

## Synthesis of Fe(III) Complexes as Antioxidants and DNA Cleavage Protectors

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### Abstract

Synthesis of quinolines containing selenium and sulfur bioactive ligands and their Fe(III) complexes is described. *In vitro* antioxidant activity were evaluated against, 1,1-diphenyl-2-picryl-hydrazil (DPPH), hydrogen peroxide, superoxide anion radical, reducing power and hydroxyl radical ( $\cdot\text{OH}$ ). At different concentrations, (20-60  $\mu\text{g/mL}$ ) the total antioxidant activity of synthesized ligands showed 71, 75 and 83% and complexes showed 76, 85, 94% of inhibition. Where at the same concentration and experimental condition the standard antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and  $\alpha$ -tocopherol exhibited 94, 95 and 63% inhibitions on peroxidation of linoleic acid emulsion. In support to antioxidants, DNA cleavage investigation of complexes leads to protective effect on DNA cleavage. Hence, this study confirms ligands and their Fe(III)-chelator as antioxidants as well as protect against DNA cleavage.

**Keywords:** Antioxidant; DNA damage; Free radicals; Peroxidase; Quinone

### Introduction

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals [1]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [2]. The most common reactive oxygen species (ROS) include superoxide ( $\text{O}_2^{\cdot-}$ ) anion, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), peroxy ( $\text{ROO}^{\cdot}$ ) radicals, and reactive hydroxyl ( $\text{OH}^{\cdot}$ ) radicals. The nitrogen derived free radicals are nitric oxide ( $\text{NO}^{\cdot}$ ) and peroxy nitrite anion ( $\text{ONOO}^{\cdot}$ ). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome [3]. In treatment of these diseases, antioxidant therapy has gained an immense importance. To overcome this problem, current research is now directed towards finding synthetic and naturally occurring antioxidants of plant origin.

Recent studies on intramolecularly stabilized organoselenium and sulfur compounds show that the Se and S interactions play an important role in the catalytic antioxidant activity [4]. Since, Se resembles sulfur (S) in many of its properties [5], they may be considered to be isosteric [6]. The biological and pharmaceutical activities of different selenium compounds are of special interest because it function found in the active site of a large selenium dependent enzymes, such as (GSH-Px) [7,8], modification of metal toxicity [9] and prevention of cancer [10].

Further, in living organisms free radical formation and the free radical defense system are closely related to metal ions and complexes [11,12]. Iron is one of the essential element involved in several biological functions, acting as a catalytic component of many metalloenzymes, including SOD. Transition metal complexes of Mn(II)/Mn(III), Cu(II) and Fe(III) have notably shown important antioxidant properties, namely SOD mimetic activity [13-15]. Fenton reaction describes Fe(II) induced hydroxyl radical formation [16-18] and other transition metal ions (V, Cr) take part in fenton-like reactions [19,20].

Previous studies have demonstrated that chemical modifications in the ring size, donor atoms and substituents on the complexes, may have profound effects both on the stability and the antioxidant activities [21,22]. These, antioxidant supplement may reduce oxidative damage [23]. Since, the damage occurs *in vivo* by hydroxyl radical generated in metal-mediated Fenton-type reactions [24]. Herein support to free radical mechanism in antioxidant activity we carried out DNA cleavage behavior in presence hydrogen peroxide, the results leads to DNA cleavage protector.

To the best of our knowledge, the metal complexes with ligand containing sulfur and selenium for antioxidants are not reported yet. So, in view of biological importance of selenium and sulfur, in the present work we describe iron(III) complexes of substituted quinoline containing sulfur and selenium focusing on their possible application as antioxidants and DNA cleavage protectors.

## Experimental

### Chemicals

All reagents and solvents used were AR grade, commercially purchased. Ammonium hexa fluoro phosphate ( $\text{NH}_4\text{PF}_6$ ), and tris-HCl buffer, Ferric chloride,  $\alpha$ -tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich, E. Merck, Himedia, Qualigens, Mumbai, India) and solution was prepared using deionised double distilled water. Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr pellets on Perkin-Elmer 157 IR spectrophotometer.  $^1\text{H}$  NMR spectra were recorded in DMSO- $d_6$  on EM-390 (300 MHz) NMR spectrometer, Electronic spectra were recorded on 835 Perkin-Elmer UV/Vis spectrophotometers using Nujol mull technique. Mass spectra were recorded on MASPEC low resolution instrument operating at 70 eV and UV-Visible spectra were recorded using SHIMADZU, UV-1650 PC model.

### General procedure for the synthesis of 2-[[2-(2-mercaptoquinolin-3-yl) methylene] amino] benzoic acid (4a) and 2-[[2-(2-selenoquinolin-3-yl) methylene] amino] benzoic acid (4b)

The mixture of 2-mercapto or 2-selenoquinoline-3-carbaldehyde (2a or 3a) (1.89 g, 2.36 g, 0.01 mol) and anthranilic acid (1.37 g, 0.01 mol) were stirred and refluxed for 1-2 hrs. The completion of reaction was monitored by TLC. Then the reaction mass cooled and was poured into crushed ice. The obtained yellow or brown colored precipitate was filtered and recrystallized from methanol.

### Spectral data of 2-[[2-(2-mercaptoquinolin-3-yl) methylene] amino] benzoic acid (4a)

Yellow solid, Yield 92%, mp.167°C; IR (v) (KBr)  $\text{cm}^{-1}$ ; 3020(C-H, Ar-H); 1668 (C=N); 1263 (COOH); 1892 (CHN); 2593 (S-H);  $^1\text{H}$  NMR (DMSO  $d_6$ ),  $\delta$  7.1-8.3 (m, 8H, Ar-H), 8.7 (s,  $^1\text{H}$ , CHN), 10.1 (s,  $^1\text{H}$ , SH), 11.2 (s,  $^1\text{H}$ , COOH), Elemental analysis, Found: C-66.21; H-3.94; N-9.10. Calculated for  $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$ : C-66.22; H-3.92; N-9.08.

### Spectral data of 2-[[2-(2-selenoquinolin-3-yl) methylene] amino] benzoic acid (4b)

Brown solid, Yield 85%, mp.192°C ; IR (v) (KBr)  $\text{cm}^{-1}$ ; 3015 (C-H, Ar-H); 1658 (C=N); 1278 (COOH); 1881 (CHN); 2585 (Se-H);  $^1\text{H}$  NMR (DMSO  $d_6$ ),  $\delta$  7.0-8.1 (m, 8H, Ar-H), 8.9 (s,  $^1\text{H}$ , CHN), 10.5 (s,  $^1\text{H}$ , Se-H), 11.8 (s,  $^1\text{H}$ , COOH), Elemental analysis, Found: C-57.45; H-3.42; N-7.88. Calculated for  $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_2\text{Se}$ : C-57.48; H-3.40; N-7.89.

### General procedure for the synthesis of 5a and 5b

The compounds 4a (3.08 g, 0.01 mol) and 4b (3.08 g, 0.01 mol) were dissolved in 20-25 ml of PPA, than refluxed at 120°C for 3-4 hrs. The cooled reaction mass poured into crushed ice and basified with sodium hydrogen carbonate. The obtained precipitate were filtered and recrystallized from methanol.

### 6-Mercapto-1H-benzazepino[3,4-c] quinolin-1-one (MBAQ) (5a)

Yellow solid, Yield 87%, mp.176°C ; IR (v) (KBr)  $\text{cm}^{-1}$ ; 3024 (C-H, Ar-H); 1672 (C=N); 1620 (C=O); 1887 (CHN); 2576 (S-H);  $^1\text{H}$  NMR (DMSO  $d_6$ ),  $\delta$  7.3-8.0 (m, 8H, Ar-H), 8.8 (s,  $^1\text{H}$ , CHN), 10.4 (s,  $^1\text{H}$ , SH), Elemental analysis, Found: C-70.32; H-3.48; N-9.64. Calculated for  $\text{C}_{17}\text{H}_{10}\text{N}_2\text{O}$  S: C-70.33; H-3.47; N-9.65.

### 6-Seleno-1H-benzazepino[3,4-c] quinolin-1-one (SBAQ) (5b)

Brown solid, Yield 79%, mp.205°C ; IR (v) (KBr)  $\text{cm}^{-1}$ ; 3019 (C-H, Ar-H); 1661 (C=N); 1631 (C=O); 1895 (CHN); 2598 (Se-H);  $^1\text{H}$  NMR (DMSO  $d_6$ ),  $\delta$  7.0-8.1 (m, 8H, Ar-H), 8.7 (s,  $^1\text{H}$ , CHN), 10.8 (s,  $^1\text{H}$ , Se-H), Elemental analysis, Found: C-60.54; H-2.87; N-8.33. Calculated for  $\text{C}_{17}\text{H}_{10}\text{N}_2\text{O}$  Se: C-60.55; H-2.89; N-8.31.

### Preparation of Fe(II) metal complexes [Fe(MBAQ) $_3$ ](PF $_6$ ) $_3$ (6a) and [Fe(SBAQ) $_3$ ](PF $_6$ ) $_3$ (6b)

**General procedure for the synthesis of complexes 6a and 6b:** Ferric(III) nitrate monohydrate (0.404 g, 0.001 M) and 6-mercapto or 6-seleno-1H-benzazepino[3,4-c] quinolin-1-one (0.870 g (5a) or 1.011 g (5b), 0.003 M) were dissolved in hot ethanolic solution in the molar ratio 1:3 and refluxed on water bath for 2 hr. The complexing mixture was cooled and precipitated by the addition of hot ethanolic solution of ammonium hexafluoro phosphate ( $\text{NH}_4\text{PF}_6$ ) to filtrate. The complex was filtered and dried under vacuum before recrystallized from acetone-ether mixture.

### Complex [Fe(MBAQ) $_3$ ](PF $_6$ ) $_3$ (6a)

Pale brown solid, Yield 81%, mp.183°C; IR (v) (KBr)  $\text{cm}^{-1}$ ; 423, 753, 837, 1105, 1620, 1645, 1875, 3025. Elemental analysis, Found: C-44.96; H-2.23; Fe-4.12; N-6.16. Calculated for  $\text{C}_{51}\text{H}_{30}\text{F}_{18}\text{FeN}_6\text{O}_3\text{P}_3\text{S}_3$ : C-44.98; H-2.22; Fe-4.10; N-6.17.

### Complex-[Fe(SBAQ) $_3$ ](PF $_6$ ) $_3$ (6b)

Brown solid, Yield 73%, mp.231°C; IR (v) (KBr)  $\text{cm}^{-1}$ ; 435, 573, 785, 839, 1055, 1620, 1854, 1640, 3096. Elemental analysis, Found: C-40.76; H-2.02; Fe-3.73; N-5.61. Calculated for  $\text{C}_{51}\text{H}_{30}\text{F}_{18}\text{FeN}_6\text{O}_3\text{P}_3\text{Se}_3$ : C-40.77; H-2.01; Fe-3.72; N-5.59.

### Invitro antioxidant activity

**Total antioxidant activity of [Fe(MBAQ) $_3$ ](PF $_6$ ) $_3$  (6a) and [Fe(SBAQ) $_3$ ](PF $_6$ ) $_3$  (6b):** The antioxidant activity of complexes was determined according to the thiocyanate method [25]. An 10 mg of both complexes was dissolved in 10 mL methanol, and a 10 mg of both ligands were dissolved in 10 mL ethanol. Concentration of 20, 40, and 60  $\mu\text{g/mL}$  of complexes and ligands or standards samples in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL linoleic acid emulsion consists of 175 mg of Tween-20, 155 mL of linoleic acid, and 0.04 M of potassium phosphate buffer (pH 7.0) in potassium phosphate buffer (0.04 M, pH 7.0). On the other hand, a 50 mL control consisted of 25 mL linoleic acid emulsion and 25 mL potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37°C. After the mixture was stirred, the peroxide value was determined by reading the absorbance at 500 nm after incubation. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without complexes, ligand or standard were used as blank

samples. All data about total antioxidant activity are the averages of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation (1):

$$\% \text{ Inhibition} = \left[ \frac{(A_0 - A_1)}{A_0} \times 100 \right] \text{ ----- (1)}$$

where  $A_0$  was the absorbance of the control reaction and  $A_1$  was the absorbance in the presence of the samples of [Fe(MBAQ)<sub>3</sub>] and [Fe(SBAQ)<sub>3</sub>] and their ligands 5a and 5b [26].

### Reducing power

The reducing power of ligands and complexes were determined according to the literature method [27]. The five concentrations of complexes (20, 40, 60 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl<sub>3</sub> (0.5 mL, 0.1%). The absorbance was measured at 700 nm.

### Superoxide anion scavenging activity

Measurements of superoxide anion scavenging activity were based on the method described [28]. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of Nitroblue Tetrazolium (NBT). In these experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 mM) solution, 1 mL NADH (78 mM) solution, and a sample solution of complexes (20, 40, 60 µg/mL) in ethanol. The reaction started by adding 1 mL of Phenazine Metho Sulfate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was recorded at 560 nm. L-Ascorbic acid was used as a control. The percentage inhibition of superoxide anion generation was calculated using the formula shown in Equation (1) [29].

### DPPH free radical scavenging activity

The free radical scavenging activity was measured against 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of [30]. Briefly, 1 ml of 0.1mM solution of DPPH in ethanol was added to 3 mL of ligands and complexes in phosphate buffer at different concentrations (20, 40, 60 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min and then the absorbance was measured at 517 nm. The DPPH concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression ( $R^2:0.9769$ ):

$$\text{Absorbance} = 104.09 \times [\text{DPPH}^*]$$

The DPPH radical concentration was calculated using the following equation (2):

$$\text{DPPH Scavenging Effect (\%)} = 100 - \left[ \frac{(A_0 - A_1)}{A_0} \times 100 \right] \text{ ----- (2)}$$

where  $A_0$  was the absorbance of the control reaction and  $A_1$  was the absorbance in the presence of the sample of complex 6a or 6b and their respective ligands.

### Scavenging of hydrogen peroxide

The ability of the scavenging effect of complexes and ligands to hydrogen peroxide was determined according to the reported method [31]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Complexes (20, 40, 60 µg/mL) in ethanol and distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution. The percentage of scavenging of hydrogen peroxide was calculated using the equation (1):

### Hydroxyl radical (HO·) scavenging assay

The ability of complexes and ligands to scavenge the hydroxyl radical generated by the Fenton reaction was measured according by modified method [32]. An 200 µL of 10 mM FeSO<sub>4</sub>·7H<sub>2</sub>O, 200 µL of 10 mM EDTA and 200 µL of 10 mM 2-deoxyribose were mixed with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 µL of different concentration of compounds. Thereafter, 200 µL of 10 mM H<sub>2</sub>O<sub>2</sub> was added to the mixture before incubation for 4 h at 37°C. Later, 1 mL of 2.8% TCA and 1 mL of 1% TBA were added and placed on a boiling water bath for 10 min. Absorbance was recorded at 532 nm.

### DNA cleavage experiments

The experiments were performed in a volume of 20 ml containing pUC19 DNA in 5 mmol/L phosphate buffer contained 10 mmol/L NaCl, pH 7.4, in the presence of different concentrations (200-400 mmol/L) of complexes. Immediately prior to irradiating the samples with UV light, H<sub>2</sub>O<sub>2</sub> was added to a final concentration of 2.5 mmol/L.

The reaction volumes were held in caps of polyethylene microcentrifuge tubes, which were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 ml of a mixture containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer (45 mmol/L Tris-borate, 1 mmol/L EDTA). Untreated pUC19 DNA was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/ml) and photographed under UV light [33].

## Results and Discussion

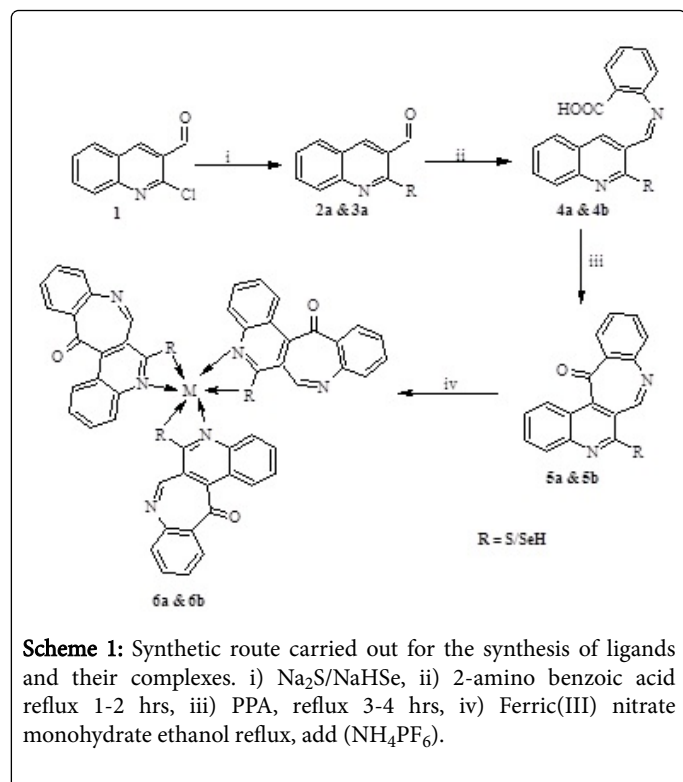
Numerous condensed quinolines have various bioactivities, which render them valuable pharmacological activities as mentioned earlier and therefore, they are a useful material in drug research. Hence, in continuation of our study in developing condensed quinolines derivatives [34] due to their significant biological activities, it appeared expedient to synthesize a series of systematically condensed quinolines used as ligands for metal complexes.

### Characterization of ligands and their complexes

The present study deals with the synthesis and evaluation of antioxidant activity of a new scaffold of iron complexes containing quinoline analogue bearing selenium and sulfur. The starting compounds for the preparation of ligands obtained according to our reported procedures [34]. The ligands were synthesized via cyclization



of 4a and 4b. The elemental analysis data and their spectral data are presented in the experimental section. The IR spectra of 5a and 5b showed an absorption at  $1672\text{ cm}^{-1}$  for  $\nu(\text{C}=\text{N})$  vibration. It also shows absorption bands at  $2576\text{-}2598\text{ cm}^{-1}$  for S/Se-H and the  $^1\text{H NMR}$  spectral data of ligands 5a and 5b showed singlet belongs to S/Se-H at  $\delta$  10.4 and 10.8 respectively, these are the evidence for the formation of expected structure. Finally, the structure was confirmed by its mass spectrum through the appearance of molecular ion peak at  $m/z$  289 [ $\text{M}^+$ ] and 336 [ $\text{M}^+$ ] (Scheme 1).

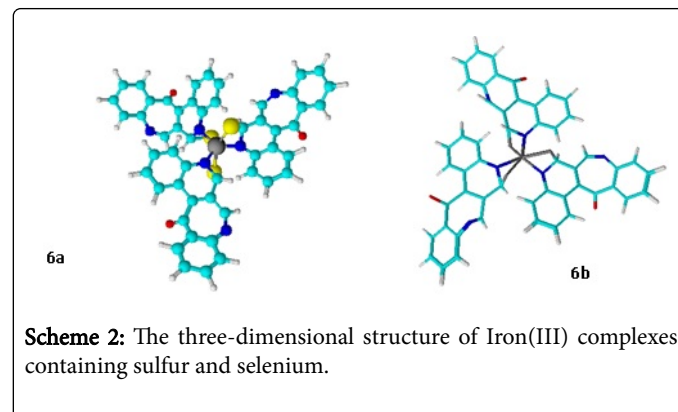


The elemental analysis data of complexes agreed with the theoretical values within the limit of experimental error. These new complexes are insoluble in water, but soluble in DMF, DMSO, and in buffer (pH 7.2) solution. The conductometric measurement values ( $28\text{-}58\text{ ohm}^{-1}\text{cm}^{-1}\text{mol}^{-1}$ ) in DMF indicate their electrolytic nature. The observed magnetic moment values ( $3.1\text{-}3.21\text{ BM}$ ) for these complexes are in the same range as reported for the octahedrally coordinated Fe(III) ion [35,36].

The IR spectra of ligands and complexes were recorded in the range of  $4000\text{-}250\text{ cm}^{-1}$  on KBr pellets. The intense bands appeared for ligands at  $1672\text{ cm}^{-1}$ , is due to  $\nu(\text{C}=\text{N})$  vibrations of quinoline nitrogen ring system. In  $[\text{Fe}(\text{MBAQ})_3] (\text{PF}_6)_3$  and  $[\text{Fe}(\text{SBAQ})_3] (\text{PF}_6)_3$  complexes, this band was shifted to  $25\text{-}35\text{ cm}^{-1}$  indicate that nitrogen atom is involved in coordination of Fe(III) ion. Besides, the complexes show low frequency in the region  $423\text{-}435\text{ cm}^{-1}$  and  $450\text{-}470\text{ cm}^{-1}$  are assigned to  $\nu(\text{M}-\text{N})$  and  $\nu(\text{M}-\text{S}/\text{Se})$  bands, respectively [35,36]. In addition, the IR spectrum of the  $\text{PF}_6$  salt of each complex showed a strong band in the region  $841\text{-}846\text{ cm}^{-1}$  ascribable to the counter anion and this band was absent for the corresponding chloride salts [37].

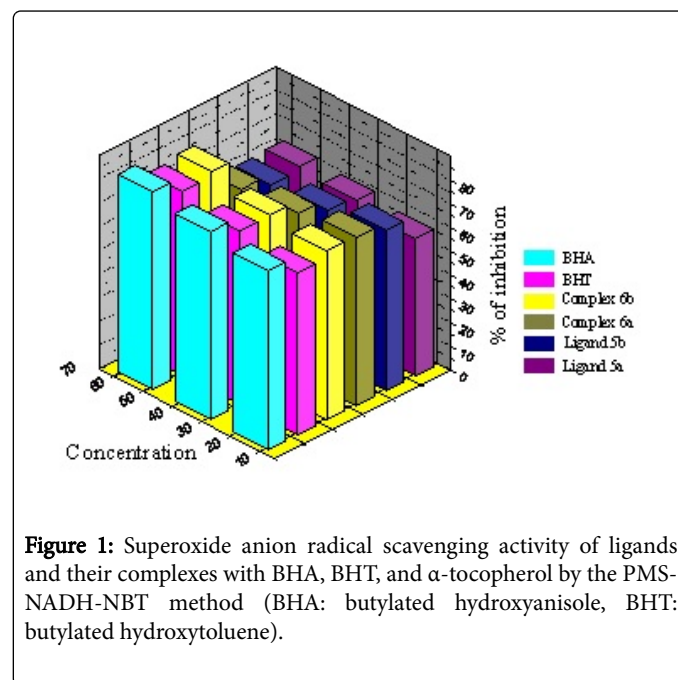
The electronic spectra of ligands was characterized by prominent bands at  $300\text{ nm}$  ascribable to the intramolecular  $\pi\text{-}\pi^*$  transition. The spectra of Fe(III) complexes exhibited a ligand to metal charge transfer band in the region  $400\text{-}420\text{ nm}$ . In addition to this, a weak band

observed in the region  $490\text{-}510\text{ nm}$  which could be assigned to  ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$  transition which suggest an octahedral configuration around the Fe(III) ion [36] (Scheme 2).



### Invitro antioxidant activities

**Superoxide anion scavenging activity:** In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The antioxidant properties of the prepared ligands and Fe(III) complexes were verified by their catalytic activities in the dismutation of superoxide radicals. The complexes showed to be active catalysts, with a better performance of scavenging ability than ligands. The SOD activity of these complexes was compared with standard BHT and BHA. The decrease of absorbance at  $570\text{ nm}$  with ligands and their complexes indicates the consumption of superoxide anion in the reaction mixture. Thus, complexes process strong superoxide radical scavenging activity than BHT, and  $\alpha$ -tocopherol (Figure 1).



The results were found statistically significant ( $p < 0.05$ ) the percentage inhibition of superoxide generated by  $60\text{ }\mu\text{g/mL}$  concentration of complexes was found to be 67% for 6a and 74% for 6b

which is greater than that of BHT and  $\alpha$ -tocopherol (35% and 28%) respectively, at same concentration.

### Scavenging of hydrogen peroxides

Hydrogen peroxide itself is not very reactive, but it can be toxic sometimes it may give rise to a hydroxyl radical [37]. Thus, removing  $H_2O_2$  is very important for protection of biological systems. Our synthesized iron complexes are capable of scavenging hydrogen peroxide in a concentration-dependent manner was determined according to the method [38]. The scavenging ability of complexes and their ligands on hydrogen peroxide is shown in Figure 2. At 60  $\mu\text{g/mL}$  concentration complex 6a and 6b exhibited 76 and 72% of scavenging effect over hydrogen peroxide. Thus, statistically these results are significant and followed this order for inhibition (complex 6a>complex 6b>BHA>BHT) for hydrogen peroxide.

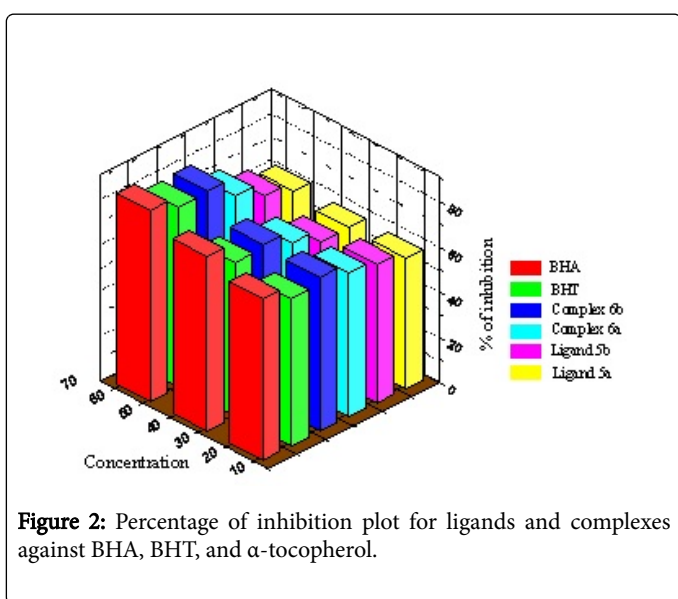


Figure 2: Percentage of inhibition plot for ligands and complexes against BHA, BHT, and  $\alpha$ -tocopherol.

### Hydroxyl radical (HO $\cdot$ ) scavenging activity

In the case of scavenging effect of hydroxyl radical we found that the complexes are good scavengers of  $\cdot\text{OH}$  radicals in a concentration-dependent manner (Figure 3). BHT and BHA are used as standard hydroxyl radical scavenging reagent. The activity of standards compared with complexes indicates the hydroxyl radical scavenging ability increases with decrease in the absorbance at 520 nm as shown in Figure 3.

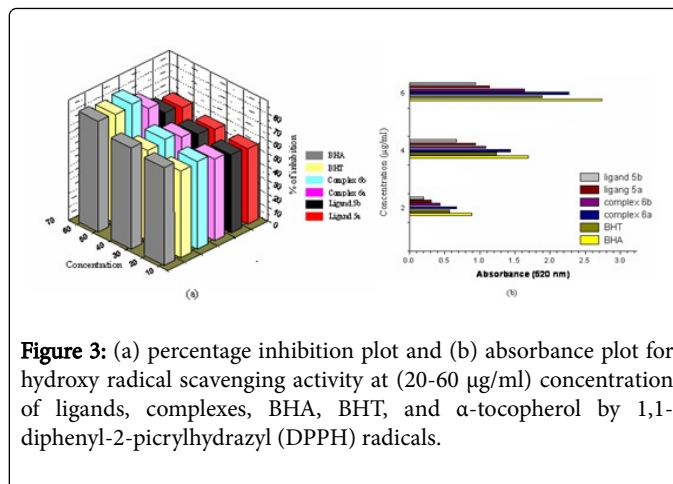


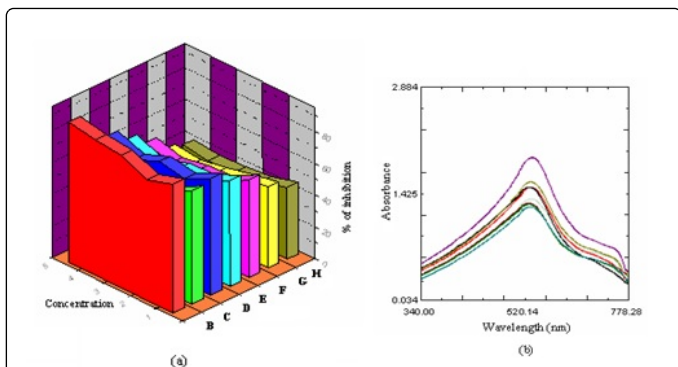
Figure 3: (a) percentage inhibition plot and (b) absorbance plot for hydroxy radical scavenging activity at (20-60  $\mu\text{g/mL}$ ) concentration of ligands, complexes, BHA, BHT, and  $\alpha$ -tocopherol by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

### Free radical (DPPH) scavenging activity

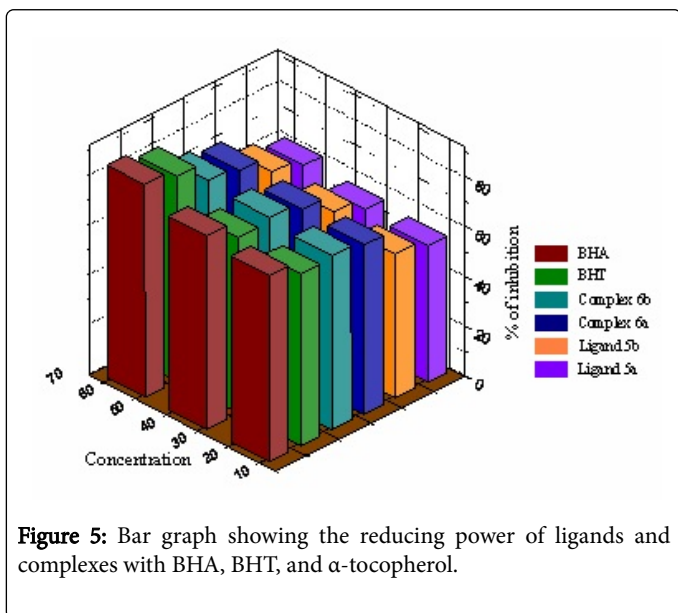
The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm leads to the activity. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between complexes and DPPH radicals progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration. Hence, DPPH is an important substrate to evaluate antioxidant activity [39,40]. Figure 4 illustrates significant ( $<0.01$ ) decrease in the concentration of DPPH radical due to the scavenging ability of complexes and ligands compared to standards. The results indicate that the complexes showed stronger DPPH scavenging activity rather than their ligands. Statistically, the scavenging effect of complexes with DPPH radical decreased in the following order of BHA>complex 6a>BHT>complex 6b> $\alpha$ -tocopherol with 64, 61, 55, 48, and 35% of inhibition, respectively.

### Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [41]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging [42]. The reducing power of complexes increased with decrease in absorbance value. Complex 6a exhibited stronger reducing power than complex 6b, may be due to the presence of selenium but this difference between the complexes with ligands were found statistically significant ( $p>0.06$ ) with standard compounds followed the order: BHA>complex 6a> BHT>ligand 5a>ligand 5b>complex 6b> $\alpha$ -tocopherol which is as shown in the Figure 5.



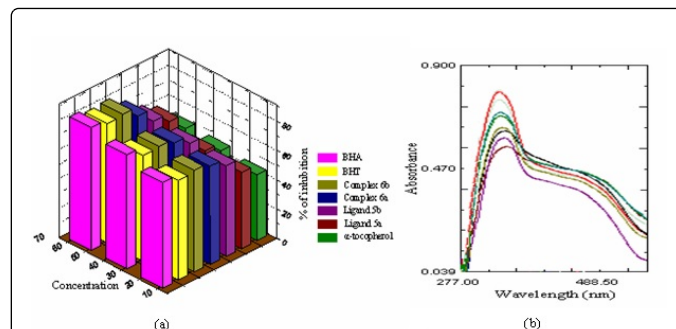
**Figure 4:** (a) Percentage of inhibition and (b) Absorption plot belongs to complexes and ligands for DPPH free radical scavenging activity at (20-60 µg/ml) concentration with BHA, BHT, and  $\alpha$ -tocopherol. Wherein % of inhibition plot B=BHA, C=BHT, D=[Fe(SBAQ)<sub>3</sub>], E=[Fe(MBAQ)<sub>3</sub>], F=5a, G=5b, H= $\alpha$ -tocopherol.



**Figure 5:** Bar graph showing the reducing power of ligands and complexes with BHA, BHT, and  $\alpha$ -tocopherol.

### Total antioxidant activity determination in linoleic acid emulsion

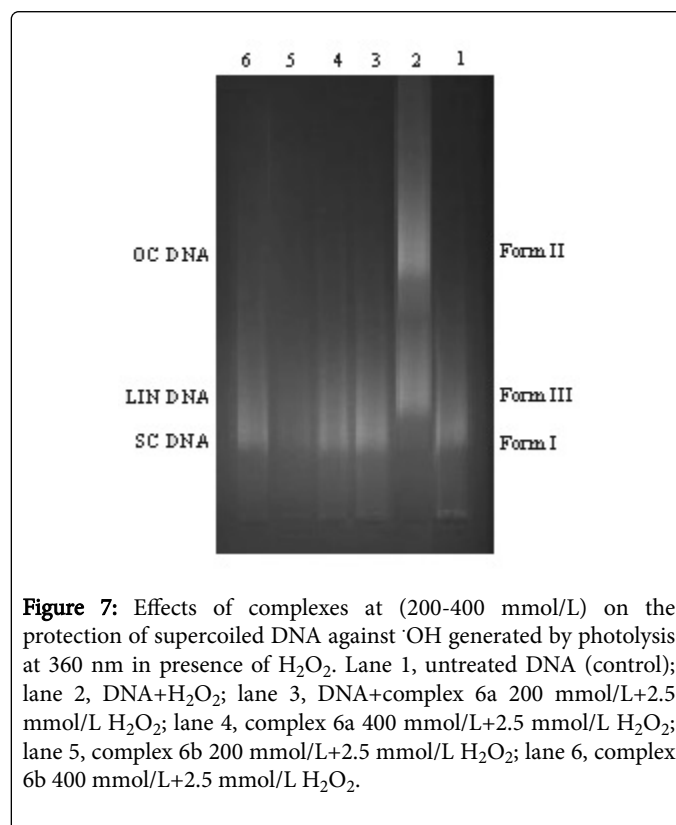
The total antioxidant activity of ligands and their complexes was determined by thiocyanate method [40,43,44]. Both complexes exhibited effective and powerful antioxidant activity whereas ligands showed comparatively less activity. Results show that the activity was increased with increasing the concentration of complexes. At 20-60 µg/mL the percentage inhibition of peroxidation in the linoleic acid system was 76, 85, and 94% for complexes and 71, 75, and 83% for their ligands, respectively which is greater than  $\alpha$ -tocopherol (63%) inhibition at 60 µg/mL. On the other hand, percentage inhibition of 60 µg/mL concentration of standard BHA and BHT was found to be 94 and 95% (Figure 6).



**Figure 6:** (a) Percentage of inhibition graph and (b) absorbance plot for the total antioxidant activity at (20-60 µg/ml) concentration of ligands and complexes with BHA, BHT and  $\alpha$ -tocopherol in the linoleic acid emulsion.

### The pUC 19 DNA cleavage studies

It is now recognized that the extremely reactive  $\cdot\text{OH}$  radical derived from  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  is a cause of DNA strand scission in cellular damage [45]. Figure 7 shows the electrophoretic pattern of DNA after UV-photolysis of  $\text{H}_2\text{O}_2$  (2.5 mmol/L) in the absence or presence of the complex.



**Figure 7:** Effects of complexes at (200-400 mmol/L) on the protection of supercoiled DNA against  $\cdot\text{OH}$  generated by photolysis at 360 nm in presence of  $\text{H}_2\text{O}_2$ . Lane 1, untreated DNA (control); lane 2, DNA +  $\text{H}_2\text{O}_2$ ; lane 3, DNA + complex 6a 200 mmol/L + 2.5 mmol/L  $\text{H}_2\text{O}_2$ ; lane 4, complex 6a 400 mmol/L + 2.5 mmol/L  $\text{H}_2\text{O}_2$ ; lane 5, complex 6b 200 mmol/L + 2.5 mmol/L  $\text{H}_2\text{O}_2$ ; lane 6, complex 6b 400 mmol/L + 2.5 mmol/L  $\text{H}_2\text{O}_2$ .

The faster-moving band corresponding to the native form of supercoiled circular DNA (scDNA) and the slower-moving band being the open circular form (ocDNA). The UV irradiation of DNA in the presence of  $\text{H}_2\text{O}_2$  (lane 2) caused the cleavage of scDNA to give open coiled DNA (ocDNA) and the linear form (lin DNA), indicating that

OH generated by UV-photolysis of H<sub>2</sub>O<sub>2</sub> produced DNA strand scission. The presence of the complexes under investigation suppressed the formation of DNA damage this shows the new complexes able to control oxidative DNA damage, which has been particularly implicated in carcinogenesis [46]. The above results indicate that radicals generated by the complexes may pair up with the ·OH generated by H<sub>2</sub>O<sub>2</sub> that might be the reason for (lanes 2-5) movement of DNA without cleavage.

## Conclusion

On the basis of the results, it is clearly indicated that the newly synthesized Fe(III) complexes [Fe(MBAQ)<sub>3</sub>] and [Fe(SBAQ)<sub>3</sub>] containing selenium and sulfur atom have significant antioxidant activity against various *invitro* antioxidant systems. The various antioxidant mechanisms of ligands and iron complexes may be attributed to their effectiveness as good scavengers of hydrogen peroxide, superoxide, and free radicals. However, the components responsible for the antioxidative activity of both complexes are clearly shows antioxidant activity. Further, these results supported by protective effect against pUC 19 DNA. Therefore, it is suggested that further work could be performed on metal complexes especially of iron as antioxidant compounds to evaluate its *invivo* effects.

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