IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 10, Issue 3 Ver. I (Mar. 2017), PP 06-14
www.iosrjournals.org

In Vitro Antioxidant evaluation and DNA binding ability of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes containing bidentate Schiff base

Mohammad Ibrahim[*1] Asif Khan[*1], Bushra Faiz[1], Muhammad Ikram[1], HazratUn Nabi[1], Muzamil Shah[1], Ahamefula A Ahuchaogu[2]
[1]*Department of Chemistry, Abdul Wali Khan University Mardan, Khyber pakhtunkhwa, Pakistan,*
[2]*Abia State University, Uturu Nigeria Department of Industrial Chemistry*

Abstract: *In the present study, the antioxidant and DNA binding properties of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes containing bidentate Schiff base for their possible free radicals scavenging properties associated with various diseases were considered. 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 withkb* $4.990\times10^{-5}M^{-1}$,$4.989\times10^{-5}M^{-1}$,$4.994\times10^{-5}M^{-1}$ *and* $5.012\times10^{-5}M^{-1}$ *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: *Free radicals, Schiff base complexes, Antioxidants, DNA binding.*

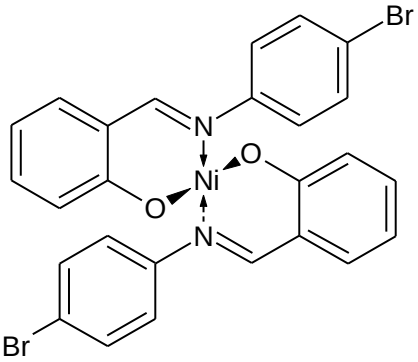
I. Introduction

A radical ("Free radical") is a species that possesses one or more ("odd or single") electrons.They are usually generated in the bodyinternally during normal metabolic activities or transferred from environment to biological system. A large number of radical and non-radical species derived from reactive oxygenspecies(ROS) such as peroxyl radicals($ROO^{\bullet}$), superoxide anion($O_2^{\bullet-}$), hydroxyl($^{\bullet}OH$), reactive hydrogen peroxide(H_2O_2) and reactive nitrogen species (RNS) like nitric oxide($NO^{\bullet}$) nitrogen dioxide($NO_2^{\bullet}$), peroxynitrite anion($ONOO^{\bullet}$) have a greater impact on humans [1-3].

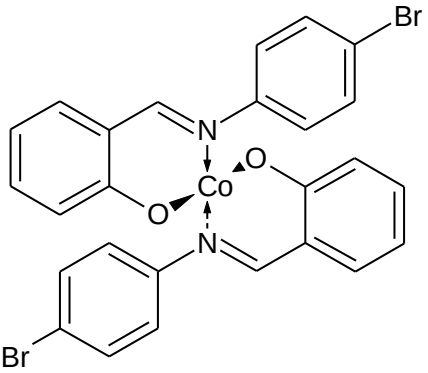
In a living system, stimulation of macrophages, leucocytes and aerobic respiration are endogenous sources, while the tobacco smokes, pollutants, ionizing radiations, organic solvents and pesticides are the major exogenous sources of free radicals production [4-5]. Excess production of these free radicals 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 and Parkinson diseases [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 and flavonoids[18], a number of synthetic antioxidants like butylatedhydroxyltoluene (BHT), butylatedhydroxyanisole (BHA) and tertiary butylhydroquinone have been prepared and their antioxidant capacity has been assessed for prevention of various diseases [19-20].

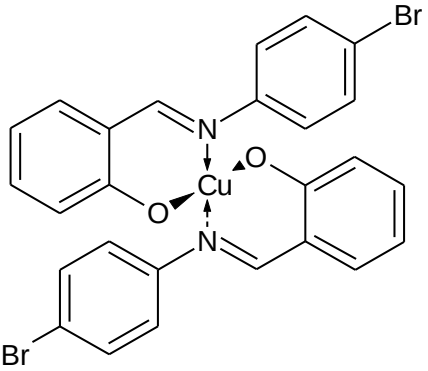
In the present study, the antioxidant activity of newly synthesized Schiff base Ni(II), Co(II), Cu(II) and Zn(II) metal complexes were evaluated *in vitro* for their possible antioxidant and DNA binding properties. The names of compounds along with their structure employed in the present work are given in Figure 1.



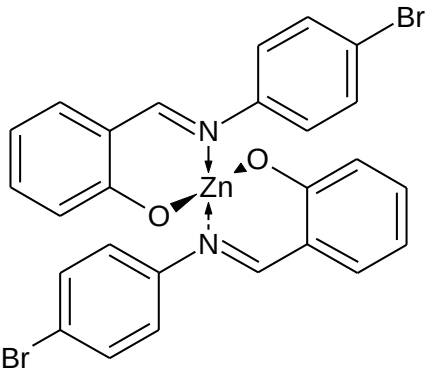
Bis(2-{(E)-[(4-bromophenyl)imino]methyl}phenolate)nickel(II)



Bis(2-{(E)-[(4-bromophenyl)imino]methyl}phenolate)cobalt(II)



Bis(2-{(E)-[(4-bromophenyl)imino]methyl}phenolate)copper(II)



Bis(2-{(E)-[(4-bromophenyl)imino]methyl}phenolate)zinc(II)

Figure 1: Ni(II), Co(II), Cu(II) and Zn(II) Schiff base metal complexes

II. Materials And Methods

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl radical(DPPH), ascorbic acid, ethylenediaminetetraacetic acid (EDTA), ferrous sulphate, trisHClbuffer, ferric chloride ($FeCl_3$), O-phenenthroline, sulfuric acid, ammonium molybdate, potassium phosphate (mono phosphate and diphosphate), hydrogen peroxide (H_2O_2), Salmon fish DNA and ethanol were analytical grade purchased from Sigma Aldrich, Pakistan.

Determination of *In-vitro* antioxidant studies

The antioxidant activity of the Ni(II), Co(II), Cu(II) and Zn(II) metal Schiff base complexes were evaluated by using rapid and simpler free scavenging assays viz, DPPH 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 Ni(II), Co(II), Cu(II) and Zn(II) metal based Schiff base complexes were assessed using the stable DPPH free radical according to Shanab*et al.* [21]. Various concentrations (30, 50, 70, 100 and 200μM) of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes were mixed with an ethanolic solution containing 85μM DPPH radical. The mixture solutions were incubated for 30 minutes at room temperature 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. The experiment was carried out in triplicate. Percentage inhibition of the drugs as well as ascorbic acid was calculated by using Equation 1.

DPPH inhibition effect (%) = $(A_c – A_s / A_c) \times 100$ (Equation 1)

Where A_c is the absorbance reading of the control and**A_s** is the absorbance reading of the sample

Ferrous ion-chelating assay

The ferrous ion chelating activity of Ni(II), Co(II), Cu(II) and Zn(II) metal based Schiff base complexes were evaluated by a standard method Puntel*et al.* [22]. Various concentrations (30, 50, 70, 100 and 200μM) of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes were mixed with 0.2 mL of 3.6 mM ferrous sulphate, 0.3 mL of 100 mMTris-HCl (pH=7.4), 0.1 mL of 9 mM O-phenanthroline and diluted up to 3.0 mL withultra-puredistilled water. The reaction mixture was shaken vigorously, incubated for 10 minutes and the decrease in absorbance was determined at 510 nm. Ethylenediaminetetraaceticacid(EDTA) 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 Equation 2.

Chelating effect (%) = $(A_c – A_s / A_c) \times 100$ (Equation 2)

Where A_c is the absorbance reading of the control and As is the absorbance reading of the sample

Ferric Reducing/Antioxidant Power Assay

The ferric reducing power of the Ni(II), Co(II), Cu(II) and Zn(II) metal based Schiff base complexes was determined as described by Kumar *et al.*[23]. Different concentrations (30, 50, 70, 100 and 200μM) of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes, 0.2 mL of 3.6 mM ferric chloride, 0.3mL of 100 mMtris buffer (pH=7.4), 0.1 ml of 9 mM O-phenanthroline and diluted up to 3.0 mL with ultra-pure distilled water. The reaction mixtures are shaken vigorously and left to stand at room temperature for 10 minutes. 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 Equation 3.

Reducing Power (%)= $(A_s – A_c / A_s) \times 100$ (Equation 3)

where A_c is the absorbance reading of the control and **A_s** is the absorbance reading of the sample

Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant capacity of Ni(II), Co(II), Cu(II) and Zn(II) metal based Schiff base complexes were evaluated by phosphomolybdenum assay assessed by Sahaa*et al*. [24]. Reagent solution containing various concentrations (30, 50, 70, 100 and 200μM) of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes aliquot in ethanol, 0.7 mL of 0.6 M sulphuric acid, 1.0 mM ammonium molybdate, 1.0 mL of 28 Mm pottacium pasphate and ultra pure distilled water was incubated at 95°C for 90 minutes. After cooling to 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 Equation 4.

Reducing Power (%) = $(A_s – A_c / A_s) \times 100$ (Equation 4)

Where A_c is the absorbance reading of the control and As is the absorbance reading of the sample

Hydroxyl radical scavenging activity

The scavenging activity of Ni(II), Co(II), Cu(II) andZn(II) metal based Schiff base Complexesfor hydroxyl radicals was measuredwith Fenton reaction [25]. Reaction mixture of various concentrations (30, 50, 70, 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 minutes 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 5.

Scavenging power (%)=$(A_s–A_c/ A_b–A_s)\times$ 100 **(Equation 5)**

Where A_c is the absorbance reading of the control, As is the absorbance reading of the sampleand 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 mMNaCl / 5mM 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 [26]. 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 4ºC 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 6to obtain the *k*b values for interaction of the complexes with DNA.

$[DNA] / (\varepsilon a-\varepsilon_f) = DNA/ (\varepsilon a-\varepsilon_f) + 1/ [kb (\varepsilon_b-\varepsilon_f)]$ **(Equation 6)**

Where εa, ε_f, and ε_b are the apparent, free and bound metal complex extinction coefficients, respectively. A plot of $[DNA]/ (\varepsilon_b-\varepsilon_f)$ versus [DNA], gave a slope of $1/ (\varepsilon_b-\varepsilon_f)$ 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®. 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.

III. Results and Discussion

DPPH radical scavenging assay

DPPH radical scavenging assay has been extensively used for screening antioxidant activity because it is sensitive enough to detect active ingredients at low concentration, the absorbance is reduced when encounter with a proton donating substance such as an antioxidant. Thus, the DPPH radicals have been widely used to investigate the scavenging activity of some synthetic compounds. In the present study, the total ethanolicsolution of all synthesized Schiff baseNi(II), Co(II), Cu(II) and Zn(II) complexes comparable with ascorbic acid were screened for *in vitro* DPPH free radical scavenging activities and all of them shows concentration dependent activities above 50% inhibition. IC_{50} values of compounds come out to be between 271.30±6.09μM and 197.44±8.84μM compared with standard ascorbic acid (IC_{50} = 76.29±14.46μM) (Table1). Ni(II) complex (IC_{50} = 271.30±6.09μM), showed comparable activity with the standard vitamin C (IC_{50} = 76.29±14.46μM). Similarly, Co(II) complexeswith IC_{50} = 208.82±7.90μM and Cu(II) complexes with IC_{50} = 224.89±7.54μM indicate moderate activity as compared with the standard ascorbic acid. Zn(II) Complexes(IC_{50} = 197.44±8.84μM) was found to be most active among all the tested complexes, comparable to the standard ascorbic acid.

In all the structure feature of metal complexes, metals are attached to two bidentate ligands (2-{(E)-[(4-bromophenyl)imino]methyl}phenolate) in which one bromine atom (Br^-) suitably located to benzene ring at para position while one oxygen atom is attached to the other benzene ring at ortho position. Although both bromine and hydroxyl groups are ortho/para directing but bromine atom withdraw the free electron from the ring acts as deactivate while oxygen atom donate the free electrons to the ring and acts as ring activating group. Due the bulky and low electronegativity nature of bromine atom the electron withdrawing power is less than oxygen atom donating potential present at ortho position. Hence the overall ligand acts as powerful antioxidant and

proton donating substituent to DPPH radical. Among all the complexes bonded to two the same ligands are depend on only the reduction potential of the central metal atom. Zn(II) Complex shows greater activities due to its greater potential power, Co(II) Complex and Cu(II) Complex are found to be moderate while Ni(II) Complex are least active as compared to the standard ascorbic acid.

Table 1:DPPH radical scavenging activity of Ni(II), Co(II), Cu(II),Zn(II) complexes and ascorbic acid.

Compounds	**%Inhibition Mean (n=3)±SEM**					**IC_{50}(μM)±SEM**
	30 (μM)	50 (μM)	70 (μM)	100(μM)	200(μM)	
Ni(II) Complex	2.33±0.88	7.00±2.00	10.66±1.76	20.66±2.02	36.66±3.52	271.30±6.09
Co(II) Complex	2.00±.57	6.33±1.33	18.00±2.64	29.66±1.85	45.33±5.36	208.82±7.90
Cu(II) Complex	0.33±1.20	5.66±0.88	15.66±1.20	25.66±0.88	42.66±1.45	224.89±7.54
Zn(II) Complex	1.33±0.33	5.66±0.33	11.33±0.33	26.33±4.17	50.00±5.13	197.44±8.84
Ascorbic acid	12.66±0.57	26.00±1.20	49.00±2.08	71.00±2.08	88.66±2.90	76.29±14.46

Ferrous ion-chelating assay

All the tested Schiff base metal complexes shows dose dependent Fe^{2+}-chelating activity comparable with standard EDTA in Table. The IC_{50} values for Fe^{2+}-chelating capability of Schiff base metal complexes of Ni(II) (272.01±5.65μM), Co(II) (252.73±6.81μM), Cu(II) (155.45±10.60μM), and Zn(II) (188.86±8.91μM) were determent and compared with standard EDTA (131.58±11.64μM). Among the metal complexes, Cu(II) metal complex was found to be better Fe^{2+}-chelating potency, Co(II) and Zn(II) complexes were moderates and Ni(II) complex was least better Fe^{2+}-chelating power.

Two bidentate ligands (2-{(E)-[(4-bromophenyl)imino]methyl}phenolate) are linked with central metal atom from both sides through oxygen and nitrogen atoms. In ligand the oxygen atom is attached to the one benzene ring at ortho position while bromine(Br^-) atom suitably located on the other benzene ring at para position while. Although both oxygen and bromine atoms are ortho/para directing but oxygen atom donates the free electrons to the ring and acts as ring activating group while bromine atom withdraw the free electrons from the ring acts as deactivating group.The bulky and low electronegativity nature of bromine atom the electron withdrawing power is less than oxygen atom having greater electrons donating potential present at ortho position. Hence, the overall complex acts as rich electrons species and acts as powerful dose dependent Fe^{2+}-chelating antioxidant. Among all the complexes bonded to two the same ligands, Cu(II) Complex shows greater iron chelation due to rich electrons cloud, Co(II) and Zn(II) Complexes were found to be moderate while Ni(II) Complex was least active Fe^{2+}-chelating antioxidant as compared to the standard EDTA.

Table 2: Ferrous ion-chelating activity of Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) complex and EDTA

Compounds	**%Chelation Mean (n=3)±SEM**					**IC_{50} (μM)±SEM**
	30 (μM)	50 (μM)	70 (μM)	100(μM)	200(μM)	
Ni(II) Complex	1.66±0.66	5.00±0.57	11.00±1.00	22.00±1.52	36.66±0.66	272.01±5.65
Co(II) Complex	1.33±0.33	2.33±0.33	14.66±0.66	21.33±1.76	38.33±4.33	252.73±6.81
Cu(II) Complex	1.00±0.00	8.33±0.33	25.66±0.33	46.00±1.73	56.33±3.18	155.45±10.60
Zn(II) Complex	5.33±0.88	8.33±0.66	13.00±1.15	24.00±1.52	54.33±5.66	188.86±8.91
EDTA	7.00±1.15	14.66±2.02	22.00±1.52	42.66±1.76	71.66±3.28	131.58±11.64

Ferric Reducing / Antioxidant Power Assay

The significant ($p<0.05$) values of ferric ion indicates the reductive capabilities of various evaluating Schiff base metal complexes. Table 3 illustrates the percentage reduction potential of different Schiff base metal complexes comparable with that of standard ascorbic acid. The reducing power increased with increasing concentration of the compounds which acts as dose dependent like the antioxidant activity. The ethanolic solution of Zn(II) complex showed the highest reducing ability (IC_{50}134.59±11.71μM) than all the tested compounds. Cu(II) and Co(II) complexes were observed almost with the same ferric reduction power as182.15±8.14μM and 182.13±11.13μM which are less than Zn(II) Complex but higher than Ni(II) Complex. Among the all metal complexes, Ni(II) Complex was measured with low ferric ion reduction (IC_{50}302.86±5.48μM).

Antioxidant can play their protective role by two mechanisms. First mechanism involves hydrogen abstraction from antioxidant (revealed in DPPH assay) while in the second mechanism, antioxidant can give an electron to the free radical and itself becomes radical cation. According to first mechanism the weaker the OH bond the more will be antioxidant activity while according to the second mechanism, lower ionization potential is responsible for more antioxidant activity. An insight to structure-activity relationship reveals the fact that the presence of electron donating group increases the antioxidant activity. All the Schiff base metal complexes contain aromatic ring which provide π electrons to the ferric ion by the process of resonance. Hence all the tested metal Schiff base complexes were determent as the same DPPH and ferrous ion chelation assay.

Table 3: Ferric ion reducing activity of Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) complexes and ascorbic acid.

Compounds	%Reduction Mean (n=3)±SEM					IC_{50} (μM)±SEM
	30 (μM)	50 (μM)	70 (μM)	100(μM)	200(μM)	
Ni(II) Complex	2.33±0.88	4.66±0.33	7.00±0.57	16.00±1.15	32.33±2.60	302.86±5.48
Co(II) Complex	6.00±.57	13.33±0.88	21.33±1.20	43.33±2.02	67.00±2.00	182.13±11.13
Cu(II) Complex	6.66±0.88	14.33±0.88	21.33±176	29.66±1.45	54.00±4.16	182.15±8.14
Zn(II) Complex	7.66±0.33	13.33±0.33	21.33±2.40	36.66±1.76	73.00±2.08	134.59±11.71
Ascorbic acid	10.33±0.88	21.00±1.73	41.33±2.60	65.00±2.08	87.33±2.90	90.07±14.11

Total antioxidant activity (Phosphomolybdenum assay)

Total antioxidant capacity of Shiff base metal complexes have been evaluated 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 ($p<0.05$) antioxidant activity and the reducing power was dose-dependent which increased with increasing concentration of the compounds. The IC_{50} was calculated for each metal Schiff base complexes as well as ascorbic acid as standard and summarized in Table 4 for molybdate radicals with ethanol solution. Among the complexes, Zn(II) Complex was found to be highest reduction power for Mo(VI) as IC_{50}134.80±10.29μM,Cu(II) and Co(II) Complexes were observed as lower antioxidant power for Mo(VI) as IC_{50}150.93±10.34 and159.58±9.93μM, respectively. Ni(II) complex was measured as weakest reducing power for Mo(VI) with IC_{50} 249.13±6.62μM comparable with standard ascorbic acid (IC_{50}83.96±15.24μM).

An insight to structure feature activity relationship reveals the fact that thepresence of electron donating group increases the antioxidant activity. All the Schiff base metal complexes contain aromatic rings which provide π electrons to reduce Mo(VI) to Mo(V) by the process of resonance. Furthermore, the order of antiradical ability for the Mo(VI) reduction was similar to those for DPPH radicals, ferrous ion chelation and ferric ion reduction of various metal Schiff base metal complexes.

Table 4: Molybdenum ion reducing activity of Ni(II), Co(II), Cu(II),Zn(II) Complex and ascorbic acid.

Drugs	%Reduction Mean (n=3)±SEM					IC_{50} (μM)±SEM
	30 (μM)	50 (μM)	70 (μM)	100(μM)	200(μM)	
Ni(II) Complex	3.00±0.57	7.00±1.15	16.00±1.73	31.66±2.02	37.00±2.30	249.13±6.62
Co(II) Complex	4.00±0.57	10.00±1.15	22.00±1.73	44.00±2.30	56.00±2.88	159.58±9.93
Cu(II) Complex	5.00±1.15	11.00±1.15	23.00±1.73	48.00±1.73	58.00±2.88	150.93±10.34
Zn(II) Complex	8.00±0.57	17.00±0.57	31.00±1.15	51.00±2.30	63.00±1.73	134.80±10.29
Ascorbic acid	11.00±1.15	20.00±2.30	44.00±1.73	67.00±1.15	94.00±2.88	83.96±15.24

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the various metal Schiff base complexes were investigated (Table 5). All the complexes exhibited strong concentration-dependent scavenging abilities for the hydroxyl radical. Ni(II) complex was found to be the most powerful scavenger of the hydroxyl radical, with an inhibition of up to IC_{50}49.41±7.05μM. Zn(II) complex was measured as slightly lower than Ni(II) complex as IC_{50}50.49±7.52μM.Cu(II) and Co(II) complexes showed the weakest scavenging activity hydroxyl radical. Ascorbic acid was treated as positive control.

Two bidentateligands (2-{(E)-[(4-bromophenyl)imino]methyl}phenolate) are linked with central metal atom from both sides through oxygen and nitrogen atoms. Around the central metal atom the ligands containing both oxygen at ortho position while bromine atom(Br^-) suitably located to benzene ring at para position. Due to less electronegativity and bulky nature of bromine atom withdraw electrons from benzene ring less effectively compared to oxygen atom having high electron donating power. Hence, the overall complex acts as powerful dose dependedhydroxyl radical scavenging antioxidant.

Table 5: Hydroxyl ion scavenging assayNi(II), Co(II), Cu(II),Zn(II)and Ascorbic acid

Drugs	% Inhibition (Mean=3))±SEM					IC_{50} (μM)±SEM
	30 (μM)	50 (μM)	70 (μM)	100(μM)	200(μM)	
Ni(II) Complex	4.464±0.78	8.99±0.78	16.60±1.25	30.19±0.56	42.79±1.39	49.41±7.05
Co(II) Complex	5.21±0.87	9.16±0.88	17.01±1.50	30.50±0.77	46.29±1.93	52.00±7.52
Cu(II) Complex	7.21±0.85	10.29±0.01	19.10±0.10	31.70±0.00	52.27±1.44	51.30±8.22
Zn(II) Complex	6.27±0.75	10.88±1.10	19.34±1.48	32.29±1.53	47.63±2.80	50.49±7.52
Vit. C	18.86±1.10	35.22±1.50	60.74±2.63	72.37±2.78	96.26±2.90	6.47±13.64

Table 6: The $IC_{50}(\mu M)$±SEM values of DPPH, FIC, FRAP, TAA and $^{\bullet}OH$ 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 $IC_{50}(\mu M)$±SEM	FIC assay $IC_{50}(\mu M)$±SEM	FRAP assay $IC_{50}(\mu M)$±SEM	TAA assay $IC_{50}(\mu M)$± SEM	$^{\bullet}OH$ assay $IC_{50}(\mu M)$± SEM
Ni(II) Complex	271.30±6.09	272.01±5.65	302.86±5.48	249.13±6.62	49.41±7.05
Co(II) Complex	208.82±7.90	252.73±6.81	182.13±11.13	159.58±9.93	52.00±7.52
Cu(II) Complex	224.89±7.54	155.45±10.60	182.15±8.14	150.93±10.34	51.30±8.22
Zn(II) Complex	197.44±8.84	188.86±8.91	134.59±11.71	134.80±10.29	50.49±7.52
Ascorbic acid	76.29±14.46	_____	90.07±14.11	83.96±15.24	6.47±13.64
EDTA	_____	131.58±11.64	_____	_____	_____

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 Ni(II), Co(II) Cu(II), and Zn(II) complexes showed absorption bands at 306, 327, 336, and 359 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 Cu(II) and Co(II) complexes in the absence and presence of Salmon fishDNA are given in Figure 2, 3, 4 and 5, respectively. With the addition of DNA, the absorption intensities gradually decreased. A total of 24% (for Cu), 13% (for Co), 11% (for Ni) and 9% (for Zn) of hypochromicity with 2.0, 1.5, 1.0 and 1.0 of red shift were obtained. The intrinsic binding constants for Cu(II), Ni(II), Co(II) and Zn(II) complexes are found to be $4.990\times10^{-5}M^{-1}$, $4.989\times10^{-5}M^{-1}$,$4.994\times10^{-5}M^{-1}$ and $5.012\times10^{-5}M^{-1}$, respectively (illustrate in Table 7) indicating a moderate intercalation between the complexes and Salmon fishDNA. 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*bwas 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 Cu(II), Ni(II), Co(II) and Zn(II) complexes.

Compound Names	λ-max		Δλ (nm)	*k*b$\times10^{-5}$ (M^{-1})
	Free	Bond		
Ni(II) Complex	305	307	2.0	4.990
Co(II) Complex	326.5	328	1.5	4.989
Cu(II) Complex	335	336	1.0	4.994
Zn(II) Complex	359	360	1.0	5.012

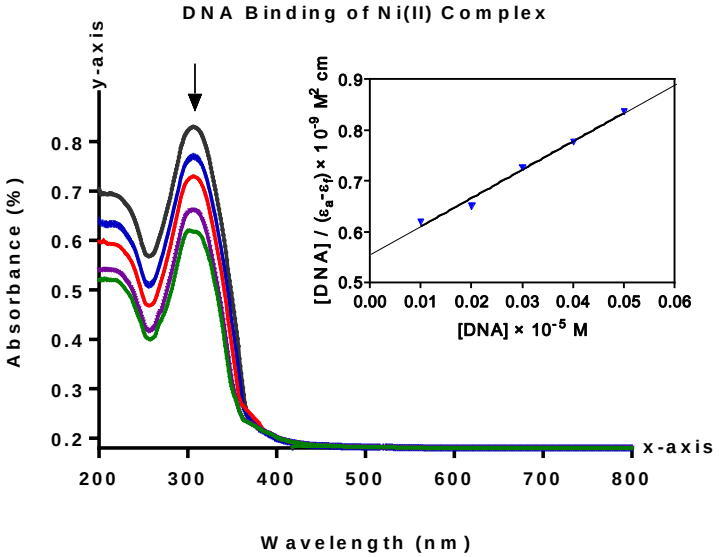


Figure 2: Absorption spectra of Ni(II) complex in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-Ɛ$_f$) × $10^{-9}M^2$cm versus [DNA] × 10^{-5} M for titration of DNA with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

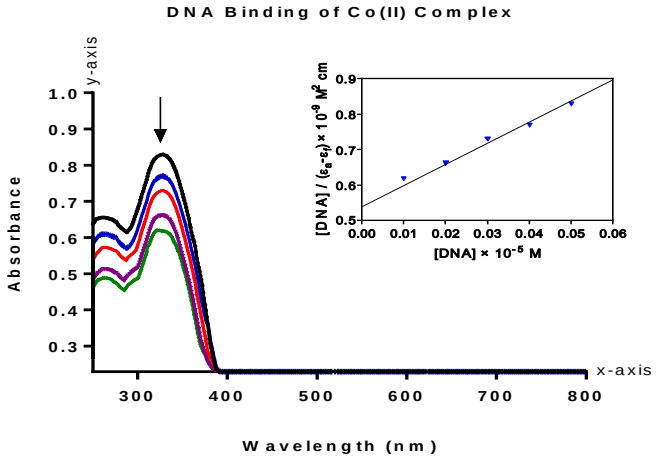


Figure 3: Absorption spectra of Co(II) complex in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-Ɛ$_f$) × 10^{-9}M^{2}cm versus [DNA] × 10^{-5} M for titration of DNA with Co(II) complex

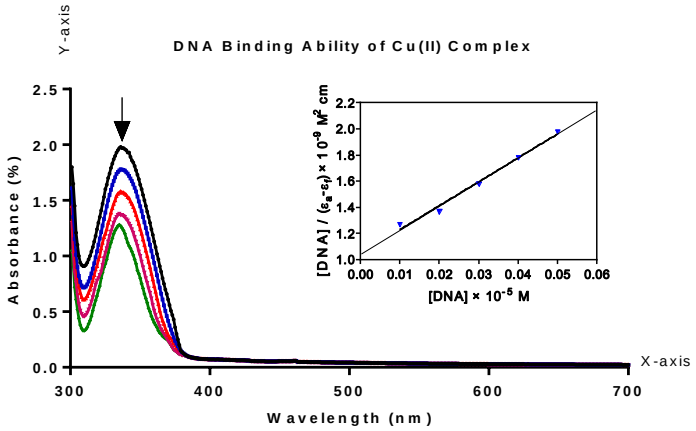


Figure 4: Absorption spectra of Cu(II) complex in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-Ɛ$_f$) × 10^{-9}M^{2}cm versus [DNA] × 10^{-5} M for titration of DNA with Cu(II) complex

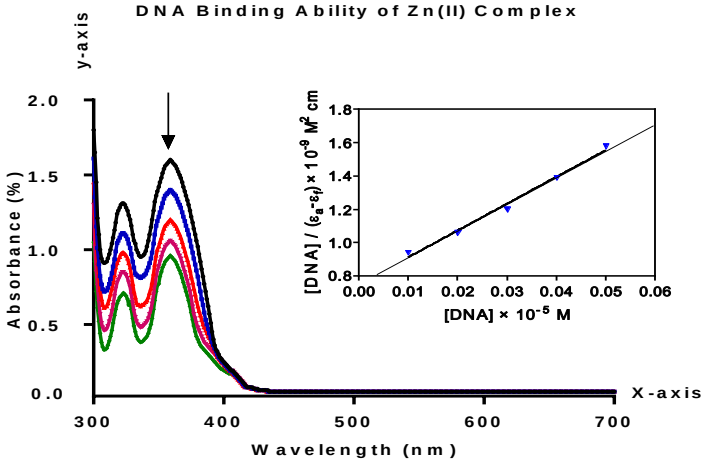


Figure 5: Absorption spectra of Zn(II) complex in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (Ɛa-Ɛ$_f$) × 10^{-9}M^{2}cm versus [DNA] × 10^{-5} M for titration of DNA with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

IV. Conclusions

The present study indicates the importance of Schiff base metal complexes as a source of synthetic antioxidants. The screening methods used in this research to evaluate the antioxidant capacity of Schiff base metal complexes are widely used. The results indicate that all compounds of Schiff base 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 metal complexes containing antioxidant parts which can donate a hydrogen atom or an electron. These four Schiff base metal complexes interact with Salmon fish DNA by intercalation modes. Their photo cleavage activities of supercoiled plasmid DNA were investigated in the presence of H_2O_2 probably via generating hydroxyl radical, the results also show efficiently oxidative cleavage activities, in conformity with DNA-binding behaviours of the complexes. In addition, Zn(II) complex exhibits a higher DNA binding affinity in contrast to Ni(II), Co(II) and Cu(II) complexes. The observations of the results obtained from the antioxidant activity and DNA binding ability of Schiff base metal complexes demonstrate that metal complexes possess effective free radical scavenging capacity and have high potent therapeutic benefits for the treatment of oxidative damage related dysfunction and diseases

References

[1] Halliwell B. Free radicals in biology and medicine. Third edition Oxford UK; Oxford University Press.1999.
[2] Valko M. Free radicals and antioxidants in normalphysiological functions and human disease. International Journal Biochemical CellBiology.2007;39(3):4484-4496.
[3] Halliwell B.Free radicals and antioxidants: a personal view. Nutrition reviews.1994;52(4):253-265.
[4] Mondal SK. Gupata M. Chakraborty G. Mazumder UK.*In-vitro* antioxidant activity of *Diospyrosmalabarica*Kostel bark. Industrial Journal of Experimental Biology.2006;44(8):39-44.
[5] Mohammad I.Chaudhuri PS.Oxidant antioxidant system, Role and significance in human body. Industrial Journal of Experimental Biology. 2002;40(1):1233-1239.
[6] Liao KL. Yin MC. Individual and combined antioxidant effects of seven phenolic agents in humanerythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. Journal of Agriculture and Food Chemistry. 2000;48(2):2266-2270.
[7] Halliwell B. Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept. NutritionReview.1999;57(4):104-113.
[8] Wu Y. Hong C. Lin S. Wu P. Shiao M. 1998. Increase of vitamin Econtent in LDL and reduction of atherosclerosis in cholesterol-fedrabbits by a water-soluble antioxidant-rich fraction of Salviamiltiorrhiza. Arteriosclerosis Thrombosisand Vascular Biology. 1998;18(5):481–486.
[9] Bandyopadhyay U. Das A.Bannerjee RK. Reactive oxygen species, oxygen damage and pathogenesis. Current Science. 1999;5(1):658-666.
[10] Lee KS. Lee SJ. Park HJ. Chung JP. Han KH.Oxidative stress effect on the activation of hepatic stellate cells. Yonsei. Medical Journal.2001;42(2):1-8.
[11] Kumaran A.Karunakaran R.J.*In vitro* antioxidant activities of methanol extracts of five *Phyllanthus*species from India. LWT-Food Science Technology.2007; 40(6):344-352.
[12] Kottaimuthu R.Ethnobotany of the valaiyans ofkarandamalaiDindigul districtTamilnadu.Ethno botanical Leaflets.2008;12(1):195-203.
[13] Tripathy S. Pradhan D. Anjana M.Anti-inflammatory and antiarthritic potential of *Ammaniabaccifera*Linn. International JournalPhytologist Bioscience.2010;1(1)1-8.
[14] Halliwell B. Aeschbach R. Löliger J. Aruoma OI. The characterization of antioxidants. Food and Chemical Toxicology.1995;33(11):601-617.
[15] Sies H. Strategies of antioxidant defense. Europium Journal of Biochemistry.1993;215(6)213–219.
[16] Tseng TH., Kao ES. Chu CY. Chou FP. Lin. Wu HW. Wang CJ. Protective effects of dried flower extracts of *Hibiscus sabdariffa L*. against oxidative stress in rat primary hepatocytes. Food and Chemical Toxicology.1997;35(4):1159- 1164.
[17] Soares JR. Dinis TCP. Cunha AP. Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free RadicalResearch.1997;26(10):469-478.
[18] Patel RP. Moellering D. Murphy-ullrich J. Jo H. Beckman JS. Darley-usmar VM. Cell signaling by reactive nitrogen and oxygen species in atherosclerosis. Free Radical Biology and Medical.2000;28(2):1780-1794.
[19] Cuzzocrea S. Riley DP.Caputi AP.Salvemini D. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. PharmacologyReview.2001;53(4):135-159.
[20] Formica JV.Regelson, W. Review of the biology of quercetinand related bioflavonoids. Food and Chemical Toxicology. 1995;33(9):1061-1080.
[21] Shanab SM. Mostafa SS. Shalaby EA. Mahmoud GI. Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. Asian Pacific Journal Tropical Disease. 2012;22(13):608-615.
[22] Puntel RL. Nogueira CW. Rocha JB. Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain *in vitro*. NeurochemistryResearch.2005;30(1):225-235.
[23] Kumar R.S. Rajkapoor B. Perumal P. Antioxidant activities of *Indigoferacassioides*Rottl. Ex. DC. Using various in vitro assay models. Asian Pacific Journal Tropical Disease.2012;2(1):256-261.
[24] Sahaa MR. Hasana SMR. Aktera R. Hossaina MM. Alamb MS. Alam MA. Mazumder MEH.*In vitro* free radical scavenging activity of methanol extract of the leaves of M*imusopselengi* Linn. Bangladesh JournalVeterinary Medicine.2008;6(2):197–202.
[25] Li P. Huo L. Su W. Lu R. Deng C. Liu L. Deng Y. Guo N. Lu C. He C. Free radical-scavenging capacity, antioxidant activity and phenolic content of Pouzolziazeylanica. Journal of the Serbian Chemical Society. 2011;76(17):709–717.
[26] Parnham MJ. Graf E. Pharmacology of synthetic organic selenium compounds. Progressive Drug Research.1991;36(4):9–47.