American Journal of Chemistry and Application
2018; 5(4): 51-57
http://www.aascit.org/journal/ajca
ISSN: 2375-3765

AASCIT American Association for Science and Technology

Antibacterial Assay of Two Synthesized Dithiocarbamate Ligands

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Citation

Gloria Ihuoma Ndukwe, James Udochukwu Nzeneri, Ovire Julius Abayeh. Antibacterial Assay of Two Synthesized Dithiocarbamate Ligands. *American Journal of Chemistry and Application.* Vol. 5, No. 4, 2018, pp. 51-57.

Received: April 2, 2018; **Accepted**: April 27, 2018; **Published**: May 31, 2018

Abstract: Dithiocarbamates are compounds that bind strongly and selectively to so many metal ions. They readily form chelates with all transition metal ions through their two donor sulphur atoms. In this study, two derivatives of dithiocarbamate (sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate) were studied to determine their effectiveness in the treatment of diseases caused by the tested organisms. Antibacterial activities of these ligands were carried out using the disc diffusion method. Antibacterial activities were exhibited by sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate, against *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis, and Salmonella typhi*. Minimum inhibitory concentration was 15 mg/ml for sodium phenyldithiocarbamate with zone of inhibition range of 8.5 mm - 19 mm and 30 mg/ml for sodium cyclohexyldithiocarbamate with zone of inhibition range of 7.7 mm - 16.3 mm. The ligands can compete favourably with gentamycin which served as the reference drug.

Keywords: Sodium Phenyldithiocarbamate, Sodium Cyclohexyldithiocarbamate, Antibacterial Activity, Dithiocarbamate

1. Introduction

Dithiocarbamates are versatile compounds with a wide range of chemistry, capable of forming complexes with most of the elements and able to stabilize transition metals in a variety of oxidation states [1-3]. This ability of stabilizing high oxidation states in metal complexes reflects strong o-bonding characteristic of these ligands [4, 5].

A very large number of dithiocarbamate complexes with transition and non-transition metal ions have been reported [2, 6-9]. Compounds with dithiocarbamate moiety have attracted attention because of their potential biological property [10, 11]. Metal complexes of dithiocarbamate present striking structural features and have many applications, such as high-pressure lubricants, fungicides, pesticides, and accelerators used in vulcanization [12, 13]. Although sulphur atoms of dithiocarbamate ligands possess o-donor and n-back-donation characteristics of the same order of magnitude, these ligands have a special characteristic in that there is an additional n-electron flow from nitrogen to sulphur *via* a planar delocalized π- orbital system [4]. The effect of the delocalized π- orbital system results in a strong electron donation and hence a high electron density on the metal leading to its next higher oxidation state [14, 5]. While dithiocarbamate complexes have been known for over the years, with many having been synthesized, majority of these contain only simple alkyl substituents such as methyl and ethyl [4, 5]. Dithiocarbamates are a class of metal-chelating, antioxidant compounds with various applications in medicine for the treatment of bacterial and fungal infections, and possible treatment of some other ailments like acquired immune deficiency syndrome [15, 5].

A lot of work has been carried out on the antimicrobial activities of dithiocarbamate. Most of the works reported on these activities are basically on the complexes of the dithiocarbamate. Mixed-ligand dithiocarbamate complexes of Ni(II) have been reported to show good activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytosa* and *Staphylococcus aureus* [16]. Likewise, 2-amino-2-methyl-1-propanol dithiocarbamate and pyridine adducts of *N*-methyl-*N*-phenyl dithiocarbamate complexes of Mn(II),

Co(II), Ni(II), and Cu(II) have shown selective activity towards some microbes and antifungal activities respectively [17, 18]. Dithiocarbamate cobalt complex has also exhibited significant antibacterial and antifungal activities with MIC of 30 µg/disc [4].

The purpose of this study is to assess the antibacterial effects of two synthesized derivatives of dithiocarbamate ligands (sodium phenyldithiocarbamate salt and sodium cyclohexyldithiocarbamate salt) for effective use as antibacterial agents.

2. Materials and Methods

2.1. Syntheses of Dithiocarbamate Ligands

Syntheses of sodium cyclohexyldithiocarbamate and sodium phenyldithiocarbamate salts have already been reported in our earlier publication [19].

2.2. Antibacterial Assay of the Ligands

The ligands were tested against five standard strain of bacteria obtained from International Centre for Drug Research, Lucknow. The microbes are: *Bacillus subtilis* ATCC 14579, *Bacillus cereus* ATCC 33923, *Pseudomonas aeruginosa* ATCC 27856, *Proteus mirabilis* ATCC 21784 and *Salmonella typhi* ATCC 2785. Antibacterial activities of the ligands were investigated using the disc diffusion method [20].

2.2.1. Preparation of Stock Solution

Stock solution of each ligand was prepared by dissolving 200 mg of the ligand in 2.0 ml of dimethylsulphoxide (DMSO), giving a concentration of 100 mg/ml. From this, other standard solutions of 30, 25 20, 10, 15, 1.0 and 0.1 mg/ml were prepared for each of the ligand using DMSO as solvent. This was done to obtain the minimum inhibitory concentrations (MIC) of the ligands. Whatman filter papers No. 2 were punched into 5 mm discs, kept in a screw-capped sample bottle and sterilized in an autoclave at 121°C for 15 minutes. Twenty (20) of the sterilized paper discs were impregnated with 0.2 ml of the various prepared concentrations of the ligands. Also, 20 sterilized paper discs were impregnated with 0.2 ml each of DMSO and gentamycin (5 µg/ml) in separate glass vials which served as the negative and positive controls respectively.

2.2.2. Preparation of Agar Plates

Nutrient agar was prepared by dissolving 28 g of the solid nutrient agar in 1L of distilled water. The prepared agar solution was stirred to homogenize, then sterilized in an autoclave at 121°C for 15 minutes, allowed to cool to 47°C and subsequently poured into Petri-dishes. The plate content was left to cool and solidify [20].

2.2.3. Preparation of Inoculums and Disc Placement

Inoculation was done using streaking method which involves streaking the inoculums around the peripheral region of the plate (Petri-dishes) using a sterilized cotton bud for each micro-organism. The Petri-dishes were divided into section with a marker indicating the various prepared concentrations of the ligands (100, 30, 25, 20, 15, 10, 1.0, 0.1 mg/ml), DMSO and gentamycin. This is to give room for the placement of the various impregnated paper discs. After the inoculation, a disc impregnated in 0.2 ml of the various prepared concentrations of each of the ligand was picked with a sterile syringe and dropped in the appropriate position (as marked) in the Petri-dish. This was done in triplicate for each bacterium to get an accurate reading. After the introduction of each of the disc, the plates were carefully and properly packed into an incubator operated at 37°C and allowed for about 24 h. Zones of inhibition were determined by measuring clear zones across the discs in mm. Mean values and standard deviations of triplicate readings were then determined and used.

3. Results and Discussion

Antibacterial activities of the ligands were determined using disc diffusion method. The activity was measured as zone of inhibition which is the diameter of the clear zone of inhibited bacterial growth. The ligands showed activity against the test organisms at concentrations of 15, 20, 25, 30, and 100 mg/ml for sodium phenyldithiocarbamate and at 30 and 100 mg/ml for sodium cyclohexyldithiocarbamate. The analysis was done in triplicate and the mean values of the results with their corresponding standard deviations are presented in Tables 1 and 2.

Table 1. *Antibacterial Activity of Sodium phenyldithiocarbamate.*

Bacteria	*Zone of inhibition (mm)									
	Ligand concentration (mg/ml)								Gentamycin (5 µg/ml)	DMSO
	100	30	25	20	15	10	1.0	0.1		
ST	19.0±0.0577	12.3±0.1000	10.7±0.1155	10.0±0.1528	8.5±0.0116	0	0	0	25.7±0.0577	0
BS	19.0±0.1000	11.7±0.1000	11.0±0.0577	9.7±0.0577	8.7±0.0000	0	0	0	25.0±0.1000	0
BC	18.7±0.1527	12.7±0.1528	11.3±0.0577	10.0±0.0577	8.7±0.1000	0	0	0	25.0±0.0577	0
PSA	19.0±0.1155	12.0±0.0000	11.0±0.0577	10.0±0.0577	9.0±0.1000	0	0	0	25.3±0.0577	0
PM	18.7±0.0577	12.3±0.0577	11.0±0.0000	10.0±0.1000	8.5±0.0577	0	0	0	25.0±0.0000	0

*The values are average values of three readings

Key: ST = *Salmonella typhi*, BS = *Bacillus subtilis*, BC = *Bacillus cereus*, PSA = *Pseudomonas aeruginosa*, PM = *Proteus mirabilis*, Gentamycin = Positive Control, DMSO = Negative Control

Chemicals or drugs are often administered to selectively eliminate or aid in the elimination of pathogenic organisms.

Substances used for the treatment of bacterial diseases are referred to as chemotherapeutic substances or agents [21]. The synthesized ligands (sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate) were studied to find out if they can be used as chemotherapeutic agents in the treatment of diseases caused by the five test organisms.

Sodium phenyldithiocarbamate was found to have antibacterial properties against all five tested organisms, with the same minimum inhibitory concentration (MIC) of 15 mg/ml. Its activities on *Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa* showed the same zone of inhibition (19.0 mm) at the same concentration (100 mg/ml), while *Bacillus cereus* and *Proteus mirabilis* also showed same zone of inhibition of 18.7 mm at the same concentration (100 mg/ml) [Table 1].

Gentamycin was found to inhibit the growth of the five tested organisms and the zone of inhibition was found to be in the range of 22.7 - 25.7 mm, while the solvent (DMSO) showed no zone of inhibition against the tested organisms. This indicates that the antibacterial activities observed in the screening of sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate were as a result of the ligands and not due to the DMSO used in the preparation of different ligand concentrations.

MIC of sodium phenyldithiocarbamate was found to be 15 mg/ml, with 8.5 mm zone of inhibition for *Salmonella typhi* and *Proteus mirabilis,* 8.7 mm zone of inhibition for *Bacillus subtilis* and *Bacillus cereus* and 9.0 mm for *Pseudomonas aeruginosa*.

Table 2. *Antibacterial Activity of Sodium cyclohexyldithiocarbamate.*

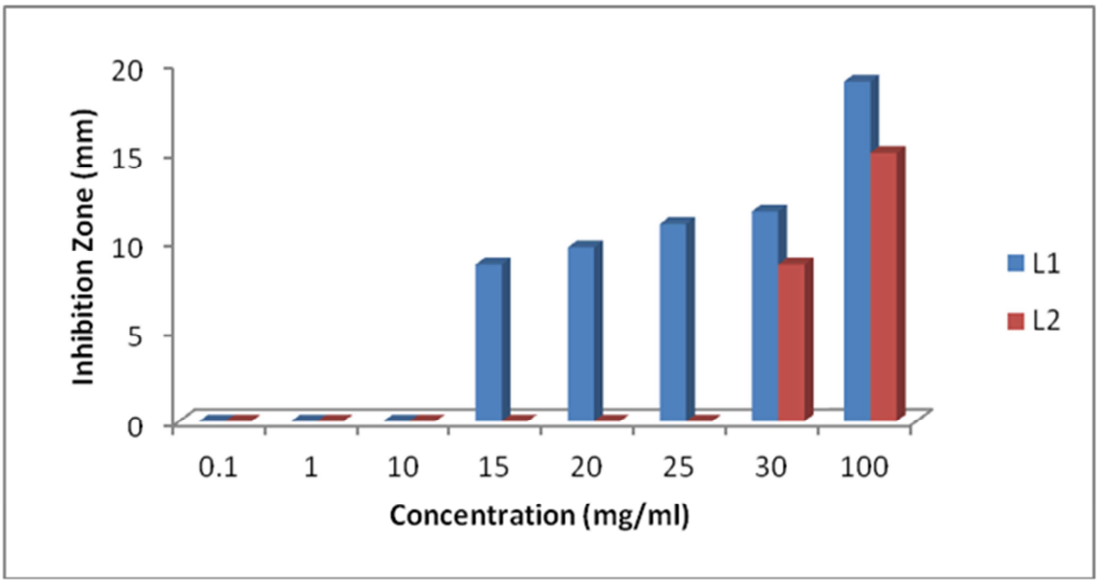
Bacteria	*Zone of inhibition (mm)									
	Ligand concentration (mg/ml)								Gentamycin (5 µg/ml)	DMSO
	100	30	25	20	15	10	1.0	0.1		
ST	16.3±0.0333	8.7±0.0577	0	0	0	0	0	0	23.7±0.0577	0
BS	15.0±0.0577	8.7±0.0000	0	0	0	0	0	0	22.7±0.1000	0
BC	15.3±0.0577	7.7±0.0577	0	0	0	0	0	0	24.3±0.0577	0
PSA	15.0±0.1000	9.0±0.1000	0	0	0	0	0	0	25.0±0.0000	0
PM	15.3±0.0577	8.3±0.0577	0	0	0	0	0	0	25.0±0.0577	0

*The values are average values of three readings

Key: ST = *Salmonella typhi*, BS = *Bacillus subtilis*, BC = *Bacillus cereus*, PSA = *Pseudomonas aeruginosa*, PM = *Proteus mirabilis*, Gentamycin = Positive Control, DMSO = Negative Control

Sodium cyclohexyldithiocarbamate was also found to have antibacterial property against the five organisms used, showing similar MIC of 30 mg/ml. Activity on *Salmonella typhi* showed the highest zone of inhibition (16.3 mm) at concentration 100 mg/ml, while other organisms showed zone of inhibition range 15.0 - 15.3 mm. Activity on *Bacillus subtilis* and *Pseudomonas aeruginosa* showed the same zone of inhibition (15.0 mm) at concentration of 100 mg/ml as shown in Table 2.

Sodium cyclohexyldithiocarbamate which showed MIC of 30 mg/ml with 8.7 mm zone of inhibition for *Salmonella typhi* and *Bacillus subtilis,* 7.7 mm for *Bacillus Cereus,* 9.0 mm for *Pseudomonas aeruginosa* and 8.3 mm for *Proteus mirabilis,* can be used for treatment of infections caused by the tested organisms.

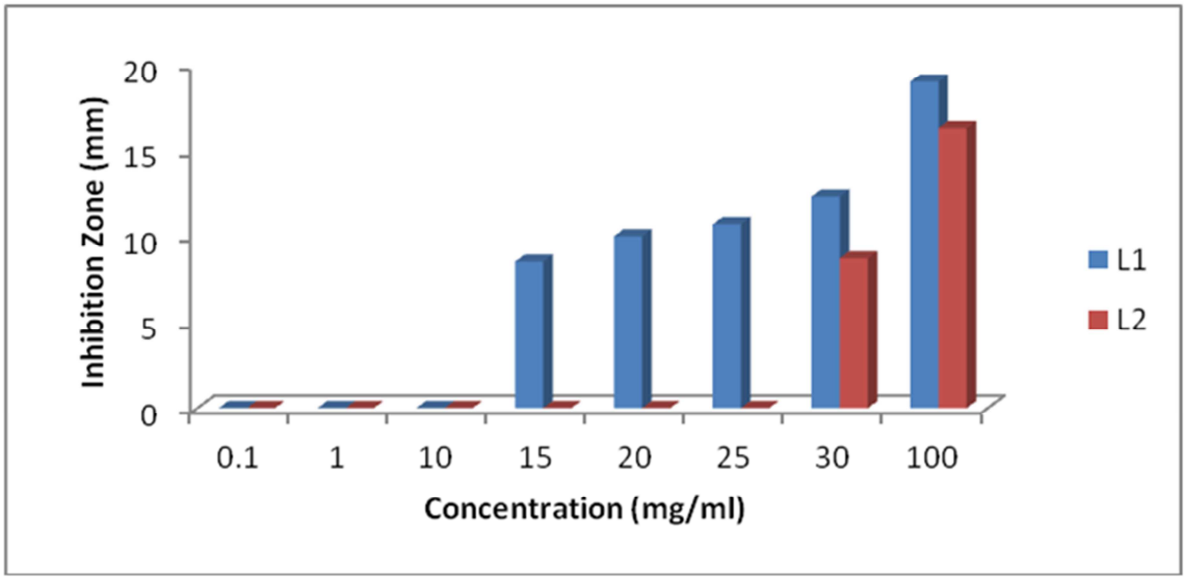


Key: L1 = Sodium phenyldithiocarbamate salt, L2 = Sodium cyclohexyldithiocarbamate salt

Figure 1. *Average zone of inhibition of both ligands on Bacillus subtilis (BS).*

The increasing number and variety of drug resistant pathogens is a serious public health problem [22]. Recent studies show that *Bacillus subtilis* grow in anaerobic conditions and use nitrite as terminal acceptor of electrons [23]. Its spores can survive the extreme heating that is often used to cook food and is responsible for causing rapines [24].

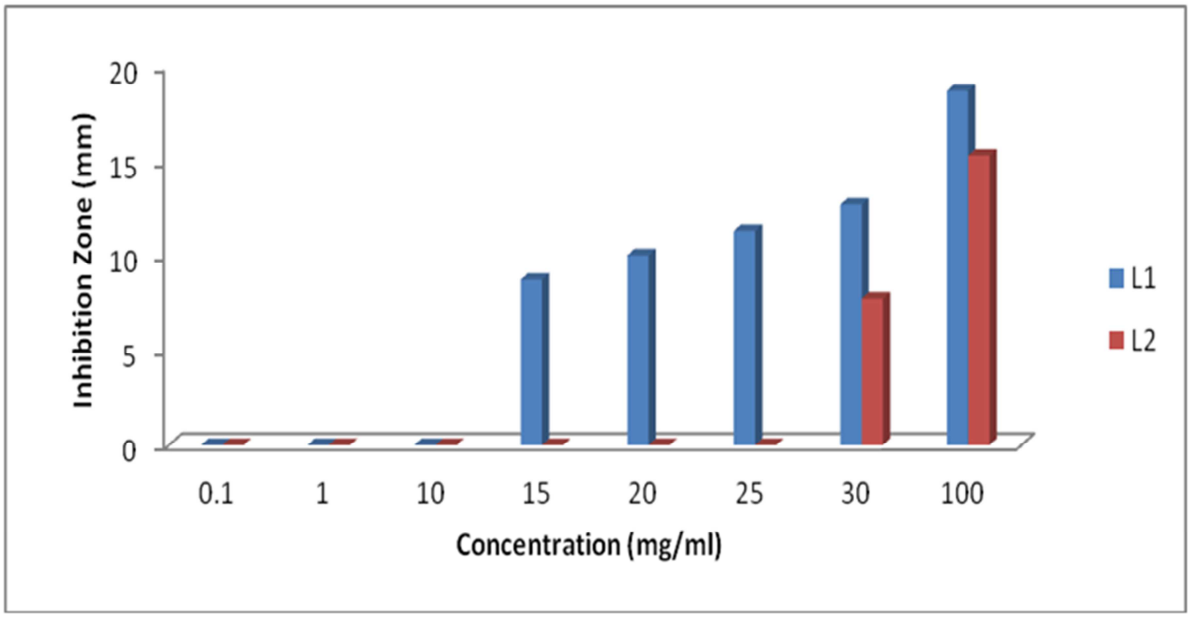
Even though *Bacillus subtilis* which is a rod-shaped Gram-positive bacterium can gain protection more quickly against many stress situations such as acidic, alkaline, osmotic or oxidative conditions and heat or ethanol [25, 26], result of this study (Table 1 and Figure 1) has shown that sodium phenyldithiocarbamate can still be effective for the treatment of diseases caused by it with more effectiveness as concentration of the ligand increases.



Key: L1 = Sodium phenyldithiocarbamate salt, L2 = Sodium cyclohexyldithiocarbamate salt

Figure 2. *Average zone of inhibition of both ligands on Salmonella typhi (ST).*

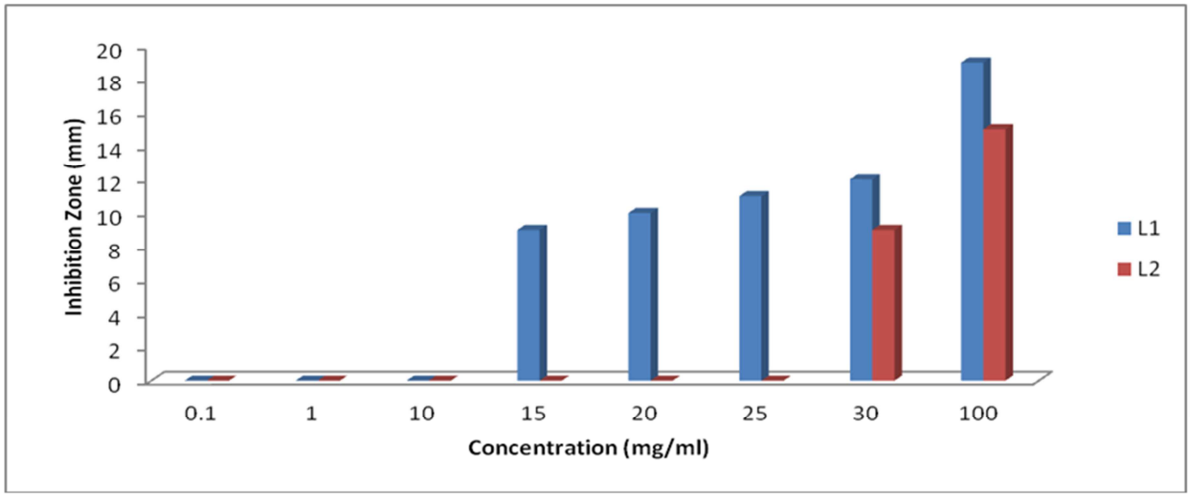
Bacillus cereus which was inhibited by sodium phenyldithiocarbamate (Figure 3) with zone of inhibition of 8.5 mm and MIC 15 mg/ml; is an endemic, Gram-positive rod-shaped beta haemolytic bacteria that causes food borne illness [27], which get compounded when food is improperly refrigerated, allowing the spores to germinate, resulting in the production of enterotoxin; and ingestion leading to illness like diarrhoea and emetic syndrome [28]. Results show that sodium phenyldithiocarbamate can be used as a remedy for the treatment of any infection caused by *Bacillus cereus*.



Key: L1 = Sodium phenyldithiocarbamate salt, L2 = Sodium cyclohexyldithiocarbamate salt

Figure 3. *Average zone of inhibition of both ligands on Bacillus cereus (BC).*

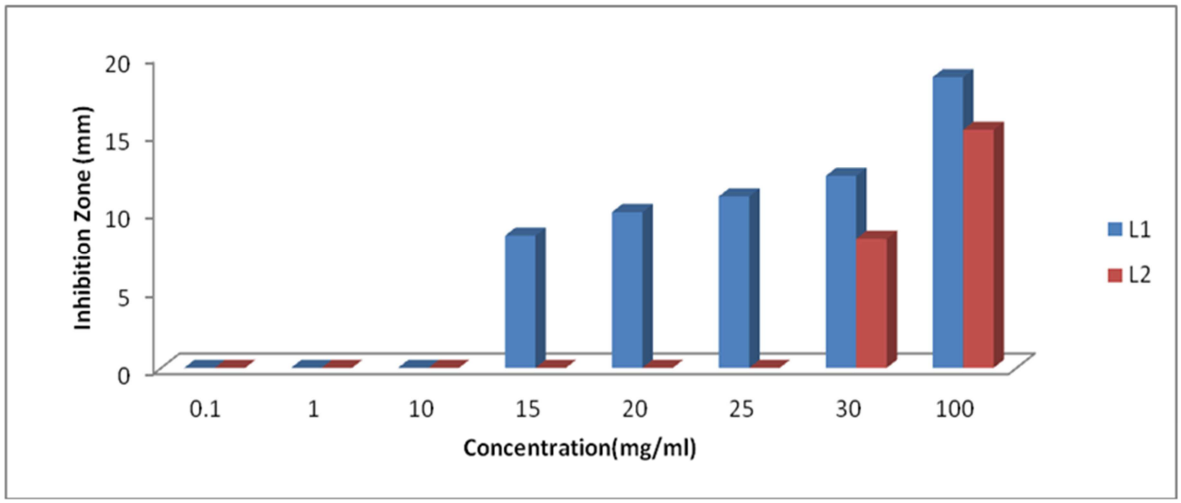
Pseudomonas aeruginosa metabolism grows in the absence of oxygen, if nitrate is available as respiratory election acceptor [29, 30]. Formation of bacteria capsule, slime layer and bio-film effectively protect cells from opsonization, antibodies, complement deposition and phagocyte engulfment [31]. Properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen [32]. The fatality rate in patients is near 50 percent [33] and it is occasionally also a pathogen of plants [34]. The combination of gentamycin and carbenicillin is frequently used for the treatment of severe *Pseudomonas* infections [35]. But, with increased concentration, these ligands can also serve as treatment for diseases caused by *Pseudomonas aeruginosa* (Figure 4).



Key: L1 = Sodium phenyldithiocarbamate salt, L2 = Sodium cyclohexyldithiocarbamate salt

Figure 4. *Average zone of inhibition of both ligands on Pseudomonas aeruginosa (PSA).*

Proteus mirabilis which was inhibited by sodium cyclohexyldithiocarbamate (Figure 5) with 8.3 mm zone of inhibition at MIC 30 mg/ml and 15.3 mm zone of inhibition at 100 mg/ml is a Gram-negative, facultative anaerobic bacterium. They inhibit growth of unrelated strains resulting in a macroscopically visible line of reduced bacteria growth where two swarming strains intersect [36]. They have the ability to produce high level of urease which hydrolyses urea to ammonia and thus makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate, and or apatite. The bacteria can be found throughout the stones, and these bacteria lurking in the stones can reinitiate after antibiotic treatment. Once the stone develop, overtime, they may grow large enough to cause obstruction and renal failures [37] and also produces a very distinct odour [38].



Key: L1 = Sodium phenyldithiocarbamate salt, L2 = Sodium cyclohexyldithiocarbamate salt

Figure 5. *Average zone of inhibition of both ligands on Proteus mirabilis (PM).*

The results (Tables 1 and 2), show that both ligands (sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate) can compete favourably with gentamycin if the concentrations are increased. For an effective treatment of bacterial infections

caused by any of these five tested organisms, concentration of not less than 30 mg/ml may be required for sodium cyclohexyldithiocarbamate and 15 mg/ml for sodium phenyldithiocarbamate.

The experimental studies (Figures 1 - 5) showed that the aromatic type of the ligand exhibited antibacterial activity for all test organisms at a lower concentration (15 mg/ml) compared to the cyclohexyl type of the ligand which showed inhibition at 30 mg/ml and above. This may be attributed to the fact that the aromatic type of the ligand is more stable with a high bonding ability [39].

The results of this study were compared to that of Jayaraju *et al.* [17], where Cu (II) and Mn (II) complexes of 2-amino 2-methyl-1-propanol dithiocarbamate inhibited the growth of four bacteria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) and two fungus (*Candida albicans* and *Candida tropicalis*) at concentration of 10 mg/ml. Cu (II) complex of 2-amino-2-methyl-1-propanol dithiocarbamate showed zone of inhibition range of 14 mm - 15 mm for both bacteria and fungi while Mn(II) complex of 2-amino-2-methyl-1-propanol dithiocarbamate showed zone of inhibition range of 13 mm - 16 mm for both bacteria and fungi. Sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate as observed from the results can only compete with the complexes used in the work of Jayaraju *et al.*, when concentrations of ≥35 mg/ml and ≥90 mg/ml respectively are used.

4. Conclusion

Moderate antibacterial activities were exhibited by both ligands against all five tested standard isolates (*Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis and Salmonella typhi)*. From the economic point of view, it is better to consider the use of the ligand itself as an antibacterial agent than their complexes which requires the addition of metals ions. Result from this study has shown that the ligands (sodium phenyldithiocarbamate and sodium cyclohexyldithiocarbamate) with increased concentrations, can compete favourably with gentamycin which served as the reference drug. Some commonly used antibiotics have side effects, hence the need to explore other alternatives. Thus, exploration of dithiocarbamate ligands as alternative antibiotics could go a long way to improving quality of health care.

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