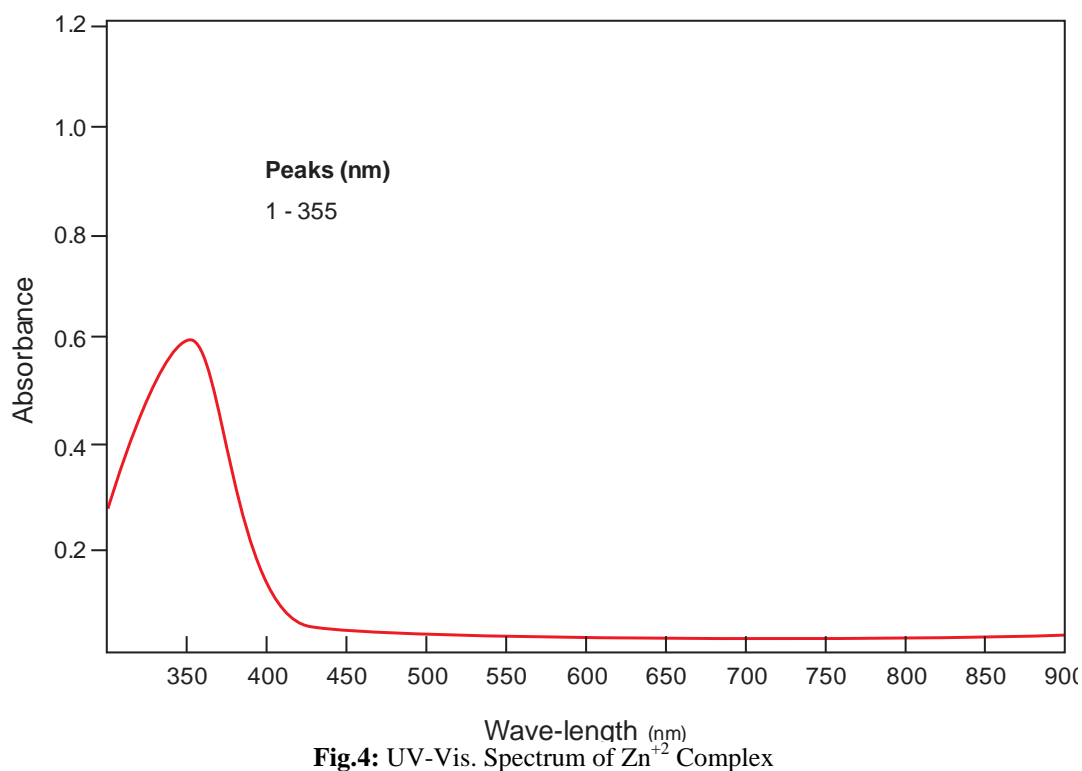
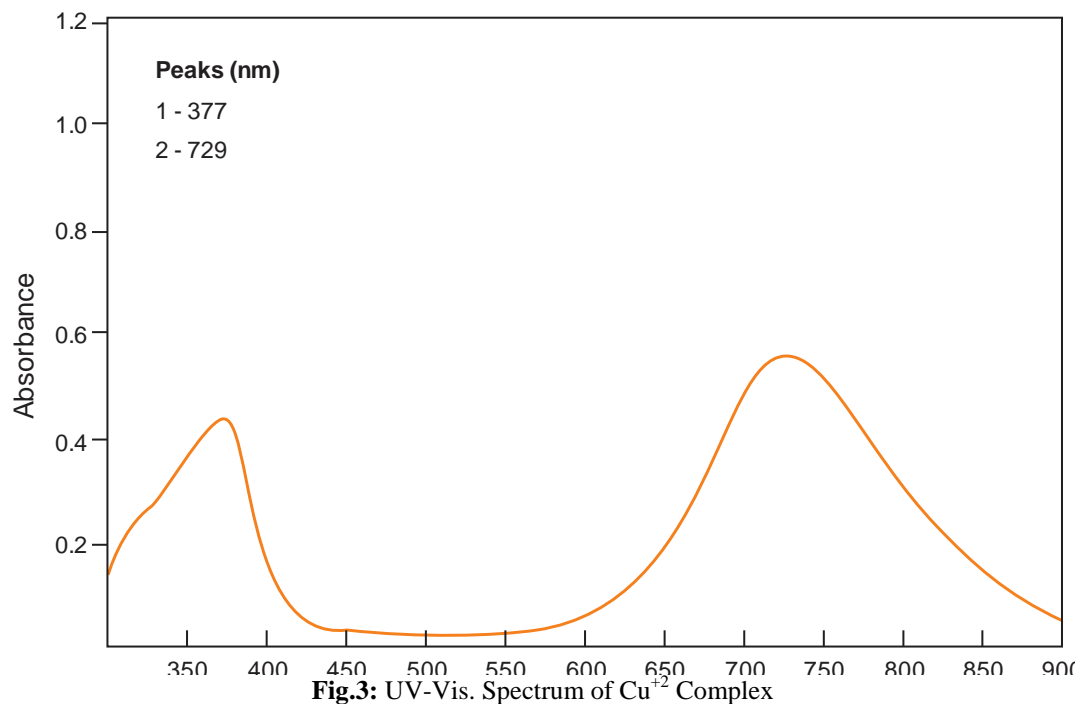


Fig.2: UV-Vis. Spectrum of Co^{+2} Complex



This data together with magnetic moment adequately support the octahedral geometry around Co⁺² and Ni⁺² complexes while tetrahedral geometry for Cu⁺² and Zn⁺² complexes. **Table-3.3.1** lists the electronic absorptions data of all four complexes.

Table-3.3.1: Electronic Data of complexes

Complexes	Absorption Maxima cm ⁻¹ (nm)
[Co(CHBH) ₂]	25546 (391), 19299 (518), 11781 (848)
[Ni(CHBH) ₂]	25468 (389), 15498 (645), 11477 (871)
[Cu(CHBH)(OAc)]	26477 (377), 13711 (729)
[Zn(CHBH)(OAc)]	28104 (355)

3.4 Infrared Spectral Data of Complexes

The broad band of hydrogen bonded OH group disappeared in the infrared spectra of metal complexes which is an indication of the deprotonating and the formation of C–O bond. A noticeable increase in stretching frequency of C–O

bond in all complexes is a distinct indication of the coordination of phenolic oxygen to the metal center. An evident decrease in stretching frequencies of C=N bond is observed in the IR spectra of all complexes which justified the involvement of aldimine nitrogen in the coordination of the ligands.

The bands corresponds to C=O considerably shifted to lower frequencies in the spectra of metal complexes providing evidence for coordination by carbonyl oxygen. The vibration characteristics of the ring (NH and C=N) however, remained unaltered which indicates that these groups did not participate in the coordination. Major IR peaks of complexes are tabulated along with the Schiff base ligand in a comparative fashion in **Table-3.4.1**.

Table-3.4.1: Major infrared absorption of ligand & complexes

CHBH	[Co(CHBH) ₂]	[Ni(CHBH) ₂]	[Cu(CHBH)(OAc)]	[Zn(CHBH)(OAc)]	Assignments
3282	---	---	---	---	phenolic ν (OH)
3286	3274	3271	3269	3267	indol ν (NH)
1715	1702	1694	1704	1699	carbonyl ν (C=O)
1650	1681	1685	1684	1686	ketimine ν (C=N)
1619	1581	1585	1579	1588	aldimine ν (HC=N)
1392	1309	1311	1311	1309	phenolic ν (C-O)
1065	1041	1043	1049	1050	hydrazinic ν (N-N)
873	841	822	832	840	chloro ν (C-Cl)
---	468	446	451	439	ν (M-N)
---	518	535	526	528	ν (M-O)

3.5 Biological Evaluation

Antimicrobial evaluation of ligand and complexes showed the same trend as was observed as in previous study i.e. the metal complexes were found to be slightly more potent antimicrobial agents than the corresponding ligands. This increased activity can be attributed to the reduced polarity of the metal upon complexation. As the polar nature of the complexes decreases, their permeation abilities through the cell membrane increases attributed to the lipophilicity and hence the binding of the metal center to other major constituents of the cell membrane including aminophospholipids and cystienyl ligands is favored. These candidate ligands competes to get bound to the less polar metal center leading to cause drastic impacts on the normal cell functions and eventually inhibit microbial growth.

Antibacterial and anti-fungal activity data for ligand and its complexes is presented in the **Table-3.5.1**.

Table-3.5.1 – Anti-microbial activity data of ligand & complexes

Compound	Anti-Microbial Zones of Inhibition (mm)								
	<i>B. subtilis</i>			<i>E. coli.</i>			<i>A. niger</i>		
	100 μM	200 μM	400 μM	100 μM	200 μM	400 μM	100 μM	200 μM	400 μM
CHBH	10	9	11	8	9	11	6	10	12
[Co (CHBH) ₂]	11	13	16	8	16	15	7	17	18
[Ni (CHBH) ₂]	13	11	23	11	18	17	7	14	15
[Cu (CHBH)(OAc)]	12	16	21	10	13	13	6	12	16
[Zn (CHBH)(OAc)]	10	15	22	12	16	17	8	13	19
<i>Streptomycin</i>	18	22	34	16	22	22	11	22	30

Table-3.5.2: Anti-oxidant activity data of ligand & complexes

Compound	IC ₅₀	% Inhibition
	μM	100 μM
CHBH	186	21.34 ± 0.24
[Co (CHBH) ₂]	N.A	N.A
[Ni (CHBH) ₂]	276	7.61 ± 0.21
[Cu (CHBH)(OAc)]	N.A	N.A
[Zn CHBH)(OAc)]	N.A	N.A
<i>BHA</i>	51	89.67 ± 0.14

*N.A = not active

Similarly for antioxidant activity, the ligand was found to be a better free radical scavenger due to the presence of OH group in its structure while upon complexation, deprotonation occurred facilitating C–O bond formation for coordination with the ligand rendering the resultant complex relatively less active (or inactive) radical scavenger than the ligand. Comparative anti-oxidant activity data for ligand and its complexes is presented in the **Table-3.5.2**.

4. CONCLUSION

This work was a continuation of a previous research and another Schiff base ligand and its metal complexes were prepared and tested for anti-microbial and anti-oxidant activities. The same trend was observed for the mode of coordination of the ligand. For the anti-microbial evaluations, the metal complexes were found more potent while for anti-oxidant activity, the ligands showed more activity than the metal complexes.

5. ACKNOWLEDGMENT

This research work was supported by the Higher Education Commission of Pakistan under the national research grants program for universities (Grant No. 1463/R&D/09).

6. REFERENCES

1. Khan, K. M., et al., *Synthesis of bis-Schiff bases of isatins and their antiglycation activity*. Bioorganic & Medicinal Chemistry, (2009), **17**(22): p. 7795-7801, <http://dx.doi.org/10.1016/j.bmc.2009.09.028>.
2. Khan, Z., et al., *Synthesis, Characterization, In-Vitro Antimicrobial and Antioxidant Activities of Co+ 2, Ni+ 2, Cu+ 2 and Zn+ 2 Complexes of 3-(2-(2-hydroxy-3-methoxybenzylidene) hydrazono) indolin-2-one*. Journal of Basic and Applied Sciences, (2015), **11**: p. 125-130, <http://dx.doi.org/10.6000/1927-5129.2015.11.17>.
3. Munde, A. S., et al., *Synthesis, characterization and thermal study of some transition metal complexes of an asymmetrical tetradentate Schiff base ligand*. Journal of the Serbian Chemical Society, (2010), **75**(3): p. 349-359, <http://dx.doi.org/10.2298/JSC090408009M>.
4. Papish, E. T., et al., *Synthesis of zinc, copper, nickel, cobalt, and iron complexes using Tris (pyrazolyl) methane sulfonate ligands: a structural model for N, N, O binding in metalloenzymes*. Inorganic chemistry, (2006), **45**(5): p. 2242-2250, <http://dx.doi.org/10.1021/ic051579a>.
5. Patel, N. H., Parekh, H. M. and Patel, M. N. *Synthesis, characterization and biological evaluation of manganese (II), cobalt (II), nickel (II), copper (II), and cadmium (II) complexes with monobasic (NO) and neutral (NN) Schiff bases*. Transition metal chemistry, (2005), **30**(1): p. 13-17.
6. Raman, N., et al., *Redox and antimicrobial studies of transition metal (II) tetradentate Schiff base complexes*. Transition metal chemistry, (2003), **28**(1): p. 29-36.
7. Gudasi, K. B., Patil, M. S. and R.S. Vadavi, *Synthesis, characterization of copper (II), cobalt (II), nickel (II), zinc (II) and cadmium (II) complexes of [7-hydroxy-4-methyl-8-coumarinyl] glycine and a comparative study of their microbial activities*. European journal of medicinal chemistry, (2008), **43**(11): p. 2436-2441, <http://dx.doi.org/10.1016/j.ejmech.2008.01.028>.
8. Kiran, G., et al., *Microwave-assisted synthesis, characterization, antimicrobial and antioxidant activity of some new isatin derivatives*. Journal of Chemistry, (2012), **2013**.
9. Bala, S., et al., *Design, characterization, computational studies, and pharmacological evaluation of substituted-N'-(1E) substituted-phenylmethylidene] benzohydrazide analogs*. Medicinal Chemistry Research, (2013), **22**(6): p. 2755-2767, <http://dx.doi.org/10.1007/s00044-012-0270-0>.
10. Alam, M. S., Choi, J.-H. and Lee, D.-U., *Synthesis of novel Schiff base analogues of 4-amino-1, 5-dimethyl-2-phenylpyrazol-3-one and their evaluation for antioxidant and anti-inflammatory activity*. Bioorganic & medicinal chemistry, (2012), 2013: p. 4103-4108, <http://dx.doi.org/10.1016/j.bmc.2012.04.058>.
11. Aslam, M., *A ONE-POT SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATIONS: SCHIFF BASES*. International Journal of Pharmaceutical Chemistry, (2012), **2**(3): p. 73-77, <http://dx.doi.org/10.7439/ijpc.v2i3.608>.
12. Sadana, A. K., et al., *Hypervalent iodine mediated synthesis of 1-aryl/hetryl-1, 2, 4-triazolo [4, 3-a] pyridines and 1-aryl/hetryl 5-methyl-1, 2, 4-triazolo [4, 3-a] quinolines as antibacterial agents*. European journal of medicinal chemistry, (2003), **38**(5): p. 533-536, [http://dx.doi.org/10.1016/S0223-5234\(03\)00061-8](http://dx.doi.org/10.1016/S0223-5234(03)00061-8).