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In vitro antioxidant, free radical scavenging and DNA binding properties of synthetic metal based imine derivatives of schiff bases

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Abstract
In the present study, the antioxidant and DNA binding properties of Schiff base ligand and their Ni(II), Co(II), Cu(II), Zn(II) metal complexes were considered for their possible free radicals scavenging properties associated with various diseases. The different models such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous ion chelation (FIC), ferric reducing antioxidant power (FRAP), total antioxidant activities (phosphomolybdenum methods) and hydroxyl radical (•OH) radical scavenging activities at different concentrations for antioxidant and DNA absorption spectroscopic analysis were assayed for DNA binding studies. The metal complexes were found to be significant dose-dependent antioxidant activities comparable with that of the classical antioxidants, ascorbic acid, ethylene diaminetetraacetic acid (EDTA) and DNA binding potential with *k*b $3.487\times10^{-5}M^{-1}$, $1.858\times10^{-5}M^{-1}$, $3.090\times10^{-5}M^{-1}$, $1.367\times10^{-5}M^{-1}$ and $9.118\times10^{-5}M^{-1}$ respectively. The compounds exhibited are very reactive towards DPPH radicals, OH radicals and Fe(II) ions and they also actively reduces Fe(III) ion to Fe(II) and Mo(VI) ion to Mo(V) form. The obtained results indicate the importance of Schiff base metal complexes as a source of synthetic antioxidants and anticancer drugs.

Keywords: antioxidants, DNA binding, free radicals, Schiff base complexes

1. Introduction

Free radicals are highly reactive molecules containing one or more unpaired electrons; they donate or take electrons from other molecules in an attempt to pair their elections and generate a more stable species. A large number of radical and non-radical species derived from reactive oxygen species (ROS) such as peroxyl radicals (ROO•), superoxide anion ($O_2^{\bullet}$), hydroxyl (•OH), reactive hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS) like nitric oxide (NO•), nitrogen dioxide ($NO_2^{\bullet}$), peroxynitrite anion (ONOO•) have a greater impact on humans [1-3]. They are usually generated in the body internally during normal metabolic activities, stimulation of macrophages, leucocytes, aerobic respiration and other metabolic processes ≥ 5% of oxygen reduced univalently to get free radicals while the tobacco smokes, pollutants, ionizing radiations, organic solvents and pesticides are the major exogenous sources of free radicals production in biological system [4].

They play a dual role (toxic and beneficial) in human. For instance, ROS/RNS are beneficially involved in many signaling pathways that control development and maintain cellular homeostasis [5] However excess production of these free radicals either internally or transferred from environment have a great impact on humans in the etiology of various diseases like cancer, cardiovascular diseases, liver injury [6], neurodegenerative, diabetes, rheumatism diseases [7], atherosclerosis [8], autoimmune disorders, aging [9], ischemia, asthma, anaemia, arthritis, monogolism, Parkinson diseases and finally may lead to oxidative stress [10-13]. Although, the body possesses defense mechanisms as antioxidant nutrients and enzymes which arrest the damaging properties of free radicals [14-15]. Continuous exposure to chemicals and contaminants may increase the amount of free radicals in the body beyond its ability to control and cause irreversible oxidative damages [16].

Therefore, antioxidants with free radical scavenging potential may be relevant in the therapeutic and preventions of diseases where free radicals are implicated [17]. In addition to natural antioxidants such as vitamin C, vitamin E, carotenoids flavonoids [18], a number of synthetic antioxidants like butylated hydroxyltoluene (BHT), butylated hydroxyanisole (BHA),

tertiary butylhydroquinone and Schiff base metal complexes have been prepared and their antioxidant capacity has been assessed for prevention of various diseases [19-21].
In the present study, the antioxidant activity of newly synthesized Schiff base Ligand and their Ni(II), Co(II), Cu(II) and Zn(II) metal complexes were evaluated *in vitro* for their possible antioxidant and DNA binding properties. The names of compounds along with their structure employed in the present work are given in Figure 1.

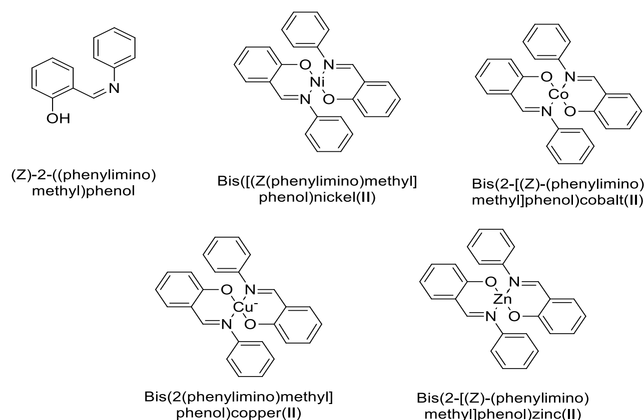


Fig 1: Structure of Schiff base Ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes

2. Materials and Methods

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ferrous sulphate, Ascorbic acid, ethylenediaminetetraacetic acid (EDTA), Tris HCl buffer, ferric chloride ($FeCl_3$), O-phenenthroline, sulfuric acid, ammonium molybdate, Potassium phosphate (mono phosphate and diphosphate), Hydrogen peroxide (H_2O_2), ethanol and Salmon fish DNA are analytical grade purchased from Sigma Aldrich Pakistan.

Determination of *In-vitro* antioxidant studies

The antioxidant activity of the newly prepared compounds were evaluated using rapid and simpler free scavenging assays viz, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, Ferrous ion-chelating assay, Ferric Reducing/Antioxidant Power Assay, total antioxidant activity by phosphomolybdenum method and hydroxyl radical scavenging assay.

DPPH radical scavenging assay

The antioxidant activity of the newly synthesized compounds were assessed using the stable DPPH free radical according to Mohammad Ibrahim *et al.* (2017) [22]. Various concentrations (50, 100, 200 and 400μM) of compounds or drugs were mixed with an ethanolic solution containing of 85μM DPPH radical. At room temperature the mixture solutions were incubated for 30 min and the decrease in absorbance was measured at 518 nm using a V-730 UV-Visible/NIR Spectrophotometer. Ascorbic acid at the same concentrations of drugs was used as a positive control. Triplicate experiment was carried out. Percentage inhibition of the drugs as well as ascorbic acid was calculated by using the following formula:

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

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

Ferrous ion-chelating assay

The ferrous ion chelating activity of newly synthesized compounds were analysed by a standard method Puntel *et al.,* 2005 [23]. Various concentrations (50μM, 100 μM, 200 μM, and 400 μM) of compounds were mixed with 0.2 ml of 3.6 mM ferrous sulphate, 0.3ml 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-pure distal 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:

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

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

Ferric reducing / antioxidant power assay

The ferric reducing power of the newly synthesized compounds were determined as described by Kumar *et al.* (2012 [24]. Different concentrations (50, 100, 200, 100 and 200 μM) of compounds, 0.2 ml of 3.6 mM ferric chloride, 0.3ml 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-pure distal water. It was shaken for 10 min vigorously and left to stand at room temperature. The increase in absorbance of the sample solution was measured at 510 nm using a V-730 UV-Visible/NIR 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:

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

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

Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant capacity of newly synthesized compounds were evaluated by phosphomolybdenum assay assessed by Sahaa *et al.*, 2008 [25]. Reagent solution containing various concentrations (50, 100, 200 and 400 μM) of compounds aliquot in ethanol, 0.7 ml of 0.6 M sulphuric acid, 1.0 mM ammonium molybdate, 1.0 ml of 28 Mm potassium phosphate and ultra-pure distal water was incubated at 95°C for 90 min. After cooling, at room temperature the increase in absorbance of the mixture is measured at 695 nm using a V-730 UV-Visible/NIR 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 formula:

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

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

Hydroxyl radical scavenging activity

The scavenging activity of Ni(II), Co(II), Cu(II) and Zn(II) metal based Schiff base Complexes for hydroxyl radicals was measured with Fenton reaction [26]. Reaction mixture of various concentrations (50, 100, 200, 100 and 200μM) of Ni(II), Co(II), Cu(II) and Zn(II) 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 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 min and the absorbance was measured at 510 nm using a V-730 **UV**-Visible/NIR Spectrophotometer. The reaction mixture without Schiff base complexes has been used as control and without Schiff base complexes and H_2O_2 as a blank. The hydroxyl radical scavenging activity of Schiff base complexes and ascorbic acid were calculated by using Equation.

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

A_c is the absorbance reading of the control,
As is the absorbance reading of the sample
A_b is the absorbance reading of the blank

DNA Absorption spectroscopic studies

The interaction between metal complexes and DNA were studied using electrochemical and electronic absorption methods. Disodium salt of Salmon fish DNA was stored at 4 ºC. Solution of DNA in the buffer 50 mM NaCl/ 5 mM Tris–HCl (pH 7.2) in water gave a ratio 1.9 of UV absorbance at 260 and 280 nm. A_{260}/A_{280} indicating that the DNA was sufficiently free from protein [27]. The concentration of DNA was measured using its extinction coefficient at 260 nm ($6600M^{-1}\ cm^{-1}$) after 1:100 dilution. Stock solutions were stored at 4 ºC and used for not more than 4 days. Doubly distilled water was used to prepare solutions. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in ethanol and diluting suitably with the corresponding buffer to the required concentration for all of the experiments. The data were then fitted to Equation 6 to obtain the *k*b values for interaction of the complexes with DNA.

$$[DNA] / (\varepsilon a-\varepsilon_f) = DNA/ (\varepsilon a-\varepsilon_f) + 1/ [kb\ (\varepsilon_b-\varepsilon_f)]$$

Where Ɛa, ε_f, and ε_b are the apparent, free and bound metal complex extinction coefficients, respectively. A plot of [DNA]/ (ε_b-ε_f) versus [DNA], gave a slope of 1/ (ε_b-ε_f) and a Y-intercept equal to $[kb/(\varepsilon_b-\varepsilon_f)]^{-1}$; *k*b is the ratio of the slope to the Y-intercept.

Statistical analysis

Linear regression analysis was used to calculate IC_{50}±SEM values from data and graphs by using Graph pad prism 6® (Motulsky and Neubig, 2001). Significant differences among the means of data were tested by the one-way ANOVA followed by the student´s t-test with significance level ($P<0.05$). All the tests were conducted in triplicate

3. Results and Discussion

DPPH radical scavenging assay

DPPH radical scavenging assay has been broadly used for screening antioxidant activity because at low concentration, the absorbance is reduced when encounter with a proton donating substance such as an antioxidant. In the present study, we investigated the Schiff base Ligand and their metal complexes for their possible antioxidant activities.

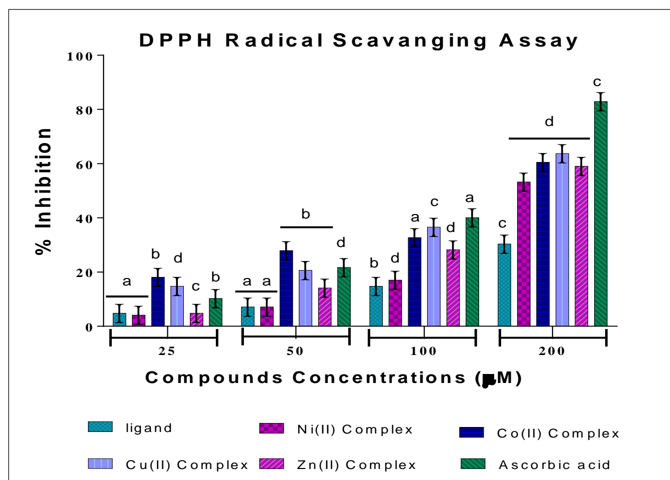
Table 1 demonstrates a significant ($p<0.01$) decline in the concentration of DPPH radicals due to the scavenging ability of Schiff base Ligand and their metal complexes and graphically represented in Figure 2. The IC_{50} values for DPPH radical with ethanolic solution of Ligand was found to be 333.58±5.771μM, which is lower scavenging stability than that of metal complexes. The IC_{50} values for Ni(II) Complex were found to be 196.70±11.30μM that shows higher scavenging properties than that of Ligand. The scavenging power of Co(II) Complex shows more noticeable ability, for which the IC_{50} values were noted to be 159.44±9.066μM. The highest scavenging ability among the metal complexes was observed for Cu(II) Complex which shows the IC_{50} values were 150.51±10.951. The IC_{50} values for Zn(II) Complex was measured to be 70.34±11.85μM, which shows better DPPH radical scavenging activity than the rest of metal complexes. The IC_{50} values for Ascorbic acid were found to 121.07±15.94. These activities were dose reliant and maximum DPPH scavenging activity was observed at higher concentrations.

Structure base study shows that the ligand (Z)-2-((phenyl-imino) methyl) phenol containing hydroxyl group located suitably at ortho position to benzene ring. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group. Hence, the overall ligand acts as a rich π electrons species and their antioxidant power. The antioxidant potential of Schiff base ligand has further enhanced by complexation with transition metals linked with central metal atom from both sides through oxygen and nitrogen atoms.

All the metal complexes containing the same two bidentate Ligands (Z)-2-((phenyl-imino) methyl)phenol attached at the same position hence the antioxidant potential depend only on central metal atom. Cu(II) Complex shows high antioxidant power due to the presence of Copper metal which has high reduction potential as compared to Ni(II), Co(II) and Zn(II) metals.

Table 1: DPPH radical scavenging activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Mean ± SEM was used to expressed the values

Drugs	Concentrations (μM), Mean±SEM				IC_{50}(μM) ±SEM
	50(μM)	100(μM)	200(μM)	400(μM)	
Ligand	4.83±0.02	7.14±0.04	14.79±0.06	30.38±0.08	333.58±5.771
Ni(II) Complex	4.09±0.03	7.11±0.05	17.00±0.05	53.25±0.09	196.70±11.30
Co(II) Complex	18.12±0.01	27.95±0.06	32.71±0.06	60.43±0.10	159.44±9.066
Cu(II) Complex	14.80±0.02	20.62±0.04	36.60±0.07	63.74±0.08	150.51±10.95
Zn(II) Complex	4.838±0.03	14.14±0.03	28.22±0.06	59.04±0.09	170.34±11.85
Ascorbic acid	10.29±0.02	21.74±0.04	40.06±0.05	82.89±0.08	121.07±15.94



Significantly $^{a}p < 0.0006$, $^{b}p < 0.0017$, $^{c}p < 0.0027$, $^{d}p < 0.0086$

Fig 2: DPPH radical scavenging activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Mean ± SEM was used to expressed the values

Ferrous ion-chelating assay

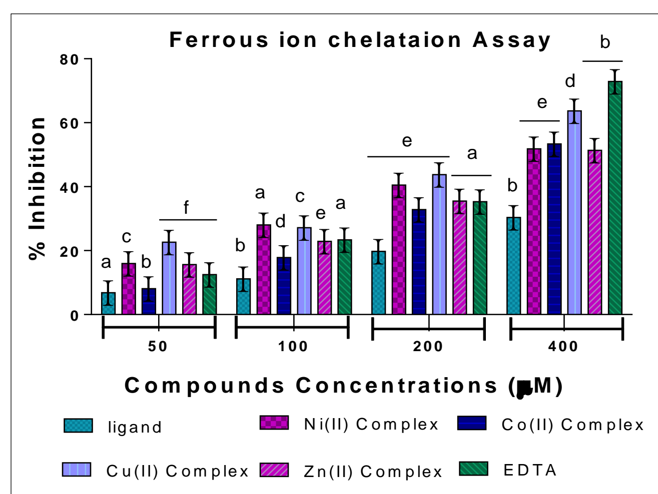
Table 2 and Figure 3 shows the Fe^{2+} chelating properties of ethanolic solution of Schiff base Ligand and their metal (Ni (II), Co (II), Cu (II), and Zn (II)) complexes and EDTA (ethylenediaminetetraacetic acid). Significant ($p<0.01$) were determined by One-way ANOVA test for samples when compared to control. The Schiff base Ligand observed at lower Fe^{2+}-chelating properties than metal complexes. The IC_{50} values for Fe^{2+}-chelating ability with ethanolic solution of ligand were found to be 297.45±5.771µM. The IC_{50} values for Ni (II) metal complex was determined to be 176.91±11.30µM and for Co (II) complex was noted to be 180.76±9.066µM. In this regard Zn(II) Complex showed marked metal chelating ability with IC_{50} values 186.83±11.85. The IC_{50} values for Ni(II) Complex was measured to be 297.45±5.771µM, which shows better Fe^{2+}-chelating activity than that of *Ligand* and Co(II) Complex but lower than Cu(II) Complex with 138.44±10.95. The IC_{50} values for EDTA (ethylenediaminetetraacetic acid) were found to be 135.05±15.94µM. These activities were dose dependent and maximum Fe^{2+}-chelating activity was observed at higher concentrations.

Two bidentate Ligands (Z)-2-((phenyl-imino)methyl)phenol are linked with central metal atom from both sides through oxygen and nitrogen atoms. Around the central metal atom the Ligands containing hydroxyl group located suitably at ortho position to benzene ring. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group. Hence, the overall Ligand acts as a rich π electrons species and their antioxidant power have further enhanced by complexation with transition metals.

All the metal complexes containing the same two bidentate Ligands (Z)-2-((phenyl-imino)methyl)phenol attached at the same position hence the antioxidant potential depend only on central metal atom. Cu(II) Complex shows high antioxidant power due to the presence of Copper metal which has high reduction potential as compared to Ni(II), Co(II) and Zn(II) metals. Hence Cu(II) metal complex has pronounce Fe^{2+}-chelation properties.

Table 2: Ferrous ion-chelating activity of Ligand and their metal complexes (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and EDTA) (Ethylenediaminetetraacetic acid). Values are expressed as mean ± SEM

Drugs	Concentrations (µM), Mean±SEM				IC_{50}(µM) ±SEM
	50(µM)	100(µM)	200(µM)	400(µM)	
Ligand	6.83±0.09	11.14±0.07	19.79±0.2	30.38±0.5	297.45±5.771
Ni(II) Complex	15.94±.08	28.00±0.1	40.52±0.3	51.85±0.6	176.91±11.30
Co(II) Complex	8.10±0.06	17.82±0.09	32.84±0.1	53.36±0.3	180.76±9.066
Cu(II) Complex	22.60±0.08	27.14±0.09	43.78±0.4	63.69±0.7	138.44±10.95
Zn(II) Complex	15.62±0.05	22.89±0.08	35.48±0.2	51.35±0.4	186.83±11.85
EDTA	12.49±0.07	23.37±0.09	35.28±0.1	72.89±0.3	135.05±15.94



Significantly $^{a}p < 0.0010$, $^{b}p < 0.0454$, $^{c}p < 0.0086$, $^{d}p < 0.0062$, $^{e}p < 0.0095$, $^{f}p < 0.0033$

Fig 3: Ferrous ion-chelating activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and EDTA) (Ethylenediaminetetraacetic acid). Values are expressed as mean ± SEM (triplicate tests were conducted for each sample)

Ferric Reducing/Antioxidant Power Assay

The compound reduction power may serve as a significant ($p< 0.01$) indicator of its potential antioxidant activity [92]. Table 3 and Figure 4 shows the reductive competences of different Schiff base ligand and their metal complexes when compared to the standard, Ascorbic acid. Like the antioxidant activity, the reducing power was dose-dependent which increased with increasing concentration of the compounds. The IC_{50} value was obtained by interpolation from linear regression analysis of concentrations vs % reduction. The ethanolic solution of Cu(II) Complex showed the highest reducing ability (IC_{50} 130.47±13.31µM) than all the other tested compounds. However, the activity was less than the standard, Ascorbic acid (IC_{50} 99.612±16.91µM)). The reducing capacity of Co(II) Complex and Ni(II) Complex also showed Significant IC_{50} values with 153.66±11.65 and 145.90±11.00µM respectively. Zn(II) Complex was determined to be 162.90±10.61µM µM that is lower among the tested Schiff base complexes but greater than ligand which showed IC_{50} 293.28±6.538µM.

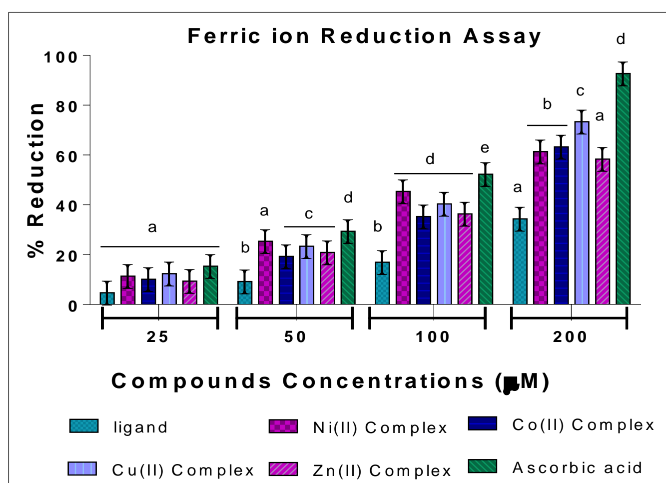
The Structure of Schiff base ligand (Z)-2-((phenyl-imino) methyl) phenol containing hydroxyl group located suitably at

ortho position to benzene ring. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group. Hence, the overall ligand acts as a rich π electrons species and their antioxidant power. The antioxidant potential of Schiff base ligand has further enhanced by complexation with transition metals linked with central metal atom from both sides through oxygen and nitrogen atoms.

All the metal complexes containing the same two bidentate Ligands (Z)-2-((phenyl-imino) methyl) phenol attached at the same position hence the antioxidant potential depend only on central metal atom. Cu(II) Complex shows high reducing power due to the presence of Copper metal atom as compared to Ni(II), Co(II) and Zn(II) metals which reduced Fe^{3+} to Fe^{+2}.

Table 3: Ferric ion reducing activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Values are expressed as mean ± SEM.

Drugs	Concentrations (µM), Mean±SEM				IC50(µM) ±SEM
	50(µM)	100(µM)	200(µM)	400(µM)	
Ligand	4.67±0.02	9.21±0.05	16.92±0.09	34.38±0.2	293.28±6.538
Ni(II) Complex	11.33±0.03	25.38±0.05	45.37±0.08	61.37±0.3	145.90±11.00
Co(II) Complex	10.10±0.02	19.27±0.06	35.29±0.09	63.26±0.2	153.66±11.65
Cu(II) Complex	12.68±0.01	23.38±0.04	40.36±0.07	73.37±0.4	130.47±13.31
Zn(II) Complex	9.33±0.03	20.89±0.06	36.33±0.08	58.39±0.3	162.90±10.61
Ascorbic acid	15.37±0.03	29.34±0.07	52.31±0.09	92.74±0.5	99.612±16.91



Significantly $^{a}p < 0.0003$, $^{b}p < 0.0379$, $^{c}p < 0.0013$, $^{d}p < 0.0009$, $^{e}p < 0.0101$,

Fig 4: Ferric ion reducing activity of Ligand and their metal complexes (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Values are expressed as mean ± SEM (triplicate tests were conducted for each sample).

Total antioxidant activity (Phosphomolybdenum assay)

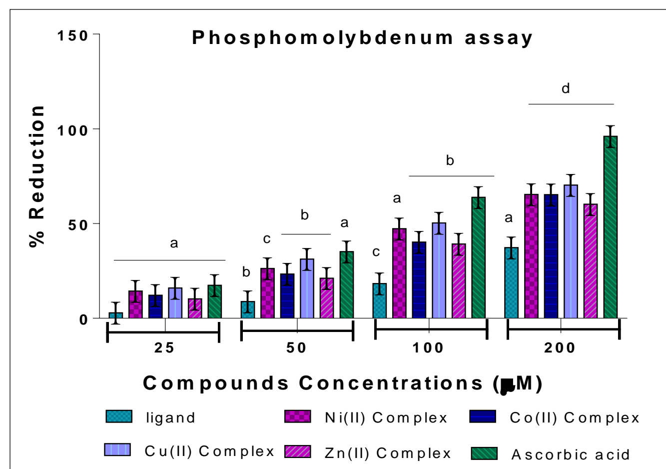
Total antioxidant capacity of Schiff base ligand and their metal complexes has been evaluate by using phosphomolybdate method with Ascorbic acid as a standard. The Mo(VI) is reduced to Mo(V), in the presence of drugs which shows maximum absorbance at 695 nm. All the compounds tested by this method possessed significant ($p<0.01$) antioxidant activity and the reducing power was dose-dependent which increased with increasing concentration of the compounds. The IC_{50} was calculated for each Schiff base ligand and their metal complexes as well as ascorbic acid as standard and summarized in table 4 and graphically represented in Figure 5. Among the drugs tested, Cu(II) complex was found to be highest antioxidant (IC_{50} 120.93±11.76µM. The reducing capacity of Ni(II) Complex and Co(II) Complex were found to be with the same antioxidant activity, IC_{50} 134.78±11.29µM, 134.42±11.55µM respectively. Ni(II) Complex & Co(II) Complex with IC_{50} 134.78±11.29µM showing lower antioxidant activity than Cu(II) Complex but higher than Zn(II) Complex with IC_{50} 155.77±11.76. The IC_{50} of ligand was found to be 264.41±7.532µM which showed lower antioxidant activity among the tested compounds. The IC_{50} values for Ascorbic acid were found to be 86.729±17.21µM.

Ligand (Z)-2-((phenyl-imino) methyl) phenol containing hydroxyl group at ortho position to benzene ring and nitrogen atom are interconnection between two rings. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group. Hence, the overall ligand acts as a rich π electrons species and their antioxidant power have further enhanced by complexation with transition metals.

All the metal complexes containing the same two bidentate ligands (Z)-2-((phenyl-imino) methyl) phenol attached at the same position and the antioxidant potential depend only on central metal atom. Cu(II) Complex shows high reducing power due to the presence of Copper metal atom as compared to Ni(II), Co(II) and Zn(II) metals which reduce Mo(VI) to Mo(V).

Table 4: Molybdenum ion reducing activity of Schiff base ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Values are expressed as mean ± SEM.

Drugs	Concentrations (µM), Mean±SEM				IC50(µM) ±SEM
	50(µM)	100(µM)	200(µM)	400(µM)	
Ligand	2.83±0.1	8.82±0.3	18.28±0.5	37.29±0.8	264.41±7.532
Ni(II) Complex	14.39±0.09	26.29±0.2	47.29±0.6	65.39±0.9	134.78±11.29
Co(II) Complex	12.20±0.2	23.33±0.1	40.22±0.5	65.28±0.7	134.42±11.55
Cu(II) Complex	15.98±0.08	31.29±0.3	50.29±0.5	70.28±0.6	120.93±11.76
Zn(II) Complex	10.22±0.07	21.10±0.2	39.22±0.4	60.19±0.8	155.77±11.76
Ascorbic acid	17.37±0.1	35.12±0.3	63.82±0.5	96.22±0.8	86.729±17.21



Significantly $^{a}p < 0.0005$, $^{b}p < 0.0268$, $^{c}p < 0.0064$, $^{d}p < 0.0156$

Fig 5: Hydroxyl radical scavenging power of Schiff base Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Values are expressed as mean ± SEM

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the Schiff ligand and their metal complexes was investigated by O-phenanthroline method. All the compounds exhibited strong concentration-dependent scavenging significant (p<0.01) properties for the hydroxyl radical demonstrated in Table 5 and graphically represented in figure 5. The Cu(II) and Zn(II) metal complexes were found to be the most powerful scavenger of the hydroxyl radical, with IC_{50} up to 36.04±12.84μM and 36.91±15.01μM respectively. Among the complexes the weakest scavenger was found to be Ni(II) metal complex with IC_{50} 39.41±14.32μM. Furthermore, the power of scavenging ability of Co(II) metal complex was noted to be 43.19±13.62μM which, shows better hydroxyl radical scavenging activity than Ni(II) metal complex but lower than Cu(II) and Zn(II) metal complexes. The ligand result showed with the IC_{50} 49.63±8.391μM is the lowest among the tested compounds. The IC_{50} values for Ascorbic acid as a standard was found to be 29.25±17.21μM.

Two bidentate Ligands (Z)-2-((phenyl-imino) methyl) phenol are linked with central metal atom from both sides through oxygen and nitrogen atoms. Around the central metal atom the Ligands containing hydroxyl group located suitably at ortho to benzene ring. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group. Hence, the overall Ligand acts as a rich π electrons species and their antioxidant power have further enhanced by complexation with transition metals.

All the metal complexes containing the same two bidentate Ligands (Z)-2-((phenyl-imino) methyl) phenol attached at the same position and the antioxidant potential depend only on central metal atom. Zn(II) and Cu(II) Complexes shows almost same antioxidant as compared to Ni(II), Co(II) metal complexes which scavenge the hydroxyl radical.

Table 5: Hydroxyl radical scavenging power of Schiff base Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Values are expressed as mean ± SEM.

Drugs	Concentrations (μM), Mean±SEM				IC_{50}(μM) ±SEM
	50(μM)	100(μM)	200(μM)	400(μM)	
Ligand	5.75±0.06	10.27±0.08	22.28±0.09	43.29±0.3	49.63±8.391
Ni(II) Complex	10.39±0.05	22.29±0.07	45.29±0.1	75.39±0.4	39.41±14.32
Co(II) Complex	9.20±0.05	20.33±0.08	40.27±0.1	71.28±0.3	43.19±13.62
Cu(II) Complex	11.98±0.04	21.22±0.07	39.29±0.09	70.28±0.4	36.04±12.84
Zn(II) Complex	12.22±0.06	24.12±0.06	48.22±0.2	80.19±0.3	36.91±15.01
Ascorbic acid	15.37±0.04	30.12±0.05	62.82±0.1	93.00±0.4	29.25±17.21

Table 6: The IC_{50}(μM)±SEM values of DPPH, FIC, FRAP, TAA and •OH assays for the radical scavenging activity of Ni(II), Co(II), Cu(II), Zn(II) Complex, ascorbic acid and standard EDTA

Compound Names	DPPH assay IC_{50}(μM)±SEM	FIC assay IC_{50}(μM)±SEM	FRAP assay IC_{50}(μM)±SEM	TAA assay IC_{50}(μM)± SEM	•OH assay IC_{50}(μM)± SEM
ligand	170.34±11.85	297.45±5.771	293.28±6.538	264.41±7.532	49.63±8.391
Ni(II) Complex	170.34±11.85	176.91±11.30	145.90±11.00	134.78±11.29	39.41±14.32
Co(II) Complex	170.34±11.85	180.76±9.066	153.66±11.65	134.42±11.55	43.19±13.62
Cu(II) Complex	170.34±11.85	138.44±10.95	130.47±13.31	120.93±11.76	36.04±12.84
Zn(II) Complex	170.34±11.85	186.83±11.85	162.90±10.61	155.77±11.76	36.91±15.01
Ascorbic acid	170.34±11.85	__________	99.612±16.91	86.729±17.21	29.25±17.21
EDTA	__________	135.05±15.94	__________	__________	__________

DNA Binding activity

The electronic absorption spectroscopy is the most common way to investigate the interactions of various Schiff base metal complexes with DNA. In general, complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism), due to the strong staking interaction between aromatic chromophore of the complex aromatic π rings and the base pairs of DNA. The ligand, Ni(II), Co(II) Cu(II), and Zn(II) complexes showed absorption bands at 331, 325, 427.5, 387 and 321 nm with increasing concentration of DNA. All the complexes showed hypochromicity and a red-shifted charge transfer peak maxima in the absorption spectra. The absorption spectra of the ligand, Ni(II), Co(II) Cu(II), and Zn(II) complexes in the absence and presence of SH DNA are given in Figure 6, 7, 8, 9 and 10, respectively. With the addition of DNA, the absorption intensities gradually decreased. A total of 10% (for ligand), 15% (for Ni), 17% (for Co), 14% (for Cu) and 16% (for Zn) of hypochromicity with 2.0, 1.0, 1.5, 2.5 and 1.0 of red shift were obtained. The intrinsic binding constants for ligand, Cu(II), Ni(II), Co(II) and Zn(II) complexes are found to be $3.487\times10^{-5}M^{-1}$, $1.858\times10^{-5}M^{-1}$, $3.090\times10^{-5}M^{-1}$,

$1.367\times10^{-5}M^{-1}$ and $9.118\times10^{-5}M^{-1}$, respectively (illustrate in Table 7) indicating a moderate intercalation between the complexes and Salmon fish DNA. These *k*b values are much smaller than the typical classical intercalators. In order to compare the binding strength of the complexes with Salmon fish DNA the *k*b were obtained by monitoring the changes in the absorbance for the complexes with increasing concentration of DNA. The *k*b was obtained from the ratio of slope to the intercept from the plot of [DNA]/(Ɛa-$Ɛ_f$) versus [DNA].

Table 7: Electronic absorption spectral properties of ligand, Ni(II), Co(II), Cu(II) and Zn(II) complexes.

Compound Names	λ-max		Δλ (nm)	*k*b $\times10^{-5}$ (M^{-1})
	Free	Bond		
ligand	329	331	2.0	3.487
Ni(II) Complex	324	325	1.0	1.858
Co(II) Complex	426	427.5	1.5	3.090
Cu(II) Complex	384.5	387	2.5	1.367
Zn(II) Complex	320	321	1.0	9.118

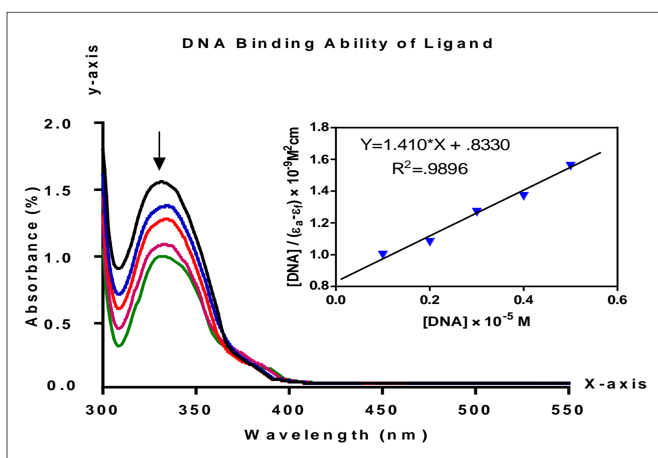


Fig 6: Absorption spectra of ligand in buffer pH 7.2 at 25 °C in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-$Ɛ_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with ligand.

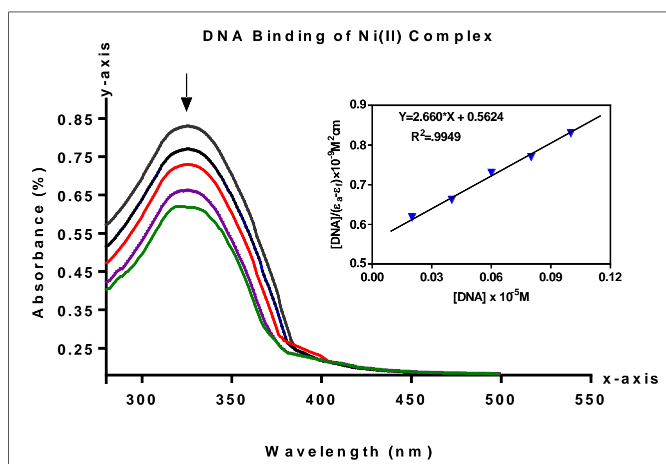


Fig 7: Absorption spectra of Ni(II) complex in buffer pH 7.2 at 25 °C in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA]/(Ɛa-$Ɛ_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with Ni(II) complex.

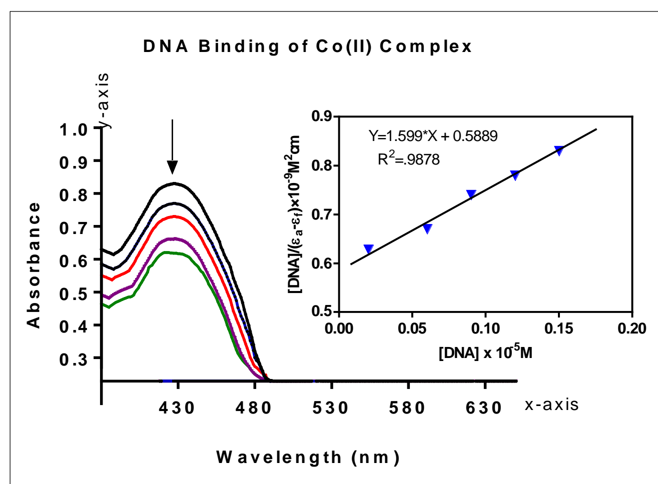


Fig 8: Absorption spectra of Co(II) complex in buffer pH 7.2 at 25 °C in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-$Ɛ_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with Co(II) complex.

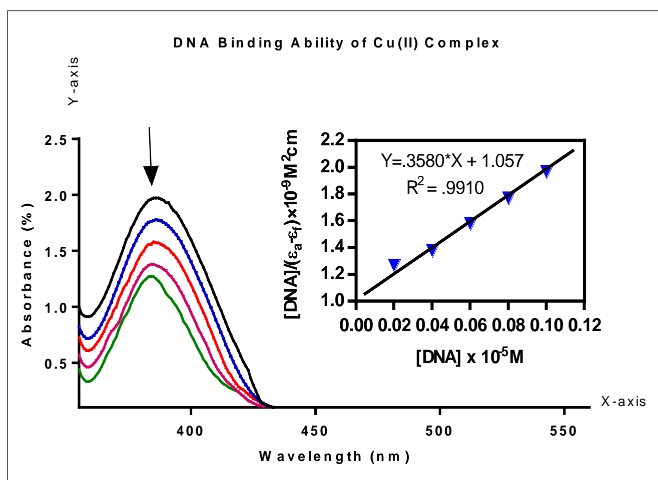


Fig 9: Absorption spectra of Cu(II) complex in buffer pH 7.2 at 25 °C in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-$Ɛ_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with Cu(II) complex

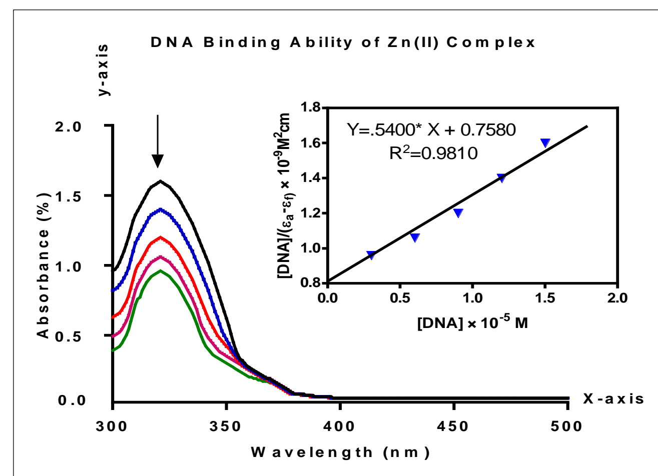


Fig 10: Absorption spectra of Zn(II) complex in buffer pH 7.2 at 25 °C in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-$Ɛ_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with Zn(II) complex.

4. Conclusion
In the present study we performed the antioxidant and DNA binding study of a Schiff base ligand and its metal complexes. This preliminarily screening of these synthetic compounds reveals interesting antioxidant and DNA binding activities, however, these results cannot be extended directly to *in vivo* systems which are differ and more complex from *in vitro* studies. Additionally, the obtained result showed that it is interesting to note that metal complexes presented higher DPPH radical and OH radicals activities, Fe+2 chelation, reducing Fe(III) to Fe(II) and Mo(VI) to Mo(V) higher than free ligand. The findings of this study clearly indicated that the antioxidant effects of Schiff base ligand and its metal complexes, presented DPPH radical-scavenging activity, demonstrating the mechanism by which these drug displayed antioxidant activities. The ability of Schiff base Ligand and its metal complexes to show significant reducing power and to hunt DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions. In conclusion the results presented in the present studies give information about the nature of antioxidants and DNA Binding intercalators found in Schiff base metal complexes.

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