Journal of Advances and Scholarly Researches in Allied Education
Vol. 12, Issue No. 2, January-2017, ISSN 2230-7540

A Study of Synthesis and Superoxide Dismutase Activity of Phthalicacid [Bis(Benzyloxy)Ethyl]Ester

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Abstract – In this paper, we report the amalgamation of another ligand phthalicacid[bis(benzyloxy)ethyl] ester by the response of phthalic anhydride and ethylene glycol. The ligand was portrayed by FTIR, *HNMR and Mass spectra. Binuclear Copper (II) complex of the ligand bound to four acetic acid derivation particles was orchestrated and described by electronic spectra, FTIR spectra, EPR spectra, thermo gravimetric (TGA) investigation and electrochemical examinations. The superoxide rummaging action of the complex is additionally announced.

Keywords: synthesis, superoxide dismutase activity, phthalicacid [bis(benzyloxy) ethyl]ester, Binuclear Copper (II).

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INTRODUCTION

Cu is a basic micronutrient required by all types of life and include in an assortment of organic procedures viz., embryonic advancement, mitochondrial breath, control of hemoglobin levels and also hepatocyte and neuronal capacities. Being a change metal, Cu gets organically changed over between various redox states to be specific oxidized Cu (II) and diminished Cu (I). This extraordinary trait has made Cu metal to get showed as an essential synergist co-factor for an assortment of metabolic responses in natural frameworks (Pederson, 1973). Buildings of Cu in oxidation state +2 are likewise naturally noteworthy in view of their cell reinforcement and against free extreme movement (Fridovich, 1978, (Mizushima, et. al., 1992). They involve a class of potential mitigating drugs with lessened symptoms, and their method of activity is ascribed to their checked superoxide dismutase (SOD) mimetic action. Binuclear Cu (II) edifices are potential models for various vital organic frameworks containing couple locales (Tabbi, et. al., 1998) and have been contemplated widely ((Halliwell and Gutteridge, 1989. Vogtle and Webber, 1986. Huber, 1980. Liczmanski, 1994. Brigelius, et. al., 1974. Goldstein and Czapski, 1983) In addition, the Cu (II) edifices of multidentate Schiff base ligands have assumed an essential job in the improvement of coordination science (Underhill, et. al., 1987. Burry, et. al., 1989. Bijloo, et. al., 1990). The bimetallic Cu (II) buildings additionally have pulled in much consideration in magneto science because of the turn communication between the copper (II) focuses.

Superoxide dismutase (SOD) is a compound associated with shielding organic cells from the dangerous impacts of superoxide (Bhirud and Srivastava, 1990). In view of the metal particles present in the dynamic site SODs have been separated into - Cu-Zn-SOD, Mn-SOD and Fe-SOD out of which the first is found in warm blooded creatures (Goldstein, et. al., 1990). Lacking level of SOD focus in human body is one reason behind infections and scatters like - diabetes, ischemia, waterfall, Parkinson's sickness, diseases and so forth (Bhirud and Srivastava, 1991. Labuda, et. al., 1991). Supplementation of cell reinforcement compounds ought to be a piece of the treatment yet organization of these proteins through oral or intraperitonial courses is seriously limited because of their fast corruption and short life time in natural frameworks (Underhill, et. al., 1993). Low atomic weight metal conjugates of SOD give preferences over the characteristic catalysts in regard of their capacity in intersection the phone films, offering no immunogenicity, having longer life-time of the dynamic structures, plausibility of oral organization and similar minimal effort.

Since copper goes about as the dynamic focus in CuZnSOD,a number of Cu edifices have been composed and blended as SOD demonstrate buildings to give important bits of knowledge into the structure and reactant capacity of the Cu, Zn-SOD dynamic site . Since the dismutation of response includes the redox cycle of Cu(II) and Cu(I), it is sensible to expect that the redox capability of Cu(II)/Cu(I) can impact the SOD - like movement, Small metal edifices having great superoxide rummaging action are potential hopeful in such manner.

Cu (II) edifices with Schiff base ligands got from different aldehydes and ketones have been accounted for to mirror SOD action (Iakovidou, et. al., 2001). Different models in such manner incorporates - imidazole connected Cu (II) buildings (Patole, et. al., 2001), planar Cu (II) complex on expansion of a base, for example, N-methyl imidazole or pyridine (Maurer, et. al., 2002), curcumin edifices of Cu(II) (Cowly, et. al., 2002) and so on. There is additionally report that the SOD movement of Cu (II) edifices with Schiff base ligands of salicylaldehyde semicarbazone could be tuned by heterocyclic bases pyridine and N-methyl imidazole (Ferrari, et. al., 2002).

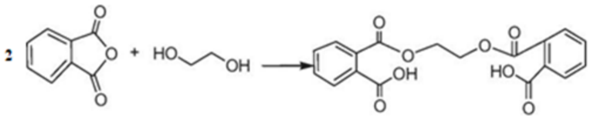
METHODOLOGY

Phthalic anhydride and ethylene glycol were acquired from Merck and Cu (II) acetic acid derivation monohydrate was obtained from Loba Chemie. All dissolvable were purged by standard strategy.

1. Synthesis

2. Phthalic corrosive bis(benzyloxy)ethyl ester (L) and its portrayal synthesis

20 mmol (2.96 g) phthalic anhydride was taken in a mortar and crushed. 10 mmol (0.6 mL) of ethylene glycol was included drop savvy with steady mixing. The response blend was warmed to 60 oC and 2-3 drops of pyridine was included. The blend was chilled to acquire a white item which was then recrystallised from methanol. The engineered way for the ligand (L) has been appeared in plan 6.1 beneath



Scheme 1: Synthetic path for the ligand (L)

Yield 85%, Melting Point = 115 ^{o}C

FTIR analysis of L: FTIR spectrum of L was recorded in KBr pallet. Peaks were observed at 3404 cm^{-1} due to u_{O-H}, 2920.7 cm^{-1} due to u_{C-H}, 1627 cm^{-1} due to $u_{C}=_{O}$, 1289.4 cm^{-1} due to u_{C-O}, 1391.3 cm^{-1} due to u_{-CH2-}, 756 cm^{-1} and 705 cm^{-1} due to aromatic C-H out of plane bending.

LC-MS analysis of L: LCMS of L was recorded in acetonitrile. The m/e value for the molecular peak (M^{+}) was observed at 381. The calculated value of molecular mass of L.H2O is 376.

^{1}HNMR of L: *HNMR of L was recorded in CDCl$_{3}$. Chemical shifts, 8 in ppm, were observed at 7.72 (2H, t, j = 5.1 Hz); 7.56 (2H, t, j = 5.7 Hz); 7.44 (4H, d, j = 4.0 Hz); 4.52 (4H, s)

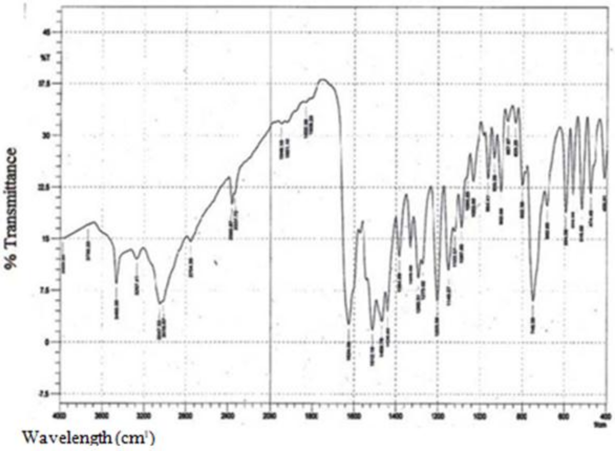


Figure 1: FTIR spectrum of L in KBr

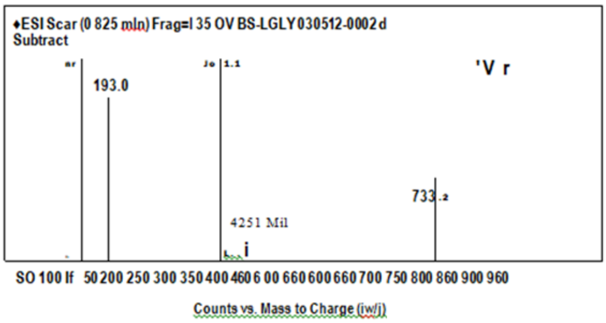
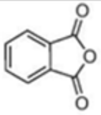
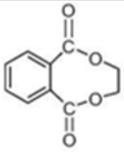
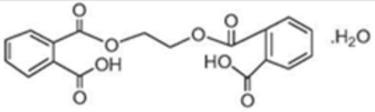


Figure 2: LC-MS spectrum of L in Ch$_{2}$CN.

Table 1: Assigned molecular fragment of L in LC-MS

m/z value	Molecular fragment
148	
193	
376	

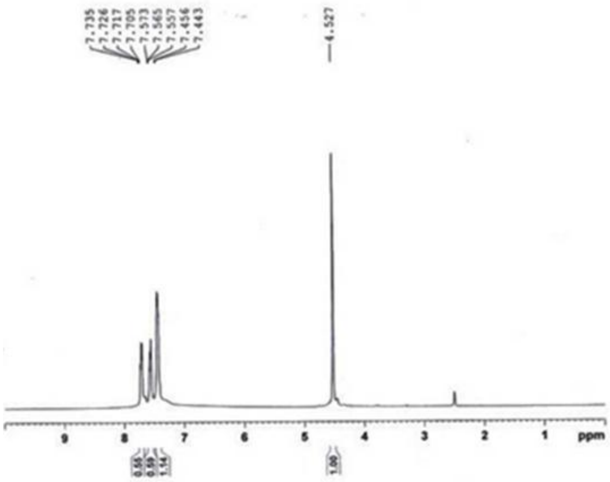


Figure 3: *HNMR of L in CDCh

Copper (II) phthalicacid [bis(benzyloxy)ethyl]ester synthesis

Cu (CH3COO) 2,1 mmol (0.199 g) was broken down in 10 mL methanol. An answer of L, arranged by dissolving 1 mmol (0.380 g) into 10 ml of methanol, was included drop shrewd with vivacious mixing. The mixing was proceeded for three hours till dim blue hasten was gotten. The accelerate was washed with diethyl ether and dried in air. The compound was recrystallised from acetonitrile and methanol blend to get blue hued gems.

RESULTS

1. UV/vis and FTIR investigation

The UV/vis spectra of the complex were recorded in acetonitrile and an expansive band was seen at Xmax 714 nm because of d ^ d change starting at Cu (II) (Fig.7.4). The elimination coefficient (s) was ascertained to be 709.68 Lmol-1cm-1. In the event of the ligand, a solid absorbance top was seen at 274nm which vanished if there should be an occurrence of the complex.

FTIR spectra for the complex blended in KBr indicated tops at 2966.5 cm-1 (u C-H of C6H5); 756 cm-1 and 705cm-1 (C-H out of plan vibration for C6H5); 1631.7cm-1 (u C=O); 1400cm (usymm. COO 1589.3 cm (uasymm. COO-); 3441 cm-1 (UO-H). The u C=O band for the carbonyl gathering was watched nearly similarly situated in both the ligand and its mind boggling, in this way showing the non inclusion of the C=O bunch in complex development with the metal particle. The presence of uCOO-method of vibration in the vibrational spectra of complex demonstrates the official of acetic acid derivation particles.

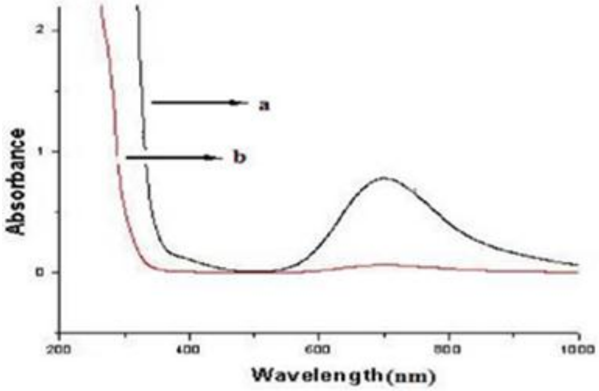


Figure 4: UV/vis spectrum of copper (II) phthalicacid [bis(benzyloxy)ethyl]ester (a) 0.0009M and (b) 0.00009M

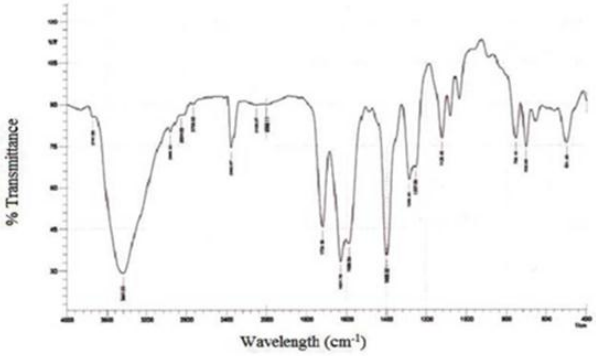


Figure 5: FTIR specture of copper (II) phthalicacid [bis(benzyloxy)ethyl]ester

$$G = \frac{(g_{\|} - 2.0023)}{(g_{\perp} - 2.0023)} = \frac{4K_{\perp}^{2} AEx_{z}}{ki^{2}\ AExy}$$

The calculated value of g tensor parameter was g|| = 2.06 and gi = 2.03. Hence g|| > gi > 2.003 which reveals that $d_{x^2-y^2}$ is the ground state (Sun, et. al., 2000). The value of G was calculated to be 2.04 which are less than 4 indicates considerable exchange interaction between the copper centres (Patel, et. al., 2005). From the observed values, it is clear that g| > gi (2.06 >2.03), which suggests elongated octahedral structure

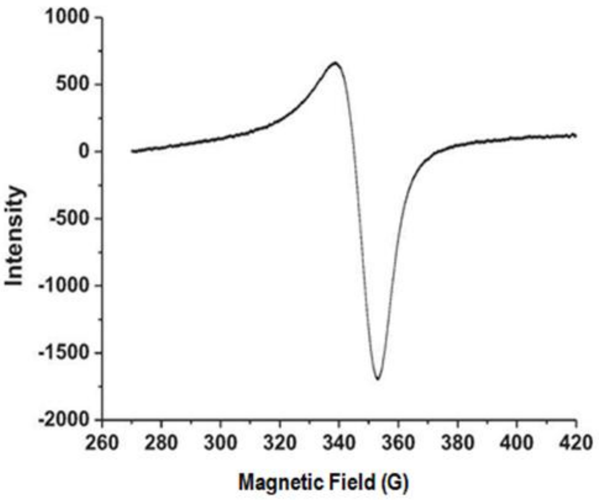


Figure 6: EPR spectra of copper (II) phthalicacid [bis(benzyloxy) ethyl]ester

2. Study of EPR spectroscopy

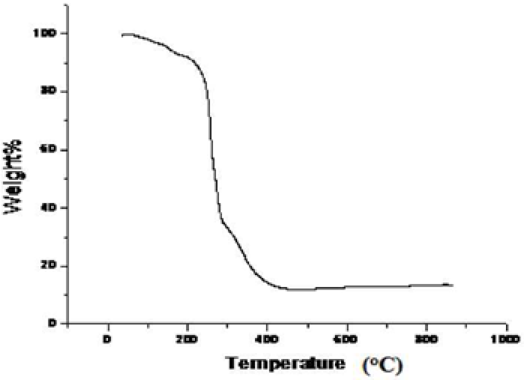
The X-band EPR spectra of the complex was recorded as the polycrystalline examples at room temperature (Fig.7.6). The giso esteem and geometric parameter G i.e. the estimation of trade association between the copper focuses was assessed by utilizing the articulation (Jouad, et. al., 2002):

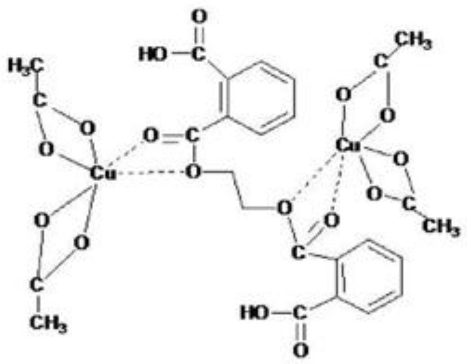
3. Magnetic moment of copper (II) phthalicacid [bis(benzyloxy) ethyl]ester

Attractive vulnerability information for the complex were gathered at room temperature. The attractive minute incentive for the homobinuclear copper edifices was observed to be 1.65 BM. This low estimation of attractive minute qualities for coupled Cu (II) particles recommends antiferromagnetic communication between Cu (II) focuses.

4. Thermo gravimetric investigations of copper (II) phthalicacid[bis (benzyloxy) ethyl]ester:

Thermogravimetric weight reduction bend for the complex has been appeared in Fig. 6.7. The weight reduction profile as a component of temperature demonstrated two stages at ca. 210 oC and 310 oC. It is notable that acetic acid derivations separate in the temperature go 200 oC to400 oC. The weight reduction profile has been broke down according to revealed writing (Chiari, et. al., 2001). The misfortune in weight in the initial step is ca. 59.50%, or, in other words fit to 4CH3COO + 0.52 L. The second drop of weight (24.48%) could be fitted to 0.19 L. The rest of the division of L represents the two oxygen molecules spent in the development of the last item polymeric (CuO2)n. This thermo gravimetric investigation results solely bolsters the structure of the mind boggling as delineated in Scheme 6.2.





5. Mass Spectroscopy study for copper (II) phthalicacid [bis(benzyloxy)ethyl]ester:

The ESI-MS spectra were measured in order to confirm the composition and purity of the compound under investigation. The spectra displayed the molecular ion peak of complex at m/z 717 which is attributable to [M+1] +. The ESI-MS spectrum of complex has been shown in Fig. 6.8. The calculated value of molecular mass of complex is 721

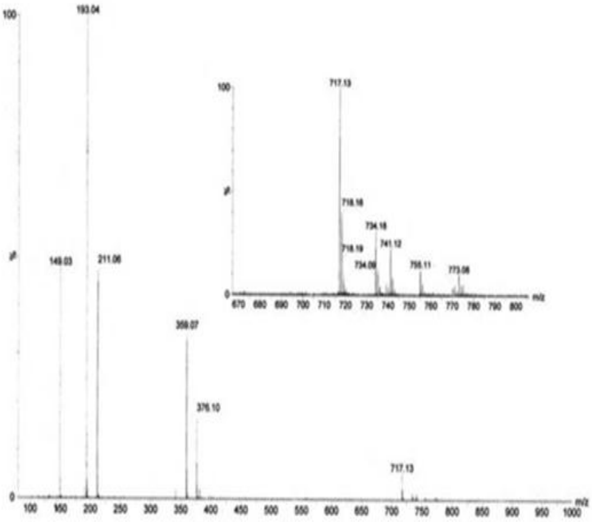


Figure 8: ESI-MS spectra of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester

6. Electrochemistry of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester

Cyclic voltammogram of the 1.0 mM arrangement of the complex in acetonitrile was endeavored on Pt plate anode. A sharp irreversible decrease crest was seen at - 0.005 V versus Ag-AgCl and sweep rate 0.100 Vs-1 (Fig.6.9). This perception is evident on the grounds that in the complex the Cu (II) particle is bound to four hard oxygen benefactor destinations which make the co-appointment exceptionally steady. On decrease of Cu (II) into Cu (I) or, in other words the four oxygen benefactor are not any more reasonable and the complex is relied upon to separate. Subsequently the irreversible decrease crest with no oxidation partner was watched

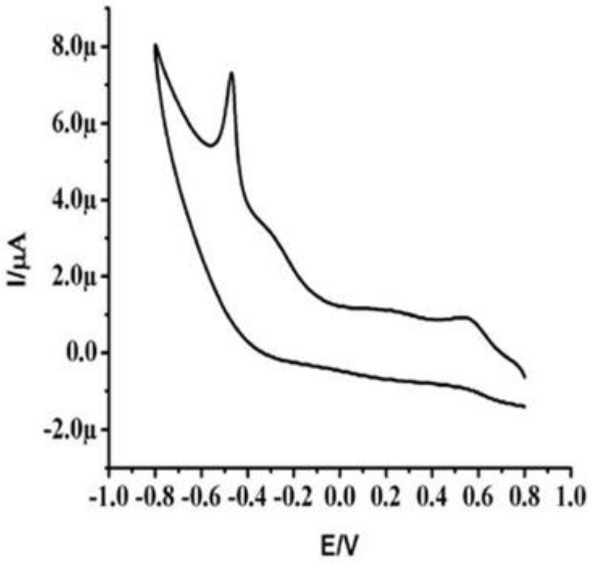
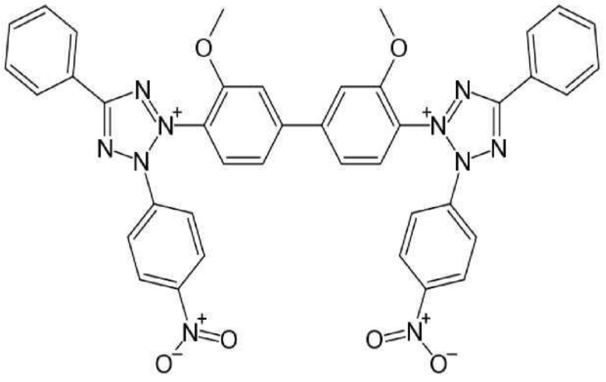


Figure 9: Cyclic voltammogram of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester in CH_3CN. Working electrode: Pt disc.reference electrode: Ag-AgCl supporting electrolyte: TBAB



Scheme: Structure of Nitroblue tetrazolium (NBT)

The SOD movement of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester has been examined by the technique for NBT decrease utilizing KO2-. as the wellspring of superoxide radical [29]. The NBT is yellow in shading. Upon decrease by KO2-DMSO arrangement a blue shaded formazon color was shaped. The assimilation of this blue shaded arrangement was estimated promptly at 560 nm against a proper clear. One unit of SOD action (IC50 esteem) was characterized as the test substance required for half restraint of NBT decrease by the superoxide anion (Ferrari, et. al., 2002). A direct connection was gotten between the centralization of the copper complex and the hindrance of the superoxide particle. The 100% of superoxide movement relates to a measure performed without complex. With the end goal to decide the grouping of the complex required to yield half hindrance of the response, we plotted the level of restraint against the metal focus and the got IC50 esteem was 8.6x10-6 M (the IC50 estimation of the local chemical is 0.72x 10-7 M (Wilkinson & Gillard, 1987). This IC50 esteem is littler than many announced ones e.g. IC50 esteems are found in the range 3.0x10-5 M to 3.7x10-5 M for Cu (II) edifices of amino acids (Pederson, 1973); 9.9x10-5 M to 2.4x10-4 M for the Cu (II) buildings with straightforward dipeptides (Mizushima, et. al., 1992). It has been recommended that just buildings with IC50 esteems beneath 20x10-6 M may turn out to be clinically fascinating (Tabbi, et. al., 1998). IC50 esteem for this complex has been contrasted and other announced one in Table 7.2. Accordingly, copper (II) phthalicacid[bis(benzyloxy)ethyl]ester satisfies this necessity and shows up as an intriguing plausibility for further examinations in the field of SOD-mimetic medications.

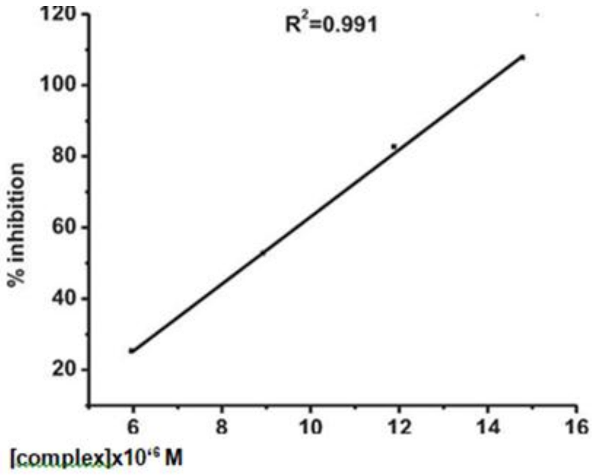
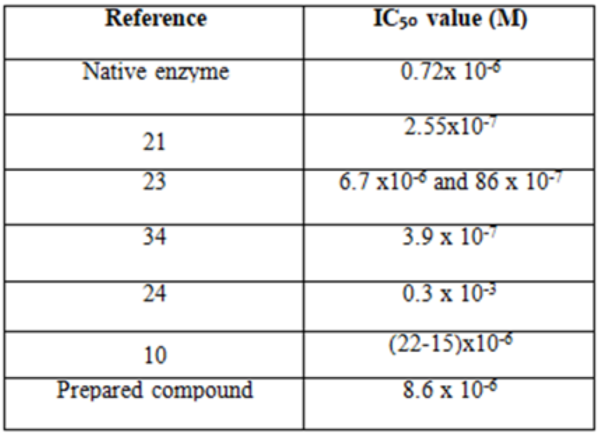


Figure 10: Plot of percentage inhibition against molar concentration of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester

Table 2: Comparison of IC_{50} value of the prepared compound with some reported ones



Reference	IC_{50} value (M)
Native enzyme	0.72×10^{-6}
21	2.55×10^{-7}
23	6.7×10^{-6} and 86×10^{-7}
34	3.9×10^{-7}
24	0.3×10^{-3}
10	$(22-15) \times 10^{-6}$
Prepared compound	8.6×10^{-6}

CONCLUSION

Another ligand framework phthalicacid[bis (benzyloxy)ethyl]ester got from phthalic anhydride and ethylene glycol has been blended and portrayed. The ligand ties to two Cu (II) particles giving another bimetallic Cu (II) complex which indicate critical superoxide dismutase action. This compound has the likelihood for further examinations in the field of SOD-mimetic medications as it satisfies all the basic prerequisites in such manner.

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