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In Vitro Antioxidant Properties of Novel Schiff Base Complexes

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Authors' contributions

This work was carried out in collaboration between all authors. Author MI guided the research and the research paper was prepared under his supervision. Author AK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MI and SR worked on drugs synthesis. Authors MS, HUN and AAA worked on literature search and review. Author AAA reviewed the study and approved the final manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: In order to identify and evaluate the metal-derived synthetic antioxidants having high free radicals scavenging capacity related to various disorders has received much interest in last decades. Recently, synthetic antioxidants are widely used instead of natural antioxidants because they are effective and cheaper.

Study Design: In the current investigation, a synthesized Schiff base ligand (2-{(*E*)-[(4-chlorophenyl)imino]methyl}phenol) and their metal complexes (Bis(2-{(*E*)[(4chlorophenyl) imino]methyl} phenol)nickel(II), Bis(2-{(*E*)[(4chlorophenyl)imino]methyl}phenol)Cobalt(II), Bis(2-{(*E*)[(4chlorophenyl)imino]methyl}phenol)copper(II), Bis(2-{(*E*)-[(4-chlorophenyl)imino]methyl}phenol) zinc(II)) have been evaluated *in vitro* for their possible antioxidant properties.

Place and Duration of Study: Department of chemistry, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan. The experiment lasted for three days (72 hours).

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Methodology: The antioxidant activity of the synthesized compounds were screened by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous ion chelation (FIC), Ferric reducing antioxidant power (FRAP), Total antioxidant activity (Phosphomolybdenum methods) and Hydroxyl radical ($^{\bullet}OH$) radical scavenging activity.
Results: The synthesized compounds showed significant dose dependent antioxidant activities comparable with that of the classical antioxidants, ascorbic acid and ethylenediaminetetraacetic acid (EDTA).
Conclusions: The compounds exhibited are very reactive towards DPPH radicals, OH radicals, and Fe^{+2} ions and it also actively reduces Fe(III) ion to Fe(II) and Mo(VI) ion to Mo(V) form. The ligand having moderate and all of the metal complexes were existed encouraging results which indicate the importance of Schiff base ligand a metal complexes as a source of synthetic antioxidants and possibly potent drugs.

Keywords: Free radicals; DPPH; antioxidants; ligand; Schiff base metal complexes.

1. INTRODUCTION

Free radicals particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) have a greater impact on humans both within the body and from the environment. In living system, during endogenous stimulation of macrophages and leucocytes, aerobic respiration and other metabolic processes ≥ 5% of oxygen reduced univalently to get free radicals endogenously. While the tobacco smokes, pollutants, ionizing radiations, organic solvents and pesticides are the major exogenous sources of free radicals production [1-3]. It is now universally accepted that free radicals have a great impact on humans in the etiology of various diseases like cancer, liver injury, cardiovascular diseases [4], diabetes, neurodegenerative and rheumatism diseases [5] atherosclerosis [6] autoimmune disorders and aging [7]. Although, the body possesses defense mechanisms as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS [8-9], continuous exposure to contaminants and chemicals may increase the amount of free radicals in the body beyond its ability to control and cause irreversible oxidative damages [10].

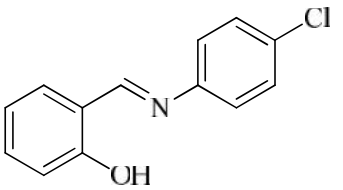
Therefore, antioxidants with free radical scavenging potential may be relevant in the therapeutic and preventions of diseases where free radicals are implicated [11]. In addition to natural antioxidants such as Vitamin E, Vitamin C, flavonoids and carotenoids [12]. Metal complexes of Schiff bases have been extensively investigated because of their industrial, antifungal, antibacterial, anticancer and herbicidal applications [13-15]. The study of Schiff bases ligands and their metal complexes is by far too large to be fully reviewed [16]. Yildiz et al. [17] summarized that Schiff bases have been used as chelating ligands in coordination chemistry, in anti-oxidative activity, anti-bacterial activity, catalysis, medicine as anti-inflammatory, antibiotics, and in industry for anti-corrosion properties.

The objective of the present study was to investigate the antioxidant activity of Schiff base ligand and their metal complexes, compared with that of the classical antioxidants, Vitamin C, for scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous ion chelation (FIC), Ferric reducing antioxidant power (FRAP), Total antioxidant activities (Phosphomolybdenum methods) and Hydroxyl radical ($^{\bullet}OH$) radical scavenging activities *in vitro.*

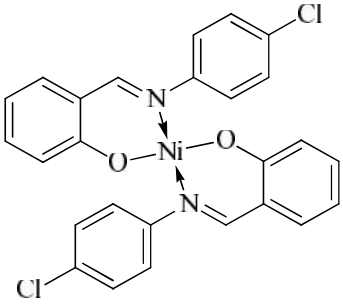
2. MATERIALS AND METHODS

2.1 Chemicals

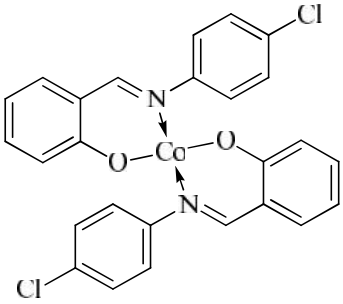
Schiff base ligand and their Nickel (II), Cobalt (II), Copper (II), Zinc (II), metals complexes (Fig. 1) were synthesized according to literature methods [18] and was dissolved in ethanol. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that the compound obtained (with 99.9% purity) presented analytical and spectroscopic data in full agreement with their assigned structure. All other chemicals, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), Ascorbic acid, ferrous sulphate, ethylene diaminetetraacetic acid (EDTA), ferric chloride ($FeCl_3$), Tris HCl buffer, O-phenenthroline, sulfuric acid, Potassium phosphate (mono phosphate and diphosphate), ammonium molybdate, Hydrogen peroxide (H_2O_2), ethanol were analytical grade purchased from Sigma Aldrich Pakistan.



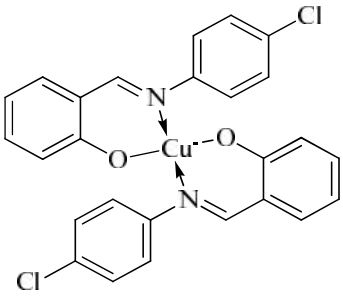
2-{(E)-[(4-chlorophenyl)imino]methyl}phenol



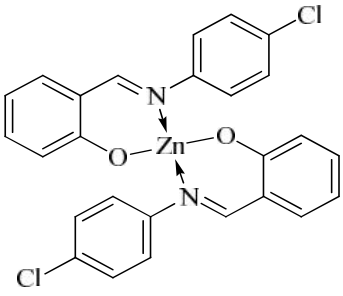
Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)nickel(II)



Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)Cobalt(II)



Bis(2-{(E)[(4chlorophenyl)imino]methyl}phenol)copper(II)



Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)zinc(II)

Fig. 1. Chemical structure of Schiff base ligand and Nickel (II), Cobalt (II), Copper (II), Zinc (II) metals complexes

2.2 Determination of Anti-oxidant Activities

2.2.1 DPPH radical scavenging assay

2.2.1.1 Principle

The free radical scavenging activity of the various concentrations of Schiff bases ligand and their metal complexes was measured *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay described by [19]. DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) with purple color is a stable free radical. On scavenging, with antioxidants (AH) the purple color of DPPH$^{\bullet}$ reduce to yellow (DPPHH) (Fig. 2) is the basic principle utilized in this assay [20].

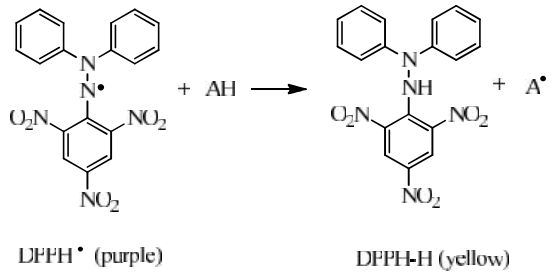


Fig. 2. Structures of DPPH radical and DPPHH

2.2.1.2 Procedure

Various concentrations (25, 50,100, 200 and 400 *µ*M) of Schiff base ligand and their metal complexes were mixed with an ethanolic solution containing 85 *µ*M DPPH radical. The mixtures were incubated for 30 minutes at room temperature and the decrease in absorbance was measured at 518 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations of drugs was used as a positive control. Percentage inhibition of the drugs as well as ascorbic acid was calculated by using the following formula:

$$DPPH\ Inhibition\ effect(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where

A_c = Absorbance reading of the control
A_s = Absorbance reading of the sample

2.2.2 Ferrous ion-chelating assay

2.2.2.1 Principle

In order to examine iron chelating properties of the Schiff base ligand and their metal complexes, O-phenantroline color method was used according to [20]. The formation of O-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents is the common principle has been utilized in this assay.

2.2.2.2 Procedure

Various concentrations (25, 50, 100, 200 and 400 *µ*M) of Schiff base ligand and their metal complexes were with0.2 mL of 3.6 mMferrous sulphate, 0.3 mL of 100 mM Tris-HCl (pH=7.4), 0.1 mL of 9 mM O-Phenanthroline and diluted up to 3.0 mL with ultra-puredistilled water. The reaction mixture was shaken vigorously, incubated for 10 minutes and the decrease in absorbance was determined at 510 nm. EDTA (ethylenediaminetetraacetic acid) at the same concentrations utilized as a reference standard and without Schiff bases complexes sample mixture as control. The Fe^{2+} chelating capacity was calculated by using the following formula:

$$Chelatingeffect(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where

A_c = Absorbance reading of the control
A_s = Absorbance reading of the sample

2.2.3 Ferricreducing/antioxidant power assay

2.2.3.1 Principle

Ferric ions reducing antioxidant potential of the Schiff base ligand and their metal complexes were investigated by O-phenanthroline color method previously described by [21]. The transformation of ortho-phenanthroline-Fe^{3+} to ortho-phenanthroline-Fe^{2+} by accepting of an electron from an antioxidant is the basic principle utilized in this assay.

2.2.3.2 Procedure

The reaction mixture containing various concentrations (25, 50, 100, 200 and 400 *µ*M) of Schiff base ligand and their metal complexes, 0.2 mL of 3.6 mM ferric chloride, 0.3 mL of 100 mM tris buffer (pH=7.4), 0.1 mL of 9 mM O-phenanthroline and diluted up to 3.0 mL with ultra-puredistilled water was shaken vigorously and left to stand at room temperature for 10 min. The increase in absorbance of the sample solution was measured at 510 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations was utilized as a reference

standard and without compounds sample mixture as control. The Reducing Power comparable with Ascorbic acid was calculated by using the following formula:

$$Reducing\ Power(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where

A_s = Absorbance reading of the sample
A_c = Absorbance reading of the control

2.2.4 Total antioxidant capacity (Phospho-molybdenum assay)

2.2.4.1 Principle

The total antioxidant potential of the Schiff base ligand and their metal complexes will be evaluated by using the phosphomolybdenum method as previously described by [22]. The total antioxidant capacity is based on the reduction of Mo(VI) to Mo(V) by accepting of an electron from antioxidant at acidic pH.

2.2.4.2 Procedure

Various concentrations (25, 50, 100, 200 and 400 *µ*M) of Schiff base ligand and their metal complexes aliquot in ethanol were mixed with 3.0 mL of reagent solution containg 0.7 mL of 0.6 M sulphuric acid, 1.0 mL of 28 mM pottacium pasphate,1.0 mM ammonium molybdate and distilled water. The reagent mixture was incubated at 95°C for 90 minutes and cooled to room temperature. The increase in absorbance of the mixture is measured at 695 nm using an UV spectrophotometer. Ascorbic acid was utilized as reference standard and without compounds sample mixture as control. The Reducing Power of drugs as well as ascorbic acid was calculated by using the following farmula:

$$Reducing\ Power(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where

A_s = Absorbance reading of the sample
A_c = Absorbance reading of the control

2.2.5 Hydroxyl radical scavenging activity

2.2.5.1 Principle

The hydroxyl radical scavenging activity of Schiff base ligand and their metal complexes was determined according to the method of [23]. The Fenton reaction is the basic principle for hydroxyl radical scavenging activity.

2.2.5.2 Procedure

Various concentrations (25, 50, 100, 200 and 400 *µ*M) of Schiff base ligand and their metal complexes, 0.1 mL of 7.5 mM O-phenanthroline, 0.5 mL of 0.2 M phosphate buffer (pH 6.6), 0.1 mL of 7.5 mM ferrous sulfate and 0.1 mL of H_2O_2(0.1%) and diluted up to 3 mL with distilled water. The reaction mixture incubated at room temperature for 30 minutes and the absorbance was measured at 510 nm using an UV spectrophotometer. The reaction mixture without Schiff bases complexes has been used as control and without Schiff bases complexes and H_2O_2 as a blank. The percentade scavanging power of Schiff base complexes and ascorbic acid were calculated by using the followingfurmula:

$$Scavanging\ Power(\%) = \frac{A_S - A_C}{A_b - A_C} \times 100$$

Where

A_s = Absorbance reading of the sample
A_c = Absorbance reading of the control
A_b = Absorbance reading of the blank

2.3 Statistical Analysis

Linear regression analysis was used to calculate IC_5±SEM values from data and graphs by using Graph pad prism 6. Significant differences among the means of data were tested by the one-way ANOVA followed by the student´s t-test with significance level ($P<0.01$). All the tests were conducted in triplicate.

3. RESULTS

3.1 DPPH Radical Scavenging Assay

In the present study, the Schiff base ligand and their metals (Ni(II), Co(II), Cu(II), and Zn(II)) complexes were investigated in comparison with the known antioxidant ascorbic acid. From the investigation it was clearly observed that Schiff base ligand and their metals complexes scavenge DPPH significantly ($p<0.01$) (Fig. 3). A considerable increase in the percent of scavenging activity is found with increase in

concentration of the compounds. The IC_{50} values for DPPH radicals with ethanolic solution of ligand and their metals complexes were found to be 557.51 ±6.067 µM, 318.03 ±9.875 µM, 302.39 ±10.68 µM, 275.40 ±11.70 µM and 326.53 ±10.17 µM respectively (Table.1). The free ligand is found less efficient in decolorizing the pink color of the DPPH solution than its their metals (Ni(II), Co(II), Cu(II), and Zn(II)) complexes. While the Cu (II) complex has shown greater scavenging activity among the tested complexes (IC_{50}=228.61 ±13.83µM). (Table. 1)

3.2 Ferrous Ion-chelating Assay

All the Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes shows dose dependent Fe^{2+}-chelating activity comparable with standard EDTA (ethylenediaminetetra-acetic acid) (Fig. 4). The IC_{50} values for Fe^{2+}-chelating capability of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes were found to be 419.56±8.071 *µ*M, 243.8947±13 *µ*M, 231.15±13.66 *µ*M, 224.11± 13.41 *µ*M, 238.26±13.48 *µ*M and standard 196.26±14.36 *µ*M respectively (Table 2).

Table 1. DPPH radical scavenging activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and ascorbic acid. % inhibition mean±SEM was used to expressed the values

Drugs	%Inhibition Mean (n=3)±SEM					IC_{50}(*µ*M) ±SEM
	12.5(*µ*M)	25(*µ*M)	50(*µ*M)	100(*µ*M)	200(*µ*M)	
ligand	1.42±0.71	4.77 ±1.16	10.86±1.65	19.76±1.27	35.13±1.79	558.31±6.051
Ni(II) Complex	3.18±0.73	7.93 ±1.24	15.76±1.86	28.28±1.28	58.33±1.90	343.81±9.869
Co(II) Complex	5.09±1.09	10.16±1.17	19.56±1.32	35.78±1.67	64.14±2.31	302.67±10.67
Cu(II) Complex	6.39±1.25	11.29±1.11	20.04±1.09	39.70±2.19	70.06±2.92	275.46±11.63
Zn(II) Complex	4.45±0.64	9.09 ±1.10	16.30±1.61	31.37±0.79	60.45±3.06	328.05±10.11
Ascorbic acid	7.03±0.93	12.42±1.85	26.05±1.74	46.39±0.69	83.08±4.04	229.29±13.81

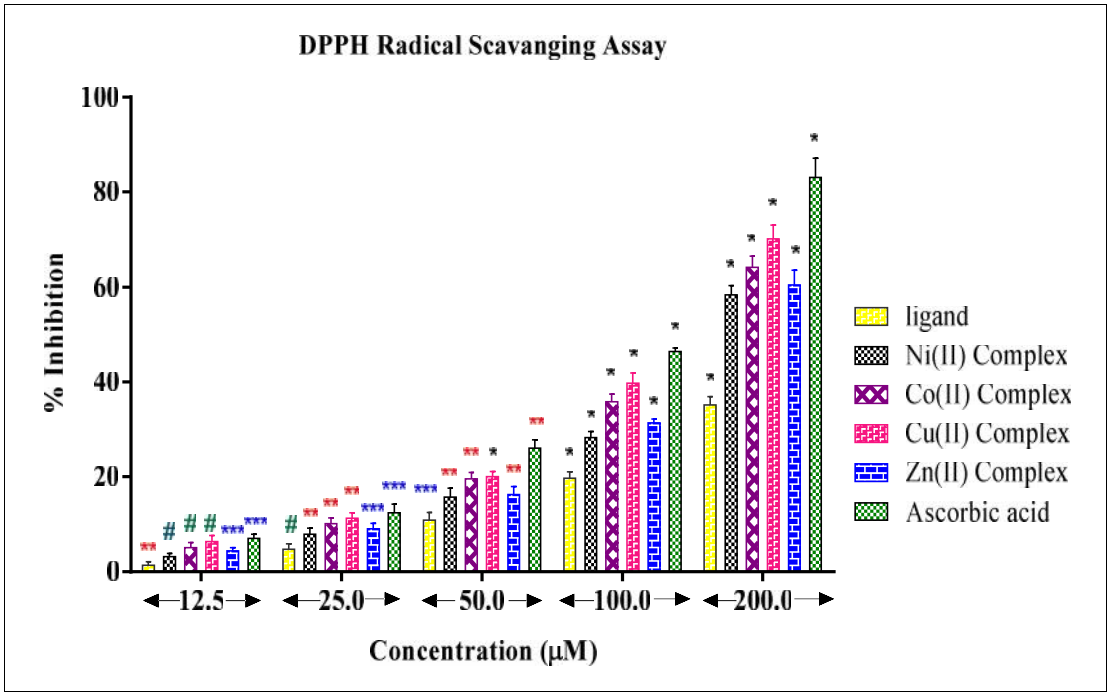


Fig. 3. DPPH radical scavenging activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and Ascorbic acid. % inhibition mean±SEM was used to expressed the values

*Significant *p<0.0001, **p<0.0012, ***p<0.0030 and #p<0.0150*

Table 2. Ferrous ion-chelating activity of Schiff base ligand and metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and EDTA (ethylenediaminetetraacetic acid). % chelation mean±SEM was used to expressed the values

Drugs	% Chelation Mean (n=3)±SEM					IC_{50}(μM) ±SEM
	12.5(μM)	25(μM)	50(μM)	100(μM)	200(μM)	
Ligand	2.53±0.52	7.34±1.09	12.78±0.95	23.63±1.68	47.87±3.07	419.56±8.071
Ni(II) Complex	3.70±1.21	11.48±0.52	24.73±1.68	44.54±2.05	78.86±4.39	243.8947±13.
Co(II) Complex	4.93±0.36	13.79±1.30	26.88±1.28	47.07±0.13	81.49±04.17	231.15±13.66
Cu(II) Complex	6.17±1.78	16.09±0.05	30.10±2.24	48.18±1.16	82.14±1.07	224.11±13.41
Zn(II) Complex	4.93±1.27	12.64±0.31	25.80±2.82	45.45±1.22	80.10±4.60	238.26±13.48
EDTA	8.64±1.16	19.20±1.51	35.15±4.22	53.63±0.63	90.25±4.67	196.26±14.36

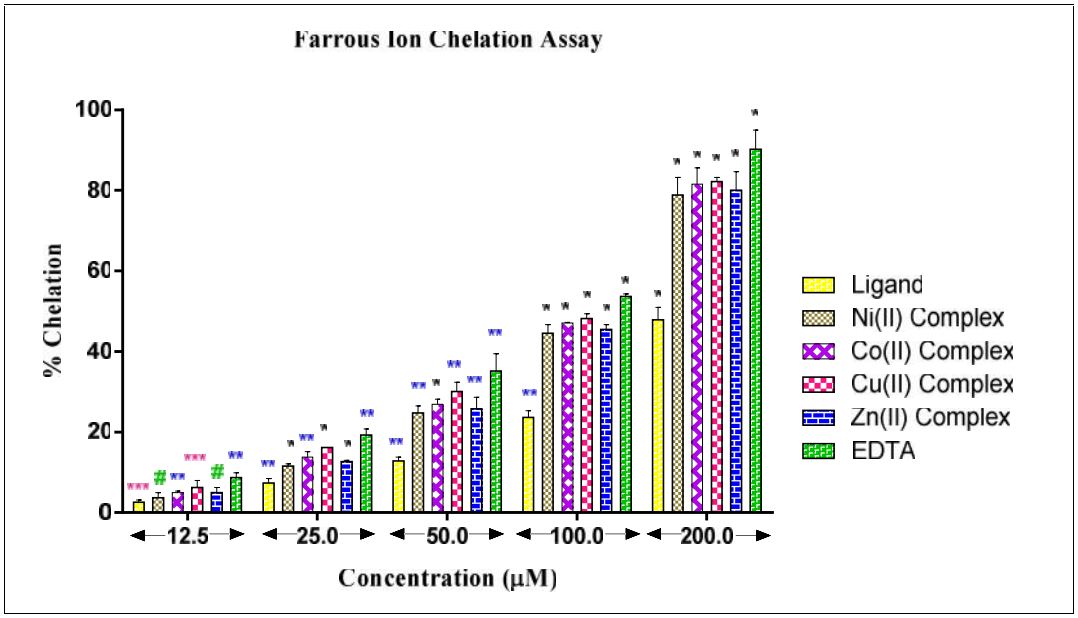


Fig. 4. Ferrous ion-chelating activity of Schiff base ligand and metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and EDTA (ethylenediaminetetraacetic acid). % chelation mean ± SEM was used to expressed the values

Significant $^{*}p<0.0001$, $^{**}p<0.0026$, $^{***}p<0.0084$ and $^{\#}p< 0.0380$

Table 3. Ferric ion reducing activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and ascorbic acid. % reduction mean±SEM was used to expressed the values

Drugs	%Reduction mean (n=3)±SEM					IC_{50} (μM) ±SEM
	12.5(μM)	25(μM)	50(μM)	100(μM)	200(μM)	
ligand	3.82±0.64	8.63±0.97	13.15±1.59	24.41±2.16	39.31±4.87	251.33±6.447
Ni(II) Complex	5.99±1.41	11.96±0.85	18.52±1.57	30.97±1.72	57.18±3.75	243.89±9.029
Co(II) Complex	8.92±1.70	11.78±1.30	19.92±1.46	33.47±2.33	60.34±3.94	231.15±9.119
Cu(II) Complex	11.14±1.48	14.34±1.27	21.83±1.89	35.49±2.15	60.84±3.81	224.11±8.939
Zn(II) Complex	6.29±0.66	13.10±1.62	19.10±1.77	32.63±3.25	58.57±3.24	238.26±9.247
Ascorbic acid	13.80±1.67	21.02±1.69	37.19±2.03	50.75±3.97	74.66±4.62	196.26±10.69

3.3 Ferric Reducing/Antioxidant Power Assay

The IC_{50} of the ethanolic solution of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II) and Zn(II)) complexes were found to be 251.33± 6.447μM, 243.89±9.029 μM, 231.15± 9.119 μM, 224.11±8.939 μM and 238.26±9.247 μM respectively (Table 3). Among the tested compounds Cu(II) complex shows the maximum reducing power.

3.4 Total Antioxidant Capacity (Phospho-molybdenum Assay)

Total antioxidant capacity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes were found to be dose-dependent, maximum Mo(VI) reducing activity was observed at higher concentrations (Fig. 6). IC50 valves of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes were summarized as 524.69±6.374 μM, 327.61±9.731 μM, 313.61± 9.895 μM, 303.29±9.857 μM, 327.48±9.776 μM respectively In (Table 4). Among the tested compounds Cu(II) complex shows the maximum total antioxidant activity.

3.5 Hydroxyl Radical Scavenging Activity

The IC_{50} valves of the Schiff ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes were found to be 612.02±5.444 μM, 377.37± 8.730 μM, 369.26±8.963 μM, 316.97±10.19 μM and 337.90±9.859μM respectively (Table.5). Standard, ascorbic acid was observed to be 235.02±12.61 μM. Among the compounds Copper (II) compound was noted to be the most powerful scavenger of the hydroxyl radical.

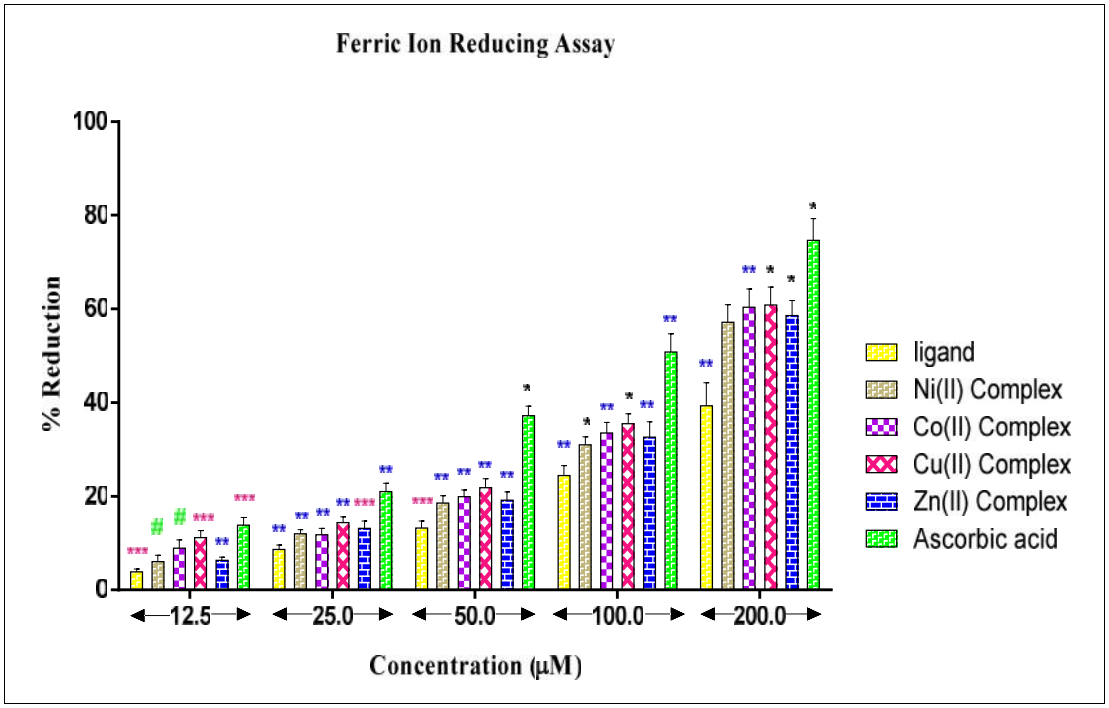


Fig. 5. Ferric ion reducing activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and ascorbic acid. % reduction mean±SEM was used to expressed the values

Significant $^{*}p<0.0001$, $^{**}p<0.0013$, $^{***}p<0.0041$ and $^{\#}p<0.0133$

Table 4. Molybdenum ion reducing activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and ascorbic acid. % reduction mean±SEM was used to expressed the values

Drugs	%Reduction Mean (n=3)±SEM					IC_{50}(μM) ±SEM
	12.5(μM)	25(μM)	50(μM)	100(μM)	200(μM)	
ligand	2.58±0.58	6.23±0.81	11.55±1.17	20.41±1.83	38.85±3.85	524.69±6.37
Ni(II) Complex	5.33±0.51	11.14±0.61	18.34±1.39	33.58±0.62	59.99±2.87	327.61±9.73
Co(II) Complex	5.86±1.20	13.31±0.40	20.39±1.34	35.90±3.15	61.24±4.02	313.61±9.89
Cu(II) Complex	7.73±0.97	14.49±0.76	22.69±1.80	36.14±2.62	62.50±0.12	303.29±9.85
Zn(II) Complex	5.69±0.42	10.37±1.16	18.56±0.58	32.61±0.62	61.56±3.12	327.48±9.77
Ascorbic acid	10.54±0.771	18.88±0.93	33.75±1.83	55.43±3.37	86.67±4.02	201.52±13.6

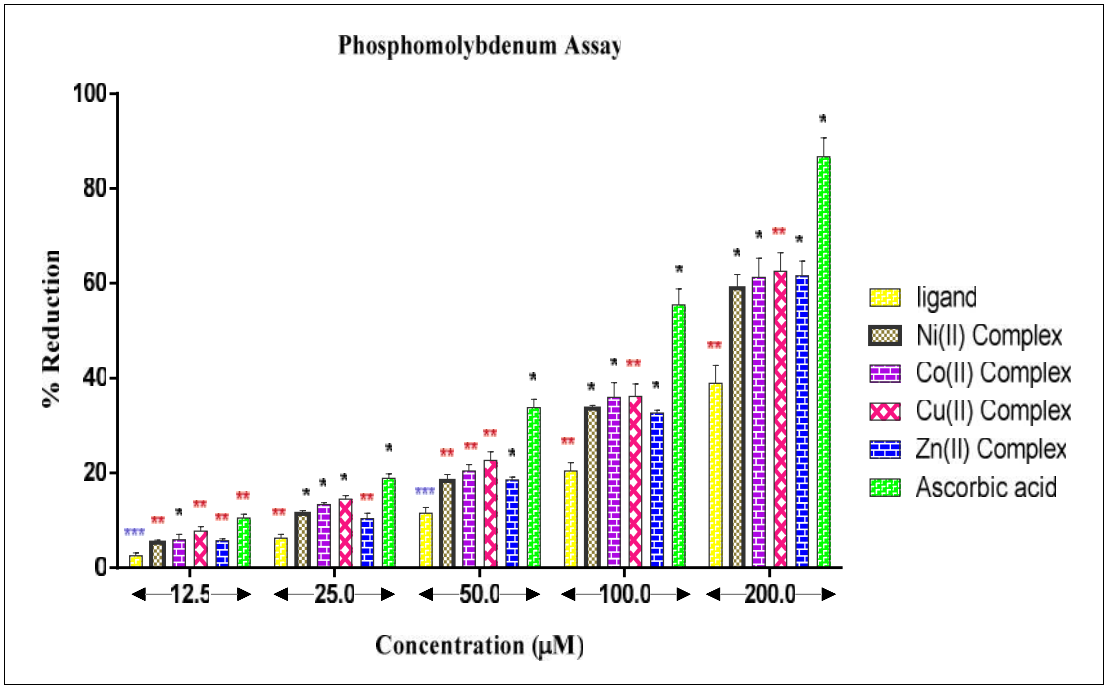


Fig. 6. Molybdenum ion reducing activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and ascorbic acid. % reduction mean±SEM was used to expressed the values

Significant $^{*}p<0.0001$, $^{**}p<0.0016$ and $^{***}p<0.0114$

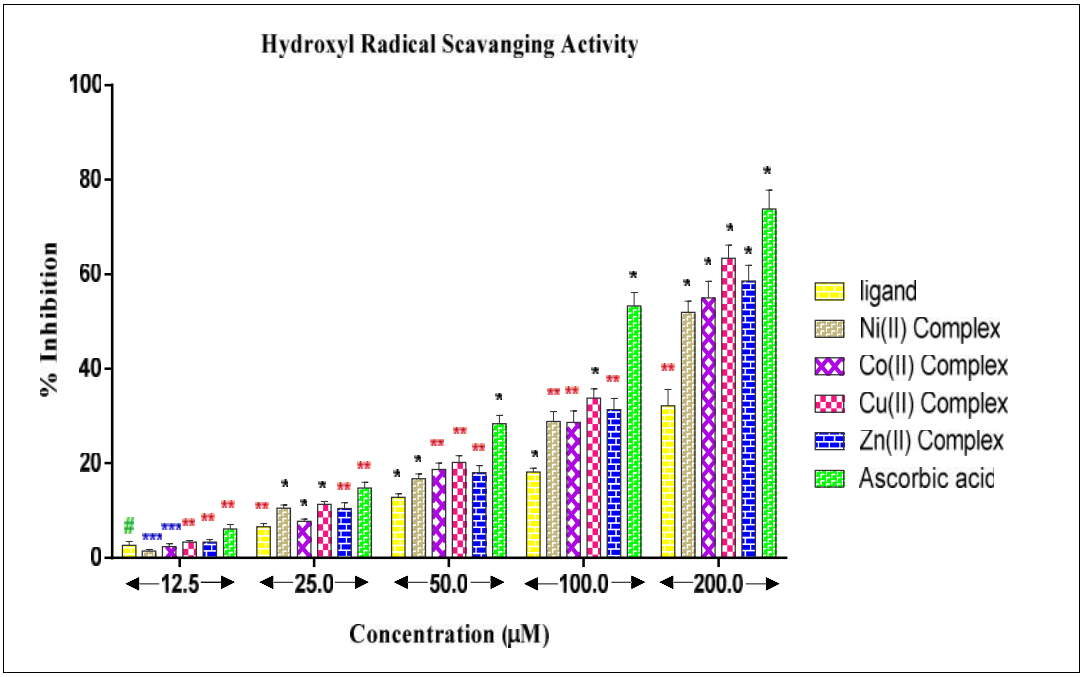


Fig. 7. Hydroxyl radical scavenging activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and Ascorbic acid. % reduction mean±SEM are used to expressed the values

Significant $^{*}p<0.0011$, $^{**}p<0.0031$, $^{***}p<0.0164$ and $^{\#}p< 0.0330$

Table 5. Hydroxyl radical scavenging activity of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and Ascorbic acid. % inhibition mean±SEM are used to expressed the values

Drugs	%Inhibition Mean (n=3)±SEM					IC_{50}(μM)±SEM
	12.5(μM)	25(μM)	50(μM)	100(μM)	200(μM)	
ligand	2.65±0.82	6.57±0.71	12.81±0.75	18.11±0.90	32.14±3.47	612.02±5.444
Ni(II) Complex	1.49±0.33	10.53±0.66	16.73±1.01	28.87±2.04	51.93±2.83	377.37±8.730
Co(II) Complex	2.39±0.60	7.75±0.44	18.72±1.38	28.64±2.50	55.02±3.52	369.26±8.963
Cu(II) Complex	3.33±0.32	11.29±0.61	20.20±1.43	33.81±1.96	63.33±2.83	316.97±10.19
Zn(II) Complex	3.34±0.52	10.40±1.24	18.302±1.46	31.32±2.35	58.59±3.31	337.90±9.859
Ascorbic acid	6.13±0.90	14.79±1.21	28.38±1.77	53.29±2.85	73.84±3.94	235.02±12.61

4. DISCUSSION

In order to elucidate the ability of the Schiff base ligand and their metals (Ni(II), Co(II), Cu(II), Zn(II)) complexes to act toward different RS, we tested some radical scavenging assay methods. Our results indicate an important H_2O_2 and $^{\bullet}OHS$ cavenging activity of the Schiff base ligand and their metal complexes at Nano molar concentrations. Besides, we observed that the ligand and their metal complexes have proton donating ability in $DPPH^{\bullet}$ assay. Together, these results indicate a strong Schiff base ligand and their metal complexes ability to act against different forms of RS such as $DPPH^{\bullet}$, $OH^{\bullet}$, Fe^{2+}, Fe^{3+} and Mo^{6+}.

The antioxidant activity of an inhibitor mainly depends on the way it participates in neutralizing the radical centers that are generated in the biological systems by donating an electron or hydrogen. The structure and properties of the inhibitor plays a prominent role in showing the activity. These results were in agreement with previous studies of metallic complexes [24] in which the ligand has antioxidant activity, and it has been found that the metal moiety had increased its activity.

Metal chelating capacity was significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (thus delaying metal-catalyzed oxidation) [25]. Since ferrous ions constitute the most effective pro-oxidants in food and biological systems, the good chelating effect would be beneficial and removal of free iron from circulation and it is a corrective approach to prevent oxidative stress-induced disorder. As seen in Table 2.

Actually, it is well recognized that iron is essential in numerous biochemical processes, including oxygen transport, cellular respiration and metabolism, drug metabolism, and DNA synthesis. However, iron is also recognized to be integrally involved in many biochemical oxidation reactions [26], which are on the basis of pathological disorders like neurodegeneration [27] and atherosclerosis [28]. Besides, compounds that interact with iron and thus block its oxidative reactions with biological components could be used as potential antioxidant agents [29-31]. The Schiff base ligand observed at lower Fe^{2+}-chelating properties than metal complexes. Among the complexes, Cu(II) metal complex was found to be better Fe^{2+}-chelator. The compounds having iron reducing activity can be very important one for a potential treatment of diseases such as in humans. Hemochromatosis can develop because of genetic mutations resulting accumulation of Fe^{3+} or overload of ferritin factors and secondary factors such as deficiency of pyruvate kinase [24] and glucose 6-phosphate dehydrogenase. Also, iron accumulation increases in cancer progression via enhancing angiogenesis. As treatment of these cases, iron chelating agents are widely used.

Thus, we believe that the Schiff base ligand and their metal complexes could be used as a further antioxidant agent in stressing conditions involving the oxidative damage induced by iron. Further tests should be conducted with Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes as an anticancer agent.

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid peroxidation. Fig. 4 shows the reductive capabilities of different Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes. Like the antioxidant activity, the reducing power was dose-dependent which increased with increasing concentration of the compounds comparable to the standard, ascorbic acid. The hydroxyl radical scavenging activity of the Schiff ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes was investigated

by O-phenanthroline method. All the drugs exhibited strong concentration-dependent scavenging properties for the hydroxyl radical (Fig. 6).

5. CONCLUSIONS

In conclusion, in the present paper we report on preliminary pharmacological properties of Schiff base ligand and their metal complexes. They all possessed antioxidant activities and are found to have enormous increase after complexation with the transition metals ions. Among these, Cu(II)-complex showed valuable radical scavenging activity compared to Schiff base ligand and their metal (Ni(II), Co(II), and Zn(II)) complexes. Moreover, Cu(II)-complex also showed the highest activity regarding Fe^{3+} reducing activity and should be tested as a drug candidate. The results indicate that all compounds of Schiff base ligand and their metal complexes exhibited is very reactive towards DPPH radicals, OH radicals, and Fe^{+2} ions. Similarly, it also actively reduces Fe(III) ion to Fe(II) and Mo(VI) ion to Mo(V) form. This shows that Schiff base ligand and their metal complexes containing antioxidant parts which can donate a hydrogen atom or an electron. Schiff base metal complexes were found to be high antioxidant potential than Schiff base ligand.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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