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ANTIOXIDANT ANALYSIS OF SYNTHESISED HYDRAZO METAL COMPLEX LIGANDS

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ABSTRACT

In biological processes, inorganic compounds play critical roles and it has been established that many organic compounds used in medicine are activated or biotransformed by metal ions metabolism. Current research endeavor ascertains to propose antioxidant activity of macrocyclic complex compounds which will certainly have specified importance in the field of materials chemistry. Antioxidant activity of few compounds can be enhanced significantly synthesis of Fe(II), metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6- methoxy-phenol (Scheme-1) and their characterization by analytical, spectral and thermal studies. Hydrazo metal complex ligands are a group of heterocyclic compoundswhich have attracted much attention as a result of their varied pharmacological properties which include antibacterial, anticoagulants, antibiotic, antifungal, anticancer, and anti inflammatory and share an important place in this regards. In the current research study, complexes are characterized by elemental analysis, IR, NMR and Chromatographic evaluation was done by Thin Layer Chromatography in order to check purity[1-4]. Antioxidant efficacy of synthesized compounds was assessed by DPPH assay 9-11. Synthesised derivatives exhibited excellent anti-oxidant property as reported by Bhat et al, they synthesized complex of benzothiazole derivatives compound shows the IC50 values at 230.69 µg/mL concentration. Finally it was concluded that the synthesised compound has the good antioxidant activity.

Keywords: Metal Complexes, Biological interest, Pharmacological interest, Antioxidant, DPPH Assay

INTRODUCTION

The living cells are disrupting by the chain reaction, that is, the attacked molecules lose its electrons and become a free radical which attacks the living cell. Natural and synthetic are the two basic types of antioxidants structures. Various alkyl substitutions containing phenolic group are in general natural antioxidants. Nitrogen compounds, phenolic compounds are categorized as synthetic antioxidants. The satirically hindered phenols and secondary aromatic amines form the primary antioxidants compounds. The hydrogen atom moves from the antioxidant molecule to radical intermediate is the first step of the radical termination.

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid oxidation. The in vitro antioxidant activity can be performed by three methods, DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide radical scavenging assay. Antioxidant activity of few compounds can be enhanced significantly synthesis of Fe(II), metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6- methoxy-phenol (Scheme-1) and their characterization by analytical, spectral and thermal studies. Hydrazo metal complex ligands are a group of heterocyclic compoundswhich have attracted much attention as a result of their varied pharmacological properties which include antibacterial, anticoagulants, antibiotic, antifungal, anticancer, and anti inflammatory and share an important place in this regards. In the current research study, complexes are characterized by elemental analysis, IR, NMR and Chromatographic evaluation was done by Thin Layer Chromatography in order to check purity[1-4]. Antioxidant efficacy of synthesized compounds was assessed by DPPH assay 9-11.

MATERIALS AND METHODS

LR/AR grade chemicals and reagents were used in standard operating procedures.

Preparation of hydrazone

2-Hydrazino-4,6-dimethyl benzothiazole

Hydrazine hydrate (4 ml of 80%) was taken in round bottom flask and kept in freezing mixture. Concentrated hydrochloric acid (4ml) was added to it. On shaking and cooling the above mixture ethylene glycol (20ml), 2-amino-4,6-dimethyl benzothiazole (0.02 mole, 3.56 gms) was added. The mixture was refluxed for 2.5 hours at 150-160°C. The solution was cooled at room temperature. The crystalline product was obtained. (Yield 71%, m.p.175°C.)

2-**[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6-methoxy-phenol:(DBYHMP)**

(4,6-dimethyl-benzothiazol-2-yl)-hydrazine (7 g, 0.005mole) was mixed with o-hydroxy-mmethoxybenzaldehyde (5.608 g, 0.005mol) in ethanol. The mixture was placed in round bottom flask and refluxed on water bath for 1 h. and then allowed to cool at room temp. The resulting light brown colored precipitate was filtered, washed several times with ethanol (Scheme-2). (Yield 80%, m.p.= 200° C)

Preparation of the complex

[Fe(DBYHMP)2]: The complex was prepared by refluxing 1:2 molar mixture of ethanolic solutions of the ligand and ferrous sulphate for 1 h, maintaining pH (~8) by adding alcoholic ammonia. The sparingly soluble, pale brown product was separated and washed with ethanol and the complex was dried over anhydrous calcium chloride in vacuum and tested with TLC (yield=69%).Chemical analysis. Found: Fe

7.82; C 57.65; H 4.57; N 11.90; O 9.08; S 9.15 %. FeC34H32N6O4S2requires: Fe 7.88; C 57.63; H 4.55; N 11.86; O 9.03; S 9.05 %.

ANTIOXIDANT ACTIVITY (DPPH ASSAY)

The DPPH assay was used to study radical scavenging activity of test samples according to Scherer and Godoy [5]. The measurement is taken at 517 nm. After the addition of an antioxidant the decrease in the absorption of the DPPH solution was taken. Standard taken is Ascorbic acid.

Principal of technique

1, 1-Diphenyl- 2-picryl-hydrazyl (DPPH) is a stable free radical and a trap ("scavenger") for other radicals, widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H. Because of a strong absorption band at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, which is measured spectro-photometrically at 517 nm in NANODROP. [5].

Required reagents, glassware and equipment –

0.1mM solution of DPPH in methanol, DMSO (Dimethyl sulfoxide), amber colored reagent bottle, test tubes, ascorbic acid, three decimal place digital balance, and NANODROP.

PROCEDURE

Preparation of Reagents and dilutions[6-10].

1. **0.1mM DPPH-**

   Stock Solution = 1mM (.02 gm of DPPH in 50 ml of methanol)

   Working Concentration = .1mM (1 ml of 1mM DPPH in 9 ml of Methanol)

2. **Positive Control = Ascorbic Acid**

   Stock Solution = 1mg/ml (10 mg in 10 ml DMSO)

   Working Concentration = different working Concentration ranging 10 µg/ml to 1000 µg/ml were prepared from stock solution

Table: 1 Dilution of Ascorbic Acid

Stock Concentration (mg/ml)	Required Concentration (µg/ml)	Ascorbic Acid	DMSO	Final Volume
1	10	10	990	1.0 ml
1	100	100	900	1.0 ml
1	250	250	750	1.0 ml
1	500	500	500	1.0 ml
1	750	750	250	1.0 ml
1	1000	1000	0	1.0 ml

Procedure (a) To determine the antioxidant of the Control[11].

1. Dilution of Ascorbic acid was prepared in DMSO solution.
2. 500 µl of dilution and 500 µl of 0.1mM solution of DPPH in methanol were added into the eppendroff in dark condition.
3. The mixture was shaken and incubated for 30 min at room temperature in the dark condition.
4. After the incubation period absorbance was measured at 517 nm in NANODROP.
5. Ascorbic Acid used as a positive control for this assay.
6. Record the absorbance of ascorbic acid as a positive control as same absorbance as test compound i.e. 517 nm.
7. Methanol serves as a blank prepared by adding 500 µl of 1mg/1ml of Ascorbic acid and 500 µl methanol.
8. Control was prepared by adding 500 µl of 0.1mM DPPH and 500 µl of DMSO.
9. The DPPH scavenging activity is calculated as follows –

$$\text{DPPH Scavenging Activity (\% Inhibition)} = \frac{(\text{OD of Control} - \text{OD of Sample}) \times 100}{\text{OD of Control}}$$

10. The DPPH antioxidant activity for the test compound was expressed in the form of IC50 value. IC50 (half maximum inhibitory concentration) value was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Procedure –(b.) To determine the antioxidant of the given compound. [12-15].

1. Dilution of test compound C1, was prepared in DMSO solution.
2. 500 µl of dilution and 500 µl of 0.1mM solution of DPPH in methanol were added into the eppendroff in dark condition.
3. The mixture was shaken and incubated for 30 min at room temperature in the dark condition.
4. After the incubation period absorbance was measured at 517 nm in NANODROP.
5. Ascorbic Acid used as a positive control for this assay.
6. Record the absorbance of ascorbic acid as a positive control as same absorbance as test compound i.e. 517 nm.
7. Methanol serves as a blank prepared by adding 500 µl of 1mg/1ml of Ascorbic acid and 500 µl methanol.
8. Control was prepared by adding 500 µl of 0.1mM DPPH and 500 µl of DMSO.
9. The DPPH scavenging activity is calculated as follows –

$$\text{DPPH Scavenging Activity (\% Inhibition)} = \frac{\text{(OD of Control – OD of Sample) x 100}}{\text{OD of Control}}$$

10. The DPPH antioxidant activity for the test compound was expressed in the form of IC50 value. IC50 (half maximum inhibitory concentration) value was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Compound dilution:

Table: 3 Amount of DMSO required to obtain certain Concentrations

Compound	**Stock Concentration**
C1	1mg/1ml

Table: 4 Dilution of concentrations with DMSO

From the stock concentration two working concentration were prepared.

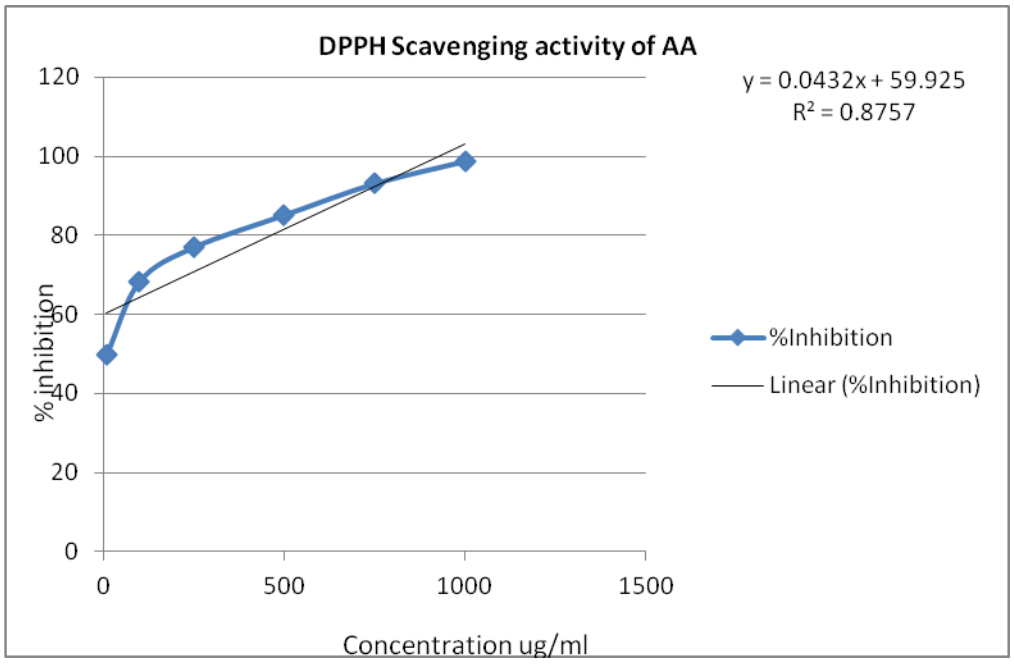
Compound	Working concentration 1	Working concentration 2
Compound 1	16.5ug/ml	8.0 ug/ml

RESULTS AND DISCUSSION

The synthesized compounds were tested for anti-oxidant activities by DPPH assay. Synthesised compounds showed good activity both in DPPH Scavenging radical assay, where as other compounds showed comparatively less activity. So, their IC50 values were calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals as shown in figure 1. The synthesized compounds have copper metal complex groups, which show very good radical scavenging activities.

Table 5- % inhibition and IC_{50} value of positive control (Ascorbic Acid)

Concentration (ug/ml)	**OD Value (517 nm)**	**%Inhibition**
10	1.011	49.851
100	0.638	68.321
250	0.463	77.010
500	0.298	85.203
750	0.137	93.197
1000	0.023	98.857
Control	2.014	-



IC value : 230.69ug/ml.

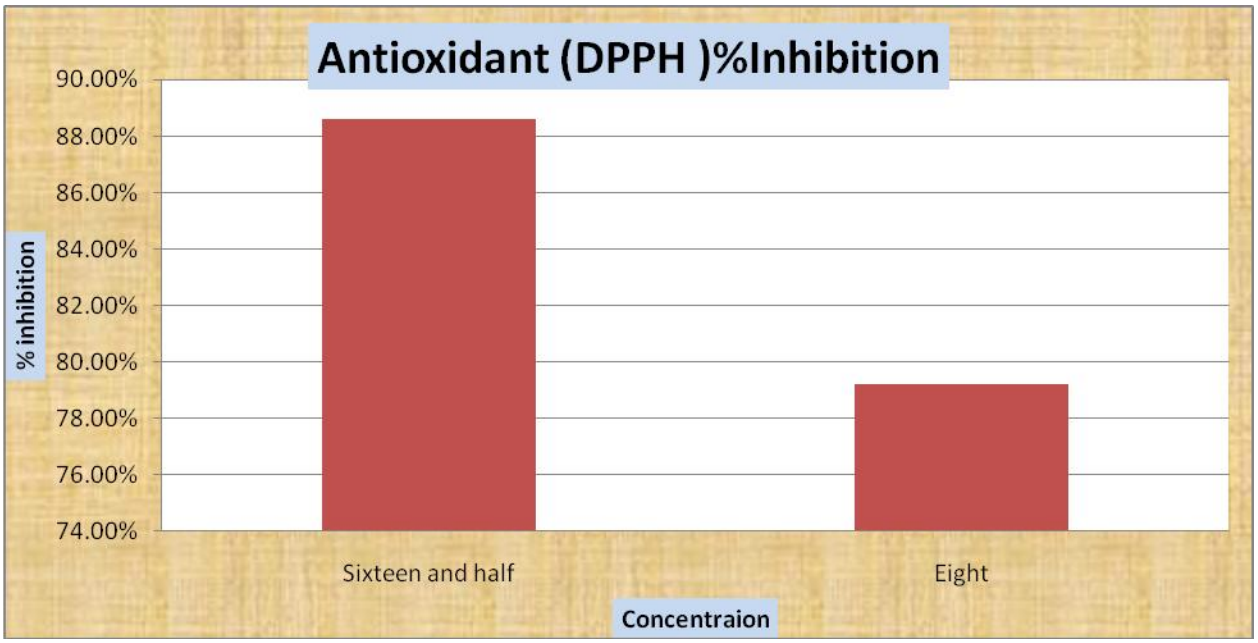
- **Calculation of IC50 value from graph :**
- Straight line equation from graph : Y=0.043x+59.92
- IC50 = (59.92-50)/0.043

Hence IC50 = 230.69ug/ml.

Test compound showed the dose dependant scavenging activity against DPPH.

Table 6:- % inhibition value of given compound.

Concentration (ug/ml)	**OD Value (517 nm)**	**%Inhibition**
Control	2.346	
16.5	0.268	88.576
8.0	0.488	79.198



Interpretation: The given compound has the % Inhibition of 88.576 and 79.198 %.

Conclusion: The given compound has the good antioxidant activity.

CONCLUSION:

Antioxidant activity of few compounds can be enhanced significantly synthesis of Fe(II), metal complexes with 2-[(4,6-dimethyl-benzothiazol-2-yl)-hydrazonomethyl]-6- methoxy-phenol (Scheme-1) and their characterization by analytical, spectral and thermal studies. Hydrazo metal complex ligands are a group of heterocyclic compoundswhich have attracted much attention as a result of their varied pharmacological properties which include antibacterial, anticoagulants, antibiotic, antifungal, anticancer, and anti inflammatory and share an important place in this regards. In the current research study, complexes are characterized by elemental analysis, IR, NMR and Chromatographic evaluation was done by Thin Layer Chromatography in order to check purity[1-4]. Antioxidant efficacy of synthesized compounds was assessed by DPPH assay 9-11. The synthesized compounds were tested for anti-oxidant activities by DPPH assay. Synthesized compounds showed good activity both in DPPH Scavenging radical assay, where as other compounds showed comparatively less activity. So, their IC50 values were calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals.

The synthesized compounds have metal complex groups, which show very good radical scavenging activities. Synthesised compounds are electron releasing which are important in radical scavenging activity.

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