



## Screening of Antimicrobial and Antioxidant Properties of Ethylenediamine Mono-dithiocarbamate to Overcome the Resistance of Microbes Against Existing Drugs

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### Abstract

Excellency of the medical treatment depends on the potentiality of drugs. The increase of the resistance of microbes against drugs is influencing this aspect. Dithiocarbamate has an application in medicine and biology. Therefore, we have synthesized ethylenediamine mono-dithiocarbamate ligand and explored its antimicrobial and antioxidant property. The ligand exhibited moderate antibacterial activity against 13 (thirteen) selected bacteria in disk diffusion method. It showed notable antifungal activity compared to the standard. Therefore, this ligand may be considered as a potential antifungal agent in antifungal drug design. The antioxidant behavior using DPPH assay revealed that this ligand has significant capability in scavenging radicals as compared to standard.

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### Introduction

The increase of the endurance of microorganisms against resistance strains of bacteria is alarming for the health issues all over the world. By changing the genotype code according to the type of the applied drugs the microorganisms are capable to survive in human body with more strength and accelerate their growth (Mehla and Ramana 2016; Hefnawy *et al.* 2018). Therefore, research with the effective microbial resisting compound is the prior concern in the present world. In this respect, dithiocarbamates may be promising therapeutic alternative that has not been adequately explored.

Dithiocarbamates (DTC) of diamine are  $>N-CS_2H$  containing compounds, capable in forming complexes with all transition metal with various oxidation states through its sulfur donor atoms (Ebony-Jewel *et al.* 2009; Nabipour 2011).

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It possesses both donation via S-donor and N-back-donation *via*  $\pi$ -orbital delocalization at the same extent. Therefore, DTC is used as fungicides, pesticides (Coucouvanis, 2003), antioxidants (Scott, 2004). Like DTC, isatin (*H* indole-2, 3-dione), an endogenous indole act as inhibitors of apoptosis (Lee *et al.*, 2001), anticonvulsants (Verma *et al.*, 2004) and other antiviral (Sriram *et al.* 2004), anti-bacterial and anti-fungal agents (Chohan *et al.*, 2004). Hence, Schiff base ligand of isatin may have medicinal value to compete with available drugs.

Schiff's and N-Mannich bases of isatin and its derivatives with 4-amino-N-carbamimidoyl benzene sulfonamide are reported have better antibacterial activity (Singhet *et al.* 2010). 3-(4-(4-dimethylaminobenzylideneamino) phenylimino) indoline-2-one showed high antioxidant activity (Prakash *et al.* 2011). Moderate antibacterial activity of Schiff bases of N-benzyl isatins with sulphanilamide and 4-methylsulphonyl aniline has been reported though they didn't exhibit any antifungal response (Shakir *et al.* 2020). The improve of antibacterial activity of chitosan/isatin Schiff base derivative than chitosan has also been reported (Omer *et al.* 2020).

Therefore, survey on literature revealed that dithiocarbamate ligands are selective in the biological activity and their intrinsic chemical interest as multidentate compound has prompted a considerable increase in the study of their coordination behavior. The antibacterial activity and bacterio antibiotic of the compound is related to cell wall structure of the bacteria. The sulphur dente of dithiocarbamate compound is expected to capable of destroying the bacterial cell. The diffusion of the drugs in the lipid layer of the microorganism may become more efficient and kill them more aggressively by deactivating the respiration process.- Consequently, systematic study of the antimicrobial and antioxidant properties of such S, N-donor containing ethylenediamine mono-dithiocarbamate ligand may have interesting research value. Therefore, this research deals with the systematic evaluation of antimicrobial and antioxidant properties of ethylenediamine mono-dithiocarbamate ligand.

## Materials and Methods

Dithiocarbamate contributes in designing and developing novel complexes having potential biological actions with fewer side effects. Previously published procedures were followed in the preparation of ethylenediamine mono-dithiocarbamate ligand (Begum *et al.*, 2017; Sarker *et al.*, 2019; Papri *et al.*, 2021) by the interaction of diamine with CS<sub>2</sub>, respectively.

### *Antibacterial screening*

The study on biological activity was done in the disc diffusion method (Papri *et al.* 2021; Uddin *et al.* 2012). Mueller-Hinton Agar was used as nutrient media for the growth of microorganism. In a sterile petri dish, the sterilized neutral media (at 25 °C maintaining pH at 7.2 ± 0.2) was incubated at 30-37 °C for 30 minutes. The pure cultured microbes were inoculated to Trypticase soy broth and incubated at 37 °C until achieving the turbidity of the 0.5 McFarland standard and then it will be dispersed on agar in a petri dish. 3 mg test sample was taken to prepare 500  $\mu$ L sample solution in DMSO. Then 50  $\mu$ L of this solution was injected in each screening test. Test sample containing discs were placed on the dry inoculated nutrient agar medium uniformly seeded with the test bacteria. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. The plates were incubated at 37 °C and inverted within 15 minutes after the discs were applied. After 16 to 18 hours of incubation the zones of inhibition produced by compounds and standards were recorded in mm and compared. The antibacterial activity of synthesized ligand was studied against some gram-positive bacteria - *S. aureus*, *B. megnaterium*, *B. creus*, *B. subtilis*, and

gram-negative bacteria - *E. coli*, *S. typhi*, *S. dysentery*, *S. boydii*, *S. paratyphi*, *S. flexneri*, *S. sonnei*, *Enteropathogenic E. coli*, *Enterotoxigenic E. coli*. Ceftriaxone was used as standard.

#### *Antifungal activity*

*A. niger*, *P. notatum*, *N. crassa*, *C. albican*, *T. harzianum*, *A. flavus* fungi were used for the test of antifungal activity of ligand using Michonazole as standard antifungal agent (Miloud *et al.*, 2020). We injected 50  $\mu\text{L}$  of 3 mg/500  $\mu\text{L}$  sample solution in each antifungal screening test. First of all we sterilized medium containing conical flasks in autoclave (at 121  $^{\circ}\text{C}$  and 15 lb psi for 15 minutes). Then, test samples (1%) were added to that sterilized medium at the point of pouring to obtain the desired concentration. Before pouring, the flask was shaken to ensure the homogeneous mixing of the chemical with the medium. The medium with definite concentration (0.1%) of chemical was then poured at the rate of 10 mL in sterilized glass Petri dish individually. Proper control was maintained separately with sterilized PDA medium without chemical and three replications were prepared for each treatment. After solidification of medium the fungal inoculum (5 mm mycelial block) was placed on the center of the Petri plates at inverted position. All the plates were inoculated at room temperature on laboratory desk for three days. Radial growth of fungal colony will be measured in mm, after three days of incubation at  $(25 \pm 2)^{\circ}\text{C}$  and an average of the three replication was taken as the diameter of the colony. The percentage inhabitation of mycelial growth of test fungi will be calculated as follows:

$$I = \left( \frac{C-T}{C} \right) \times 100 \dots\dots\dots(1)$$

where,

$I$  = Percentage of inhibition

$C$  = Diameter of the fungal colony in control ( $\text{CHCl}_3$ )

$T$  = Diameter of the fungal colony in treatment.

#### *Antioxidant activity*

DPPH radical scavenging ability of the prepared ligands was utilized to determine their antioxidant activity (Tabrizi *et al.* 2019). 500mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH, 3 mg) solution was prepared in methanol. solution of ascorbic acid was used as standard in this experiment. A variable amount (7, 15, 30, 60, 125, 250  $\mu\text{L}$ ) of a methanolic solution (1.2 mM in sample) was placed in a cuvette, and 4 mL of prepared DPPH solution was added. The % of inhibition was measured at from the variation of the UV absorbance at 517 nm wavelength that is accompanied by the colour change of the DPPH solution. Initial absorbance was measured immediately. The decrease in absorbance was recorded continuously every certain time (minutes) until the completion of the reaction at time  $t$ . Methanol was used for the baseline in this case. The absorbance of the DPPH radical without an antioxidant, i.e. the control was measured and concentration was calculated. The percentage of inhibition (% In) of the DPPH radical by each sample will be calculated by:

$$\% \text{ In} = \frac{A_0 - A_t}{A_0} \times 100 \dots\dots\dots(2)$$

where =  $A_0$  = absorbance of the control (DPPH radical) at time 0

$A_t$  = absorbance of the mixture DPPH–antioxidant at time  $t$ .

Ascorbic acid was used as a standard in this experiment.

The obtained data was fitted using dose response fitting function under growth/sigmoidal model. After nonlinear curve fitting, the half maximal effective concentration ( $EC_{50}$ ) was evaluated.

## Results and Discussion

### Antibacterial screening

The antibacterial activity of the Ethylenediamine mono-dithiocarbamate ligand was examined against gram-positive bacteria - *S. aureus*, *B. megnerium*, *B. creus*, *B. subtilis*, and gram-negative bacteria - *E. coli*, *S. typhi*, *S. dysentery*, *S. boydil*, *S. paratyphi*, *S. flexneri*, *S. sonnel*, *Enteropathgenic. E. coli*, *Enterotoxigenic E. coli* shown in Fig. 1. The ligand exhibits positive response against selected all bacteria. But compared to the standard antibacterial agent (Ceftriaxone) it shows less inhibition capability against all bacteria at similar concentration except *B. subtilis*. As a ligand Ceftriaxone possess N, S, O available dents. The beta-lactam part help Ceftriaxone to binds with the bacterial membrane forming enzymes and inhibit the cell division (Lemke and Williams 2013; Sivapalasingam and Steigbigel 2015). At the same concentration of our studied bi-dentate ligand contain N and S dente where basically S is the active site for binding with bacterial cell. Therefore, less antibacterial activity of the studied ligandis observed than that of standard. ENDTC shows good antibacterial activity that is comparable to the standard against *B. subtilis*.

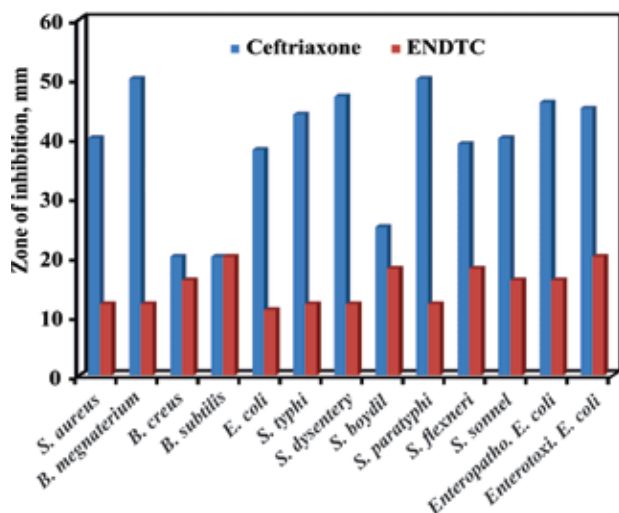


Fig. 1. Variation of antibacterial activity of Ethylenediamine-mono-dithiocarbamate against studied bacteria compared to the standard Ceftriaxone.

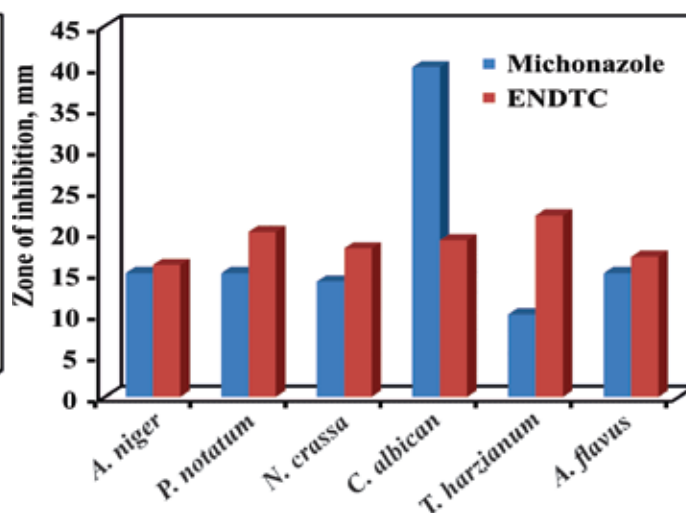


Fig. 2. Variation of antifungal activity of Ethylenediamine-mono-dithiocarbamate against studied bacteria compared to the standard Michonazole.

### Antifungal activity

Antifungal activity of Ethylenediamine mono-dithiocarbamate ligand was performed against six fungal strains *A. niger*, *P. notatum*, *N. crassa*, *C. albican*, *T. harzianum*, *A. flavus* using Michonazole as standard antifungal agent shown in Fig. 2. The ligand exhibits notable antifungal capability against selected fungal

stain. From this study it is observed that at the same concentration, ENDTC shows higher antifungal property than that of standard against five of studied six fungi viz. *A. niger*, *P. notatum*, *N. crassa*, *T. harzianum*, *A. flavus* and the activity is less against *C. albican*. ENDTC shows good antifungal activity against all the studied fungi (viz. *A. niger*, *P. notatum*, *N. crassa*, *T. harzianum*, *A. flavus*) except *C. albican*.

### Antioxidant property

The assessment of the antioxidant property of ENDTC was done using DPPH free radical-scavenging assay. The calculated % of inhibition data against various concentrations of the sample was shown graphically in Fig. 3. From this scheme it is observed that the DPPH scavenging is notable at lower concentration of sample. But with the increase of the concentration of the sample the gradual increasing trend to the ligand become shallower.

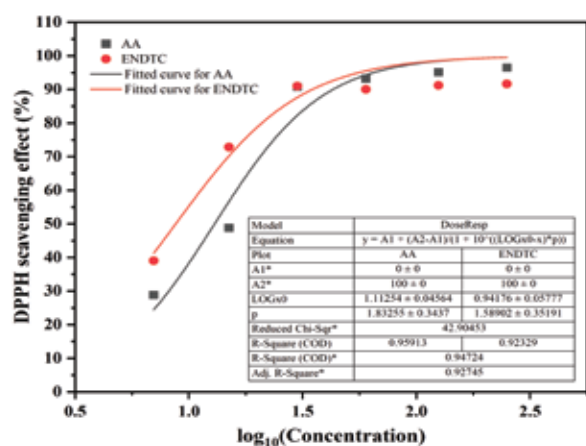


Fig. 3. Graphical presentation of antioxidant response of ENDTC with respect to standard ascorbic acid (AA) using DPPH radical-scavenging assay (ENDTC).

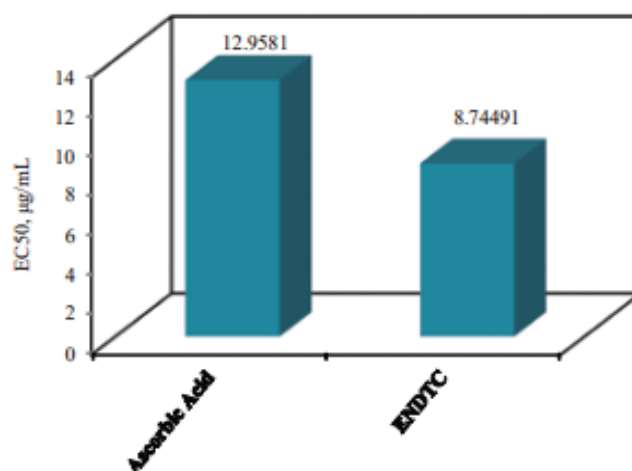
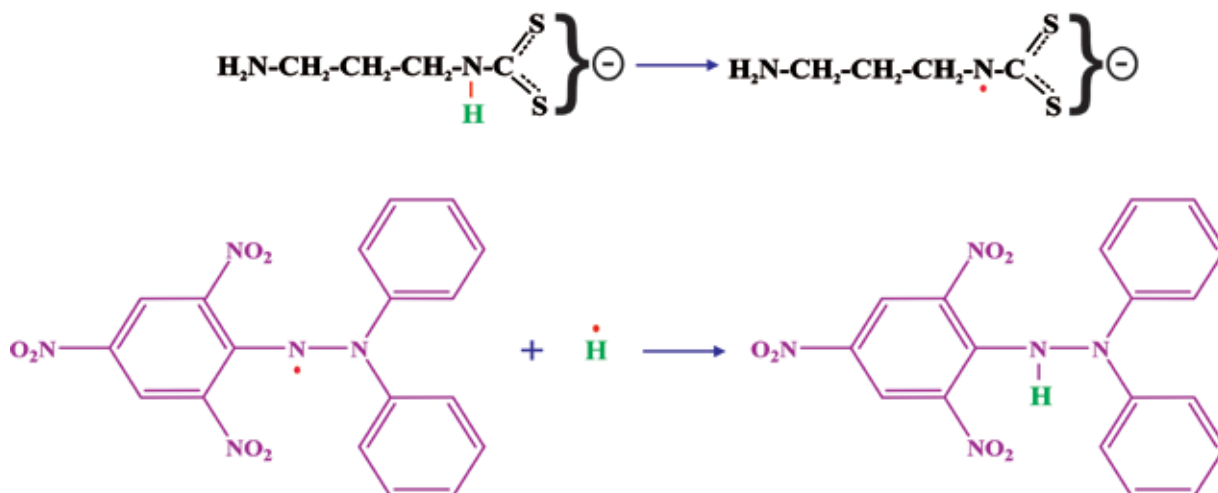


Fig. 4. Calculated half maximal effective concentration (EC50) of ENDTC with respect to standard ascorbic acid using DPPH radical-scavenging assay.



Scheme 1. Probable mechanism of DPPH radical scavenging of ENDTC.

The half maximal effective concentration ( $EC_{50}$ ) of the ligand and standard were calculated from the fitted curves of Fig. 3 and are shown in Fig. 4. It can be observed that ENDTC possesses  $EC_{50}$  value which is lower than the standard (ascorbic acid). This reveals that  $8.74 \pm 1.16 \mu\text{g/mL}$  concentration of ENDTC was required for the half of the maximum anti-oxidizing effect. Therefore, lower  $EC_{50}$  value of ENDTC indicates it's more anti-oxidizing efficiency than selected standard. This may be due to the availability of hydrogen in DTC for scavenging DPPH. In this case the delocalization of  $\pi$  electrons between C=S and C-SH provide the stability of the DTC structure.

From the above study the possible mechanism of radical scavenging may be as scheme 1. Further studies have to be done to understand the exact mechanism to properly explain the antioxidant property of the synthesized ligand.

## Conclusions

The antimicrobial and antioxidant behavior of ethylenediamine mono-dithiocarbamate ligand were investigated in this research. The microbial screening of this ligand showed that the ligand is bioactive and has toxicity against microbial cell. Comparable study of antibacterial property revealed that ENDTC has less antibacterial activity than standard. The antifungal screening of ENDTC indicated the significant capability of inhibiting most of the studied fungal strain as compared to the standard. The ligand was also applied for the study of antioxidant property. The less  $EC_{50}$  value of the studied ligand indicated that ENDTC has greater potentiality in DPPH radical scavenging than the standard. Further investigation is necessary to establish the accurate mechanism of antioxidant property.

### *Statement of Authors' credit*

A. Sarker: Writing-original draft manuscript, Visualization, Data analysis, M.Irfan Ali and T. T. Arzina: Investigation and data collection, M. Lokman Hossain: Writing-reviewing, editing and Supervision, M. Aminul Haque: Antimicrobial analysis and discussion and A. K. M. Lutfor Rahman: Conceptualization, writing-reviewing, editing and Supervision. All authors have read manuscript and approve it to submit for the publication.

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### *Conflict of interest*

The authors declared that they have no conflict of interest.

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