

***In Vitro* Antioxidant Properties of Novel Schiff Base Complexes**

ABSTRACT

Studies 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. In the current investigation, a newly synthesized Schiff base ligand (2-*[(E)-[(4-chloro phenyl) imino]methyl}phenol*) and their metals 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. The antioxidant activity of the newly 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 ($\cdot\text{OH}$) radical scavenging activity. The synthesized compounds showed significant dose dependent antioxidant activities comparable with that of the classical antioxidants, ascorbic acid and ethylenediaminetetraacetic acid (EDTA). 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, Schiff base complexes, DPPH, Antioxidants, Ligand.

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 $\geq 5\%$ 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.* (2004) 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 [17].

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 ($\cdot\text{OH}$) radical scavenging activities *in vitro*.

Materials and methods

Chemicals

Schiff base ligand and their Nickel (II), Cobalt (II), Copper (II), Zinc (II), metals complexes (Scheme 1) were synthesized according to literature methods [18], and were dissolved in ethanol. Analysis of the ^1H 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, ethylenediaminetetraacetic acid (EDTA), ferric chloride (FeCl_3), TrisHCl buffer, O-phenanthroline, sulfuric acid, Potassium phosphate (mono phosphate and diphosphate), ammonium molybdate, Hydrogen peroxide (H_2O_2), ethanol were analytical grade purchased from Sigma Aldrich Pakistan.

67 **Determination of Anti-oxidant Activities**

68 **DPPH radical scavenging assay**

69 **Principle:** The free radical scavenging activity of the various concentrations of Schiff bases
 70 ligand and their metal complexes was measured *in vitro* by 2,2-diphenyl-1-picrylhydrazyl
 71 (DPPH) radical scavenging assay described by (Ogunmoyole et al.2013)[19]. DPPH[•] (2,2-
 72 diphenyl-1-picrylhydrazyl) with purple color is a stable free radical. On scavenging, with
 73 antioxidants (AH) the purple color of DPPH[•] reduce to yellow (DPPHH) (Fig.2) is the basic
 74 principle utilized in this assay. [20]

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

$$DPPH\ Inhibition\ effect\ (\%) = \frac{A_c - A_s}{A_c} \times 100$$

81 Where A_c = Absorbance reading of the control

82 A_s = Absorbance reading of the sample

83 **Ferrous ion-chelating assay**

84 **Principle:** In order to examine iron chelating properties of the Schiff base ligand and their
 85 metal complexes, O-phenantroline color method was used according to (Milleretal.2000)
 86 [20].The formation of O-Phenanthroline-Fe²⁺ complex and its disruption in the presence of
 87 chelating agents is the common principle has been utilized in this assay.

88 **Procedure:** Various concentrations (25, 50, 100, 200 and 400 μ M) of Schiff base ligand and
 89 their metal complexes were mixed with 0.2 mL of 3.6 mM ferrous sulphate, 0.3mL of
 90 100mM Tris-absorbance was determined at 510 nm. EDTA (ethylenediaminetetraacetic acid)
 91 at the same concentration utilized as a reference standard and without Schiff bases complexes
 92 sample mixture as control. The Fe²⁺ chelating capacity was calculated by using the following
 93 formula:

$$\text{Chelating effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

94 Where A_c = Absorbance reading of the control

95 A_s = Absorbance reading of the sample

96 **Ferric Reducing / Antioxidant Power Assay**

97 **Principle:** Ferric ions reducing antioxidant potential of the Schiff base ligand and their metal
 98 complexes were investigated by O-phenanthroline color method previously described by
 99 (Benzie et al.1999) [21]. The transformation of ortho-phenanthroline-Fe³⁺ to ortho-
 100 phenanthroline-Fe²⁺ by accepting of an electron from an antioxidant is the basic principle
 101 utilized in this assay.

102 **Procedure:** The reaction mixture containing various concentrations (25, 50, 100, 200and
 103 400μM) of Schiff base ligand and their metal complexes, 0.2 mL of 3.6 mM ferric chloride,
 104 0.3mL of 100mM tris buffer (pH=7.4), 0.1 mL of 9mM O-phenanthroline and diluted up to
 105 3.0 mL with ultra pure distil water was shaken vigorously and left to stand at room
 106 temperature for 10 min. The increase in absorbance of the sample solution was measured at
 107 510 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations was
 108 utilized as a reference standard and without compounds sample mixture as control. The
 109 Reducing Power comparable with Ascorbic acid was calculated by using the following
 110 formula:

$$\text{Reducing Power (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

111 Where A_s = Absorbance reading of the sample

112 A_c = Absorbance reading of the control

113

114 **Total antioxidant capacity (Phosphomolybdenum assay)**

115 **Principle:**The total antioxidant potential of the Schiff base ligand and their metal complexes
 116 will be evaluated by using the phosphomolybdenum method as previously described by
 117 (Uyohet al. 2013) [22]. The total antioxidant capacity is based on the reduction of Mo (VI) to
 118 Mo (V) by accepting of an electron from antioxidant at acidic pH.

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 3.0mL of reagent solution containg 0.7mL of 0.6M sulphuric acid, 1.0mL of 28mM pottacium pasphate,1.0 mM ammonium molybdate and distal water. The reagent mixture was incubated at 95°C for 90 min 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:

$$\text{Reducing Power}(\%) = \frac{A_c - A_s}{A_c} \times 100$$

Where A_s = Absorbance reading of the sample

A_c = Absorbance reading of the control

Hydroxyl radical scavenging activity

Principle:The hydroxyl radical scavenging activity of Schiff base ligand and their metal complexes was determined according to the method of (Li et *al.*2011) [23].The Fenton reaction is the basic principle for hydroxyl radical scavenging activity.

Procedure: Various concentrations (25, 50, 100, 200 and 400 μ M) of Schiff base ligand and their metal complexes, 0.1mL 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 mLof H₂O₂(0.1%) and diluted up to 3 mL with distilled water. The reaction mixture incubated at room temperature for 30 min 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 H₂O₂ as a blank. The percentade scavanging power of Schiff base complexes and ascorbic acid were calculated by using the following formula:

$$\text{Scavanging Power}(\%) = \frac{A_s - A_c}{A_b - A_c} \times 100$$

Where A_s = Absorbance reading of the sample

A_c = Absorbance reading of the control

A_b = Absorbance reading of the blank

Statistical analysis

Linear regression analysis was used to calculate $IC_{50} \pm SEM$ 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.

RESULTS

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 $557.51 \pm 6.067 \mu M$, $318.03 \pm 9.875 \mu M$, $302.39 \pm 10.68 \mu M$, $275.40 \pm 11.70 \mu M$ and $326.53 \pm 10.17 \mu M$ 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 ($IC_{50} = 228.61 \pm 13.83 \mu M$). (Table. 1).

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.

Drugs	Concentrations (μM), Mean $\pm$ SEM					$IC_{50}(\mu M) \pm SEM$
	25(μM)	50(μM)	100(μM)	200(μM)	400(μM)	
ligand	1.27 ± 0.01	4.77 ± 0.02	10.86 ± 0.03	19.76 ± 0.04	35.13 ± 0.05	558.31 ± 6.051
Ni(II) Complex	3.18 ± 0.01	7.90 ± 0.03	15.76 ± 0.04	28.43 ± 0.06	58.33 ± 0.10	343.81 ± 9.869
Co(II) Complex	5.09 ± 0.02	10.16 ± 0.04	19.56 ± 0.05	35.78 ± 0.07	64.21 ± 0.13	302.67 ± 10.67
Cu(II) Complex	5.73 ± 0.02	11.29 ± 0.03	20.10 ± 0.05	39.70 ± 0.07	70.09 ± 0.15	275.46 ± 11.63
Zn(II) Complex	4.45 ± 0.01	9.09 ± 0.02	16.30 ± 0.03	31.37 ± 0.05	60.78 ± 0.12	328.05 ± 10.11
Ascorbic acid	7.00 ± 0.03	12.42 ± 0.04	26.08 ± 0.05	47.05 ± 0.09	83.01 ± 0.16	229.29 ± 13.81

Ferrous ion-chelating assay

169 All the Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes
 170 shows dose dependent Fe^{2+} -chelating activity comparable with standard EDTA
 171 (ethylenediaminetetra- acetic acid) (Fig.4). The IC_{50} values for Fe^{2+} -chelating capability of
 172 Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), and Zn(II)) complexes were found
 173 to be $419.56 \pm 8.071 \mu\text{M}$, $243.8947 \pm 13 \mu\text{M}$, $231.15 \pm 13.66 \mu\text{M}$, $224.11 \pm 13.41 \mu\text{M}$,
 174 $238.26 \pm 13.48 \mu\text{M}$ and standard $196.26 \pm 14.36 \mu\text{M}$ respectively (Table 2).

175 **Table 2: Ferrous ion-chelating activity of Schiff base ligand and their metal (Ni(II),**
 176 **Co(II), Cu(II), Zn(II)) complexes and EDTA(ethylenediaminetetra- acetic acid).**

Drugs	Concentrations (μM), Mean $\pm$ SEM					$\text{IC}_{50}(\mu\text{M}) \pm \text{SEM}$
	25(μM)	50(μM)	100(μM)	200(μM)	400(μM)	
Ligand	2.46 ± 0.01	7.34 ± 0.03	12.78 ± 0.03	23.63 ± 0.04	47.87 ± 0.06	419.56 ± 8.071
Ni(II) Complex	3.70 ± 0.02	11.49 ± 0.04	24.73 ± 0.05	44.54 ± 0.07	78.89 ± 0.10	$243.8947 \pm 13.$
Co(II) Complex	4.93 ± 0.02	13.79 ± 0.04	26.88 ± 0.06	47.27 ± 0.08	81.49 ± 0.12	231.15 ± 13.66
Cu(II) Complex	6.17 ± 0.03	16.09 ± 0.05	30.10 ± 0.06	48.18 ± 0.07	82.14 ± 0.11	224.11 ± 13.41
Zn(II) Complex	4.93 ± 0.02	12.64 ± 0.03	25.80 ± 0.05	45.45 ± 0.06	80.19 ± 0.10	238.26 ± 13.48
EDTA	8.64 ± 0.04	19.54 ± 0.05	35.48 ± 0.07	53.63 ± 0.08	90.25 ± 0.14	196.26 ± 14.36

178 Ferric Reducing / Antioxidant Power Assay

179 The IC_{50} of the ethanolic solution of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II)
 180 and Zn(II)) complexes were found to be $251.33 \pm 6.447 \mu\text{M}$, $243.89 \pm 9.029 \mu\text{M}$,
 181 $231.15 \pm 9.119 \mu\text{M}$, $224.11 \pm 8.939 \mu\text{M}$ and $238.26 \pm 9.247 \mu\text{M}$ respectively (Table 3). Among the
 182 tested compounds Cu(II) complex shows the maximum reducing power.

183 **Table 3: Ferric ion reducing activity of Schiff base ligand and metal (Ni(II), Co(II),**
 184 **Cu(II), Zn(II)) complexes and Ascorbic acid.**

Drugs	Concentrations (μM), Mean $\pm$ SEM					$\text{IC}_{50}(\mu\text{M}) \pm \text{SEM}$
	25(μM)	50(μM)	100(μM)	200(μM)	400(μM)	
ligand	3.81 ± 0.01	7.50 ± 0.03	13.00 ± 0.04	24.81 ± 0.06	39.18 ± 0.08	251.33 ± 6.447
Ni(II) Complex	5.93 ± 0.02	12.08 ± 0.04	18.01 ± 0.06	31.00 ± 0.07	56.86 ± 0.10	243.89 ± 9.029
Co(II) Complex	8.89 ± 0.03	11.25 ± 0.04	19.20 ± 0.05	34.44 ± 0.07	58.19 ± 0.11	231.15 ± 9.119
Cu(II) Complex	10.59 ± 0.03	13.75 ± 0.03	21.60 ± 0.07	35.55 ± 0.08	59.53 ± 0.12	224.11 ± 8.939
Zn(II) Complex	6.77 ± 0.02	12.91 ± 0.04	18.40 ± 0.06	33.33 ± 0.07	58.52 ± 0.11	238.26 ± 9.247

Ascorbic acid	14.40±0.05	21.25±0.07	36.80±0.09	50.00±0.10	74.24±0.14	196.26±10.69
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Total antioxidant capacity (Phosphomolybdenum 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 values 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.

Table 4: Molybdenum ion reducing activity of Schiff base ligand and metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and Ascorbic acid.

Drugs	Concentrations (μM), Mean±SEM					IC ₅₀ (μM) ±SEM
	25(μM)	50(μM)	100(μM)	200(μM)	400(μM)	
ligand	1.96±0.00	6.54±0.03	11.60±0.04	20.00±0.05	38.16±0.09	524.69±6.37
Ni(II) Complex	4.90±0.02	11.21±0.04	18.75±0.06	33.33±0.08	59.54±0.11	327.61±9.73
Co(II) Complex	5.88±0.02	13.08±0.04	20.53±0.07	35.00±0.08	61.83±0.12	313.61±9.89
Cu(II) Complex	7.88±0.03	14.01±0.05	22.32±0.07	35.83±0.09	63.38±0.12	303.29±9.85
Zn(II) Complex	5.88±0.02	10.28±0.03	18.75±0.06	31.66±0.07	60.30±0.10	327.48±9.77
Ascorbic acid	10.78±0.04	18.69±0.06	33.92±0.08	55.00±0.10	86.25±0.15	201.52±13.6

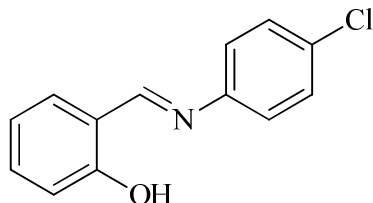
Hydroxyl radical scavenging activity

The IC₅₀ values 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±9.859 μM 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.

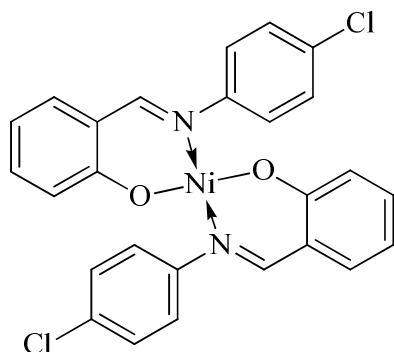
Table 5: Hydroxyl radical scavenging activity of Schiff base ligand and metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes and Ascorbic acid

Drugs	Concentrations (μM), Mean±SEM					IC ₅₀ (μM) ±SEM
	25(μM)	50(μM)	100(μM)	200(μM)	400(μM)	
ligand	1.13±0.01	6.53±0.03	11.47±0.03	17.65±0.04	32.75±0.06	612.02±5.444
Ni(II) Complex	1.90±0.00	9.46±0.04	16.94±0.04	28.75±0.06	51.87±0.10	377.37±8.730
Co(II) Complex	2.38±0.01	7.98±0.03	18.76±0.05	28.83±0.06	53.12±0.11	369.26±8.963
Cu(II) Complex	3.87±0.02	10.9±0.04	19.83±0.05	34.87±0.08	61.24±0.13	316.97±10.19
Zn(II) Complex	3.59±0.02	8.34±0.03	17.37±0.04	30.76±0.09	58.54±0.13	337.90±9.859
Ascorbic acid	5.79±0.02	14.82±0.04	27.47±0.06	53.78±0.11	73.87±0.16	235.02±12.61

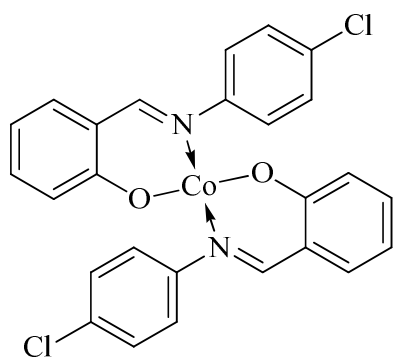
Fig.1(A)Chemical structure of Schiff base ligand (2-{(E)-[(4-chlorophenyl)imino]methyl}phenol), i.e. (2-{(E)-[(4-chlorophenyl)imino]methyl}phenol) and (B) (Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)nickel(II), (C) Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)Cobalt(II) (D) Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)copper(II) (E) Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)zinc(II)).



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



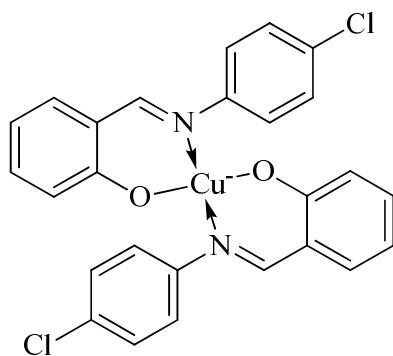
Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)nickel(II)



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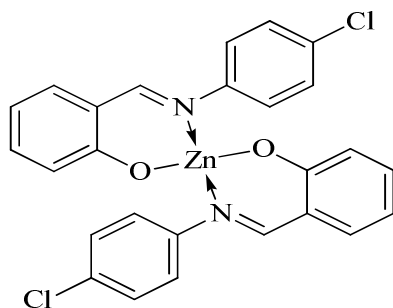
Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)cobalt(II)



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



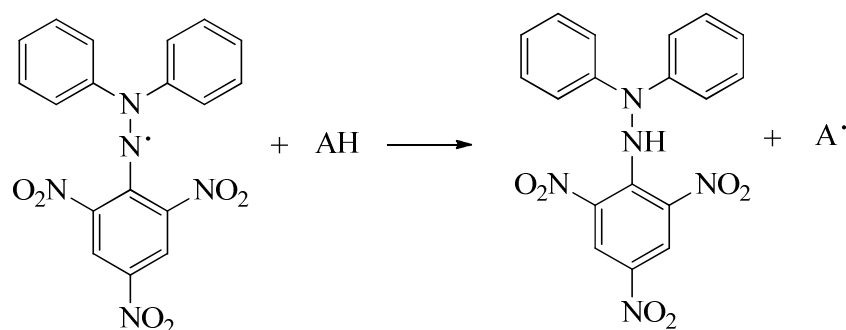
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Bis(2-{(E)-[(4-chlorophenyl)imino]methyl}phenol)zinc(II)

229

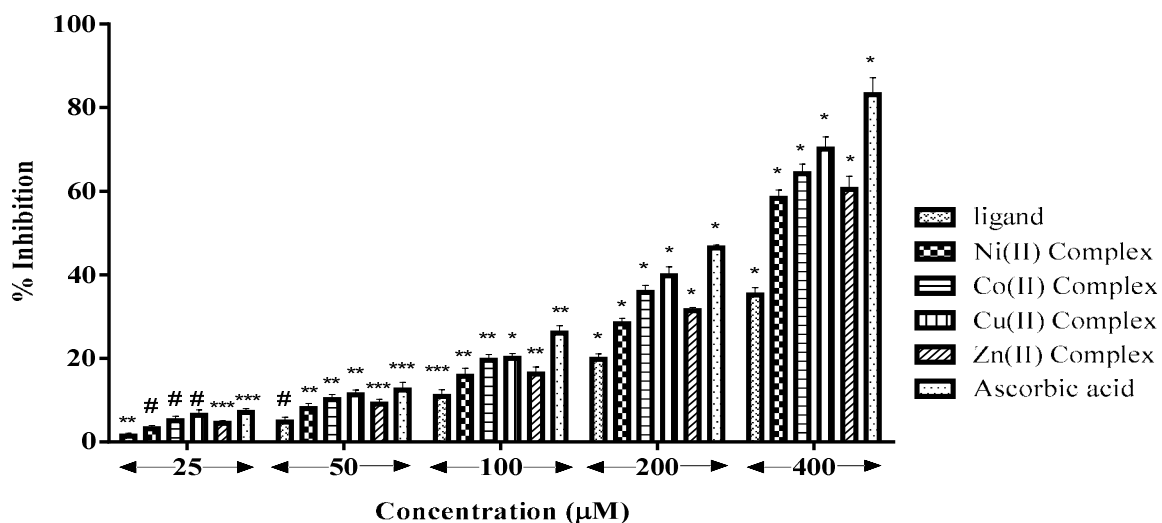
Fig. 2: Structures of DPPH $\cdot$ radical and DPPHH



DPPH[•] (purple)

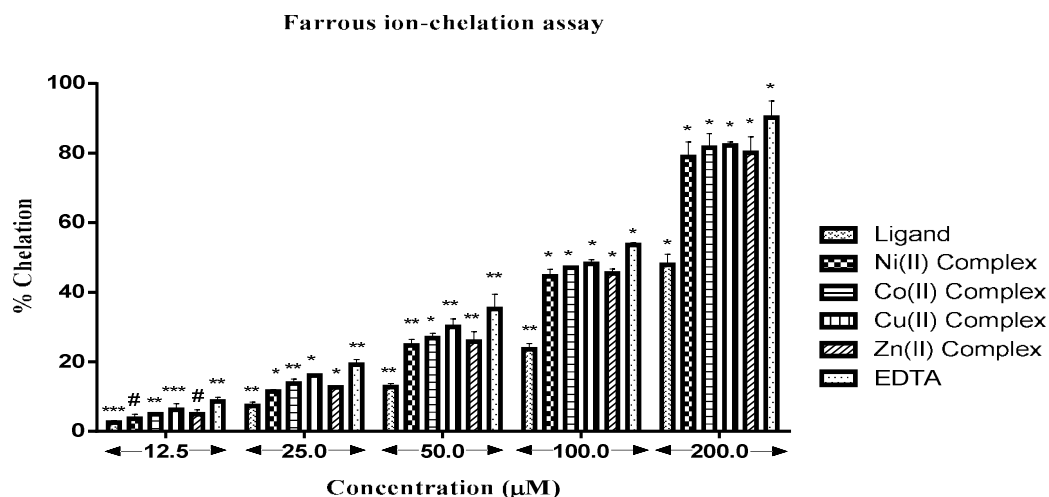
DPPH-H (yellow)

DPPH Radical Scavenging assay



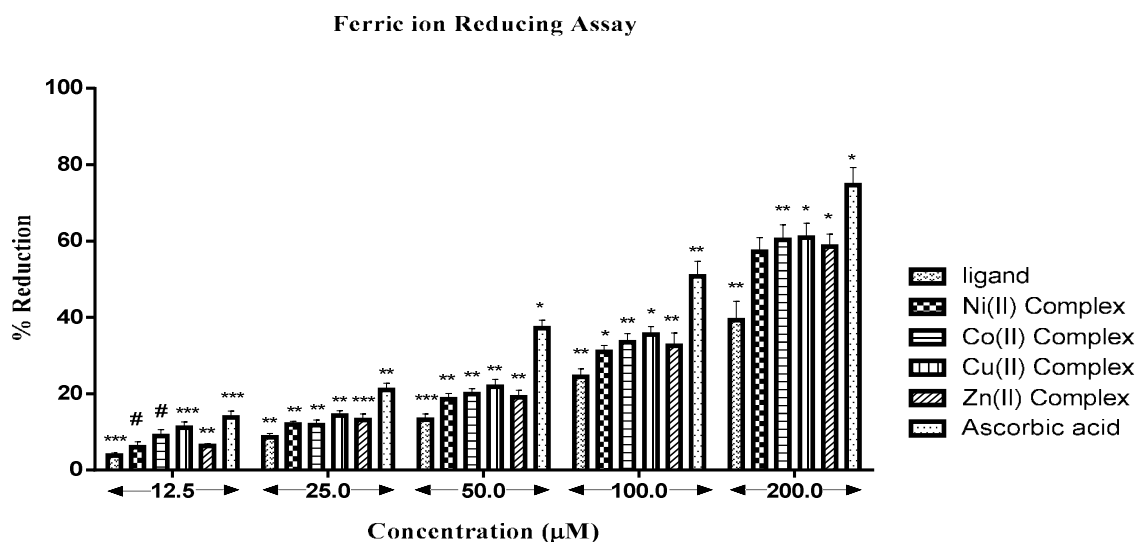
Significant * $p < 0.0001$, ** $p < 0.0012$, *** $p < 0.0030$, # $p < 0.0150$

Fig.3. Scavenging activity of the Schiff base ligand and their metals, Ni(II), Co(II) Cu(II) and Zn(II) complexes, on DPPH radical. Different concentrations of the compounds were incubated with 85μM DPPH ethanolic solution for 30 min. Ascorbic acid was used as positive control. Results are expressed as percentage of control (vehicle DMSO). Each point represents the Mean $\pm$ SEM of three to four independent experiments. (*) denotes $p < 0.05$ as compared to control (vehicle).



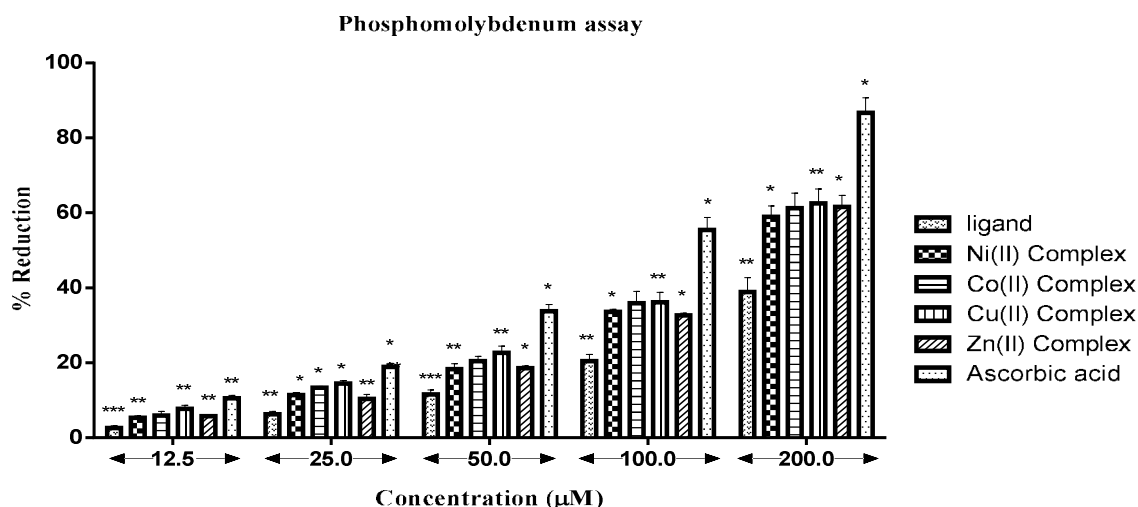
Significant * $p < 0.0001$, ** $p < 0.0026$, *** $p < 0.0084$, # $p < 0.0380$

Fig.3.1. Effects of the Schiff base ligand and their metals, Ni(II), Co(II) Cu(II) and Zn(II) complexes, on iron chelating assay. Each point represents the Mean $\pm$ SEM of three to four independent experiments. (*) denotes $p < 0.05$ as compared to control (vehicle).Data are presented as Mean $\pm$ SEM ($n = 3$).



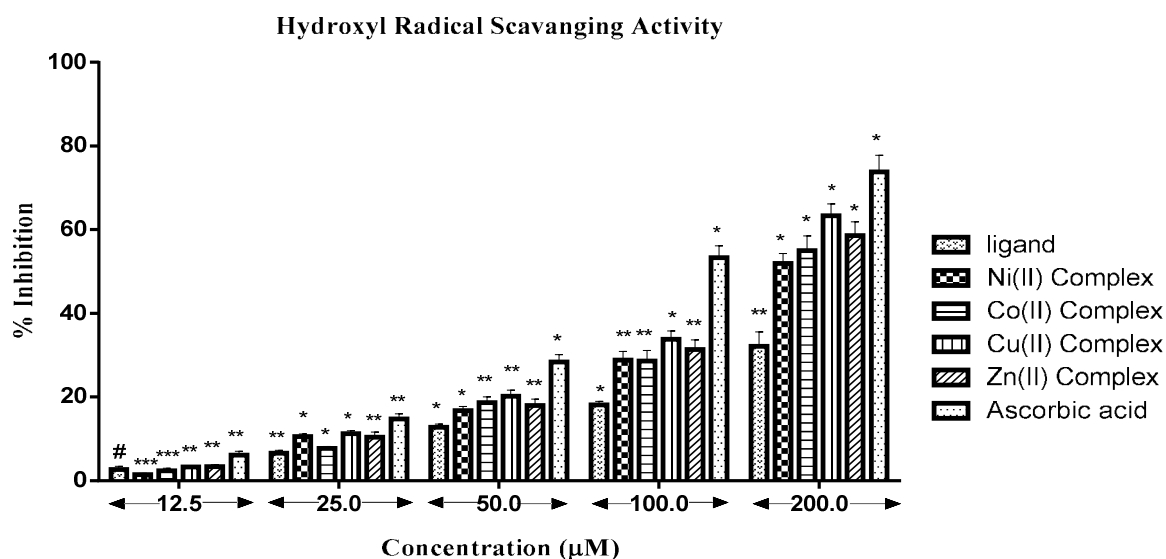
Significant * $p < 0.0001$, ** $p < 0.0013$, *** $p < 0.0041$, # $p < 0.0133$

Fig.4. Reducing power of the Schiff base ligand and their metals, Ni(II), Co(II) Cu(II) and Zn(II) complexes. Each point represents the Mean $\pm$ SEM of three to four independent experiments. (*) denotes $p < 0.05$ as compared to control (vehicle).Data are presented as Mean $\pm$ SEM ($n = 3$).



Significant * $p < 0.0001$, ** $p < 0.0016$ and *** $p < 0.0114$

Fig.5. The hydroxyl radical scavenging activity of Schiff base ligand and their metal Ni(II), Co(II) Cu(II) and Zn(II) complexes were measured. Each point represents the Mean $\pm$ SEM of three to four independent experiments. (*) denotes $p < 0.05$ as compared to control (vehicle). Data are presented as Mean $\pm$ SEM ($n = 3$).



Significant * $p < 0.0011$, ** $p < 0.0031$, *** $p < 0.0164$, # $p < 0.0330$

Fig.6. Total antioxidant activity of Schiff base ligand and their metal Ni(II), Co(II) Cu(II) and Zn(II) complexes were measured by the phosphomolybdenum assay. Each point represents the Mean $\pm$ SEM of three to four independent experiments. (*) denotes $p < 0.05$ as compared to control (vehicle). Data are presented as Mean $\pm$ SEM ($n = 3$).

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. The results indicate an important H_2O_2 and $\text{OH}^\cdot$ scavenging 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 $^\cdot$ assay. Together, these results indicate a strong Schiff base ligand and their metal complexes ability to act against different forms of RS.

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 to 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, it is 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. Figure 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).

Conclusions

In conclusion, in the present paper we report on preliminary pharmacological properties of four new 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 are 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 can donate a hydrogen atom or electron. Schiff base metal complexes were found to possess high antioxidant potential than Schiff base ligand.

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