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In vitro microbial study of metal complexes [Cu (II)] with reference to schiff base ligand and antibiotics

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Abstract
In Vitro antibacterial activity of ligand and its metal complexes were evaluated using Well disc & Diffusion method and compared to the standard drug like Amikacin, Ceftriaxone, Gentamycin and Levofloxacin. The antibacterial activity was examined against Escherchia Coli (E-Coli) as gram negative bacteria (GNB) and Bascillus Subtilis as gram positive bacteria (GPB).Tested complexes followed the order in GPB and GNB :$[Cu(L)_2Cl_2]$ > $[Cu(L)_2Br_2]$ > Ligand and the antibiotic followed the order in GPB and GNB: Ceftriaxone >Amikacin > Levofloxacin and Gentamycin resisted. The Minimum Inhibition Concentration (MIC) or Zone of Inhibition (ZI) diameter in the range of 6-20 mm and clearly explained by Conceptual Model of Inhibition (CMI), Tweedy's Chelation Theory (TCT) and Overton's Concept Theory (OCT).

Keywords: gram positive bacteria (GPB), gram negative bacteria (GNB), inoculation, microbial study, FTIR, NMR, minimum inhibition concentration (MIC), overton's concepts (OC), tweedy's chelation theory (TCT), conceptual model (CM), biological evolution, macconkey agar media

Introduction
Metal Based Drug [MBD] derived from transition metal have been extensively studied for their biological activity [1]. Enhancing their bioactivity is key to designing new and more effective medical drug. Metal based drug bioactivity can be increased by modulating the metal chelation, which in turn decreases their toxicity, and enhances their lipophilicity, absorbance, and stability [2]. Some transition metals are essential component of biological system, playing diverse roles including as co-factors in the enzyme oxidation process, controlling the geometry of the enzyme active sites and activating enzymatic reaction [3]. Moreover, they are present in vitamins such as Co (II) in B12. Copper is essential for nervous system development and it exists in many enzymes and proteins with a wide range of function such as in energy metabolism [4]. Zinc is the most abundant transition metal in human body playing several important roles including in immunity and metabolism and is crucial for the function of more than 300 enzymes [3, 5]. Medicinal inorganic chemistry is comparatively a new discipline which developed after the serendipitous discovery of the antitumor activity of cis-platin [6- 8].

Aim
The interest of this study is to examine the modification that the metal can make to the properties of an organic molecules. When the latter is co- ordinated to it. Copper complexes have attracted great deal of attention due to their therapeutic application as anti-microbial and anti-oxidant so we have been interested in the study of complexation of this metal and in vitro microbial, spectral study of Cu (II) ions.

In Vitro Biological Evolution Methodology
1. Solubility Test
The Synthesized Schiff Base and its metal (II) complexes are only soluble in DMSO and sparingly Soluble (SS) in MeOH, Acetone, Chloroform, Methyl Cyanide etc.

2. Preparation of Stock Solution
A stock solution of 100 µg/ml was made by dissolving 10 mg of each compound in dimethyl sulphoxide (100ml).

3. Preparation of Agar Plates Media
Nutrient MacConkey Agar (nearly 40 gm) was used for the growth of specific bacterial species. The agar plates were suspended in freshly distilled water [500 ml] allowed to soak for 15min and then boiled on a water bath until the agar was completely dissolved. The mixture was auto calved for 10 min at 120^{o}C and then poured into previously washed and sterilized petri discs and stored at 40^{o}C for inoculation.

4. Preparation of Disc
The compound [10 µl] was applied to a paper disc (filter paper, Whatmann No.4 disc, 6mm diameter) using a micropipette. The discs were left in an incubator for 48 hours at 37^o C and then applied to the bacteria grown on agar plates.

5. Process of Inoculation
Inoculation was done with the help of Pt. (Platinum) wire loop which was made red hot in a flame, cooled and then used for the application of bacterial strains.

6. Application of Discs
Sterilized forceps were used for the application of the paper disc on the previously inoculated agar plates and then the plates were incubated at 37^{o}C for 24 hours. The Zone of Inhibition [ZI] or Minimum Inhibition Concentration (MIC) was then measured (in mm) around the disc and the results are tabulated for discussion. Amikacin, Gentamycin, Ceftrixone, Levoflaxacin was used as standard drug. The complexes were compared in terms of Zone of Inhibition [ZI] or MIC.

7. Microbial Study of the Compound

Using the above procedure, microbial study of the compounds were done on GNB & GPB by Well disc and Diffusion Method.

Result & Discussions

Generally antibacterial agents are more effective against Gram positive bacteria [GPB] which is due to the difference in the composition of the cell wall of GPB and Gram Negative Bacteria [GNB] [9]. The cell wall of GNB is mainly composed of Lipopolysaccharides which presents accumulation of compounds in the cell membrane for this reason, GPB are more sensitive compared to GNB [10] [Fig.6] due to the nature of the cell membrane and their compositions. GNB have three other components outside the peptidoglycon [Lipopolysaccharide, Phospholipids, Periplasmic space] for defence GPB lack these protective coats outside peptidoglycon layer which then make them more vulnerable to foreign attack [11] [Fig 6].

From the structure of Schiff Base Ligand [SBL], [Fig.7] the nitrogen is not linked to the central metal atom (CMA), so the ligand may cause toxicity after entering the body and interfere the metabolic reaction. While in the case of Schiff base complex [SBC] the nitrogen (imine gr, >C=N) are directly linked with the CMA [Fig, 8] that significantly reduces the amount of toxicity. Thus appropriate amount of dose of SBC prepared may be chosen to kill bacteria in the body.

The higher activity in the antimicrobial activity of the complexes over the SBL, can be explained on the basis of chelation theory [12-15]. The enhancement in the anti – microbial activity may be rationalized on the basis that the complex mainly possess azomethine [>C=N] group. The FTIR data of imine group delivers a peak at ν 1513(S) & 1512(S) cm^{-1} for [Cu(DMBAP)2Cl2]&[Cu(DMBAP)2Br2] respectively and NMR data clearly predicts the protons of imine group shifted downfield indicating the coordination of ligand to metal ion through nitrogen atom of imine group (Table 3). The imine group [>C=N] present in the complexes shows a good impact on biological activity [16-18]. against E – coli, Bacillus beside the other parameters.

In metal complexes the +ve charge of the metal ion is shared with the hetero donor atom [N] present in the ligand and there may be π-electron delocalization over the whole chelating system [19]. Hence the increase in the Lipophilic character of the metal chelates favour its permeation through the lipid layer of the bacterial membrane and blocking of the metal binding site in the enzyme of microorganism.

The present investigation clearly depicts metal complexes may serve as a vehicle for activation of ligands as the principal cytotoxic species [20]. The minimum inhibitory concentration [MIC] value of the compound against the respective bacteria (E- coli), Bascillus Substillis are Summarized in [Table 2], [Fig.1-4].

A comparative study of growth inhibition zone (GIZ) values of schiff base and its complexes indicate that metal complex exhibit higher anti – bacterial activity than the free ligand and the same is indicated from the result given in [Table 2]. This is probably due to the greater liphophilic nature of the complex such increased activity of the metal chelates can be explained on the basis of Overton's Concept and Tweedy's Chelation Theory [TCT] [21].

According to Overton Concept of cell permeability the lipid membrane that surrounds the cell favours the passage of only lipid soluble material due to which liposolubility is an important factor which controls the anti – microbial activity.

On Chelation, the polarity of the metal ion will be reduced to a greater extent due to overlap of the ligand orbitals and partial sharing positive charge of metal ion with donor group [22-23] Further it increase the delocalization of π – electron over the whole chelate ring and enhances the lipophilicity of the complex this increased lipophilicity enhance the penetration of the complex into lipid membrane and blocks the metal binding sites on enzymes of microorganism [24] These metal complexes also disturb the respiration process of the cell and thus blocks the synthesis of proteins which restricts further growth of the microorganism. [25] From the observed data [Table 2], [Fig.1-4], It is found that metal complexes have higher anti-bacterial activity than the free Ligand. Hence complexation increases the microbial activity [26] such increased activity of metal complexes was explained on the basis of chelation [27].

According to Tweedy`s Chelation Theory [TCT], chelation reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π – electron delocalization within the whole chelate ring system that is formed during co-ordination. Such chelation could enhance the lipophilic character of the central metal atom [CMA- Cu (II)] and hence increasing the hydrophobic character and liposolubility of the complexes favoring its permeation through the lipid layers of the cell membrane. This enhances the rate of uptake/ entrance and thus the antimicrobial activity of the testing compounds. Accordingly, the antimicrobial activity of the complexes can be referred to the increase of their lipophilic character which in turn deactivate enzyme responsible for respiration process and probably other cellular enzymes which play a vital role in various metabolic pathways of the tested Microorganism.

The antibacterial activity of the compound followed the order as;

$[Cu(L)_2Cl_2]$> $[Cu(L)_2Br_2]$ > Antibiotic (Except Gentamycin) > Ligand

Conceptual Model of Inhibition

The mechanism of inhibition may also be explained by the conceptual model. In GPB, the complex inhibits as a pocket via scheme 1 [Fig.9] and covers the whole surface in an irregular manner / eclipsed form in case of $[Cu(L)_2Cl_2]$ while the inhibition effect of the complex $[Cu(L)_2Br_2]$ takes place via scheme 2 [Fig. 10] in an concentric circular shaped, meniscus shaped.

The order of inhibition of the tested compound may be due to the higher electronegativity, lesser atomic size, higher electron affinity and lesser ionic radius of chlorine atom than bromine atom [Table 1]. Although, the higher the molecular weight of complex, $[Cu(L)_2Br_2]$ to $[Cu(L)_2Cl_2]$. The effect of MIC is just reverse due to being small size of complex 2 than complex 3 [Table 1], so complex 2 will enter easily in the bacterial cell wall [Fig.5], and thus MIC value is greater & the order is:

$[Cu(L)_2Cl_2]$ > $[Cu(L)_2Br_2]$ > Antibiotic (Except Gentamycin) > Ligand

Table 1: Atomic Number, Ionic Radius, Electro negativity, Ionization Potential, Electron Affinity of the Investigated Metal ions [Cu (II)] with Halogen.

Compound	At. No	I.R	E.N	E.A	A.R	2nd I.E.	Mol. Wt.
Cu (Metal ion)	29	71	2.00	118.4	128	1958	63.5
Cl2	17	0.181	3.00	- 349	99	2298	70.90
Br2	35	0.19	2.8	- 325	114	2103	159.8
[Cu (L)2Cl2]	-	-	-	-	-	-	618.446
[Cu(L)2Br2]	-	-	-	-	-	-	707.346

Table 2: Anti Microbial Effect of Ligands [L], Cu (II) Complexes and Some Antibiotic

SL. No.	Compounds	Gram Positive Bacteria [Bascillus Subtillis] [GPB] (mm)	Gram Negative Bacteria [E – coil] [GNB] (mm)
1	Ligand,[L] Or [DMBAP]	6 [Resist] [W]	6 [Resist] [W]
2	[Cu(DMBAP)2Cl2]	20 [S]	18 [S]
3	[Cu(DMBAP)2Br2]	19 [S]	18
4	Amikacin	16 [S]	15
5	Ceftrixone	17 [S]	16
6	Gentamycin	6 [Resist] [W]	6 [Resist]
7	Levofloxacin	14 [M]	13 [M]

Note:- 10 < Weak [W] ; >10 Moderate [M] ; >16 Significant [S].

Table 3: FTIR & NMR Data of Ligand and Complexes

SL. No.	Compounds	FTIR Data, ν (cm^{-1})	1H NMR Data (δ), (ppm)
1	Ligand[L] [DMBAP]	1586 (S)	9.732
2	[Cu(DMBAP)2Cl2]	1513 (S)	9.816
3	[Cu(DMBAP)2Br2]	1512 (S)	9.811

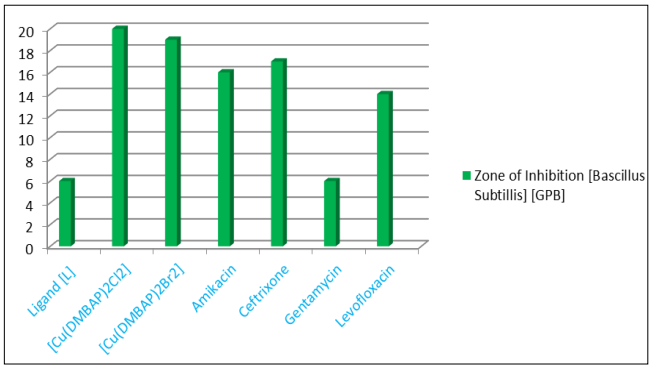

Fig 1: Zone of Inhibition of Compound Vs GPB in mm

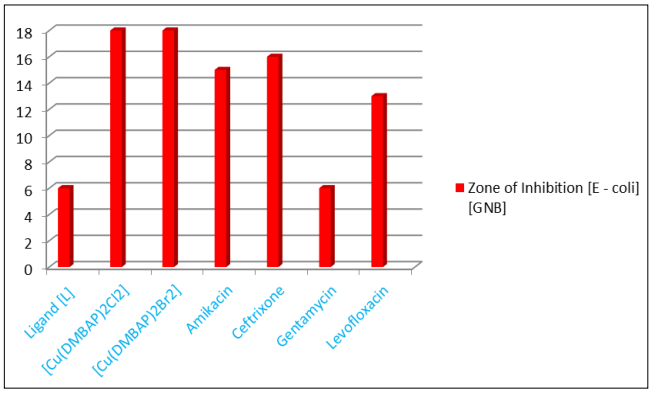

Fig 2: Zone of Inhibition of Compound Vs GNB in mm

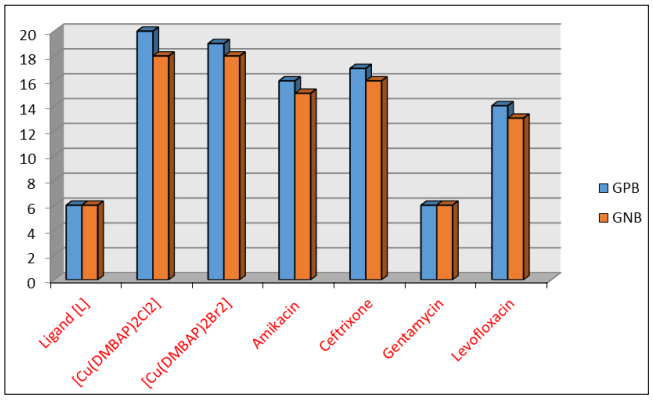

Fig 3: Zone of Inhibition of Compound Vs GPB & GNB

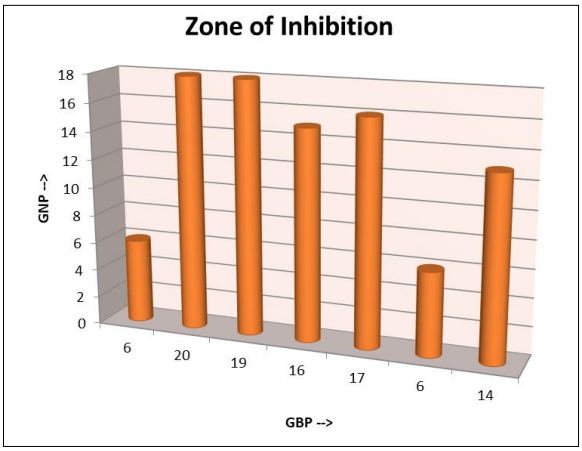

Fig 4: Zone of Inhibition of GPB Vs GNB in mm

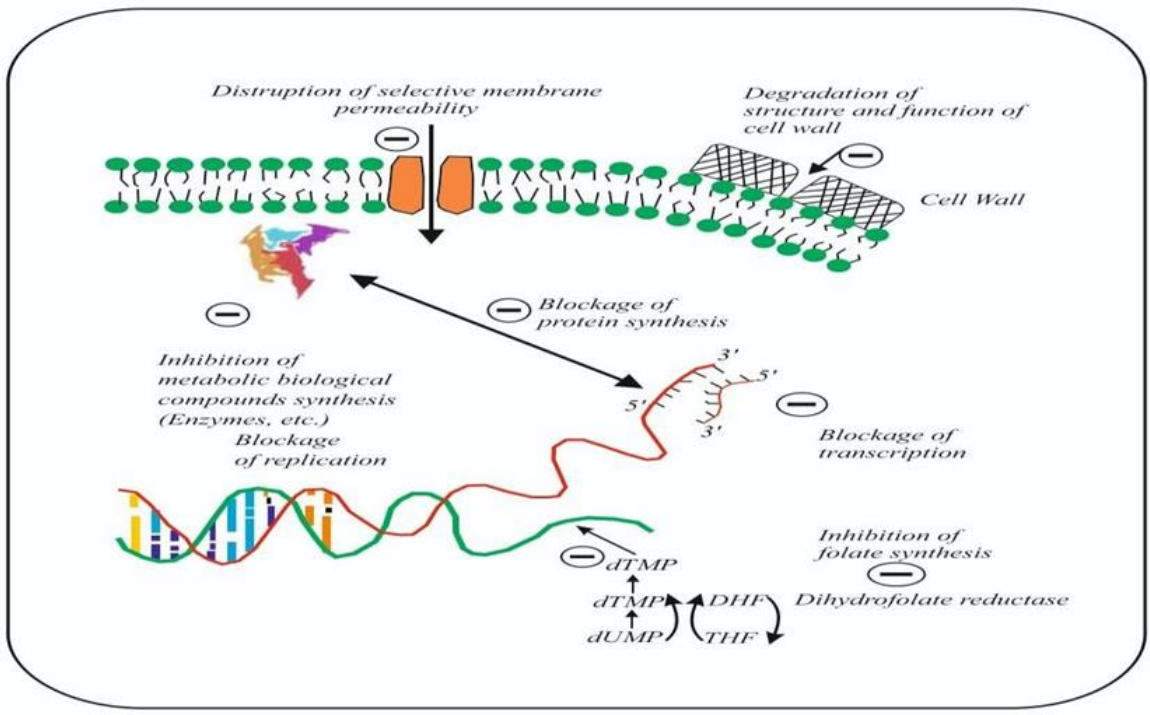

Fig 5: Treatment of Bacterial Infection by Target of Antimicrobial (Compounds) (Modified)

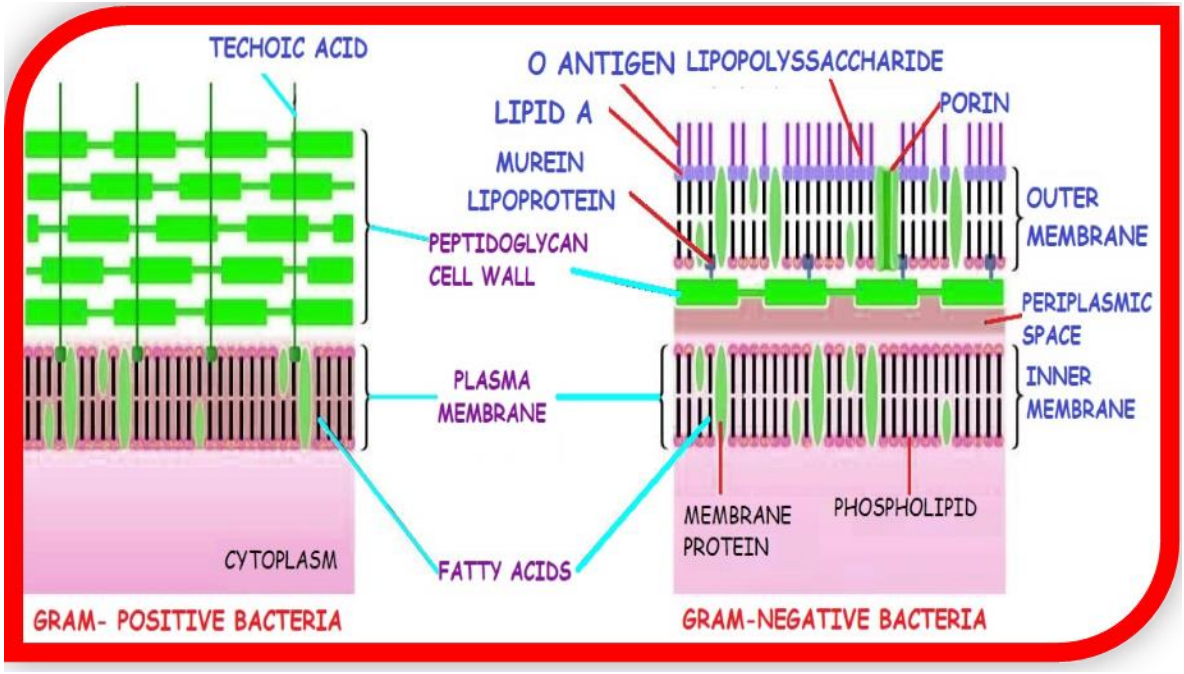

Fig 6: (Modified) Cell Wall Structure of GPB and GNB

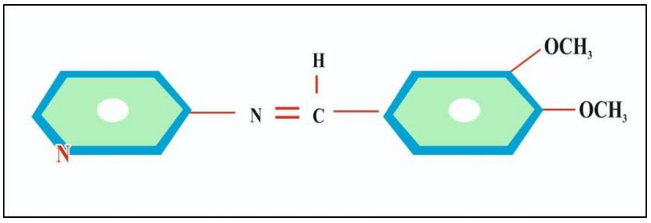

Fig 7: Proposed Structure of Ligand [L], [N-(3,4-dimethoxybenzylidine)-3-aminopyridine] [DMBAP]

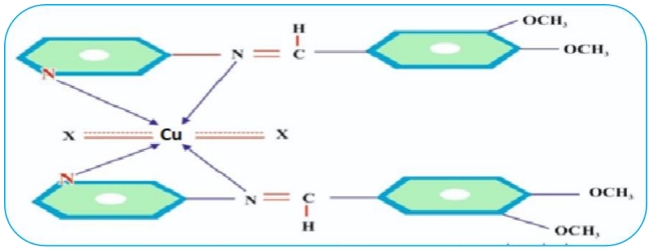

Fig 8: Proposed Structure of $[Cu(L)_2X_2]$; [X = Cl^-, Br^-] Shape – Octahedral

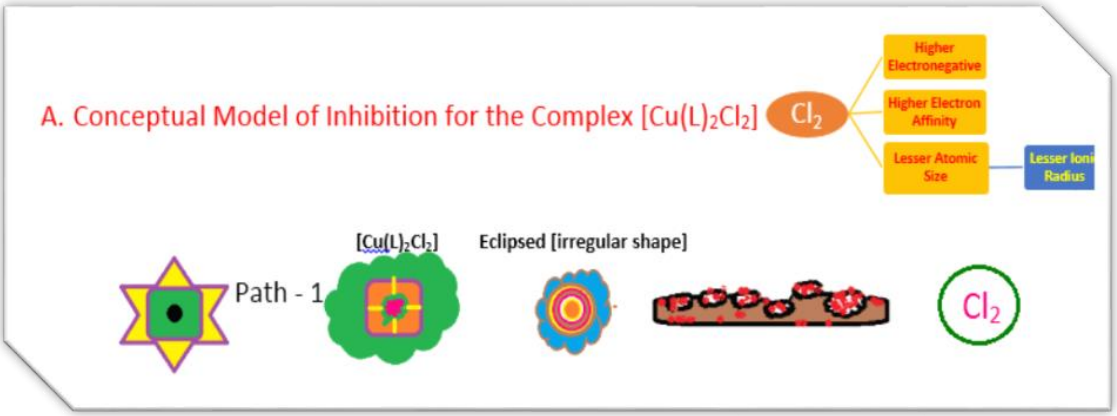

Fig 9: Scheme – 1 Conceptual Model of Inhibition of the Compound

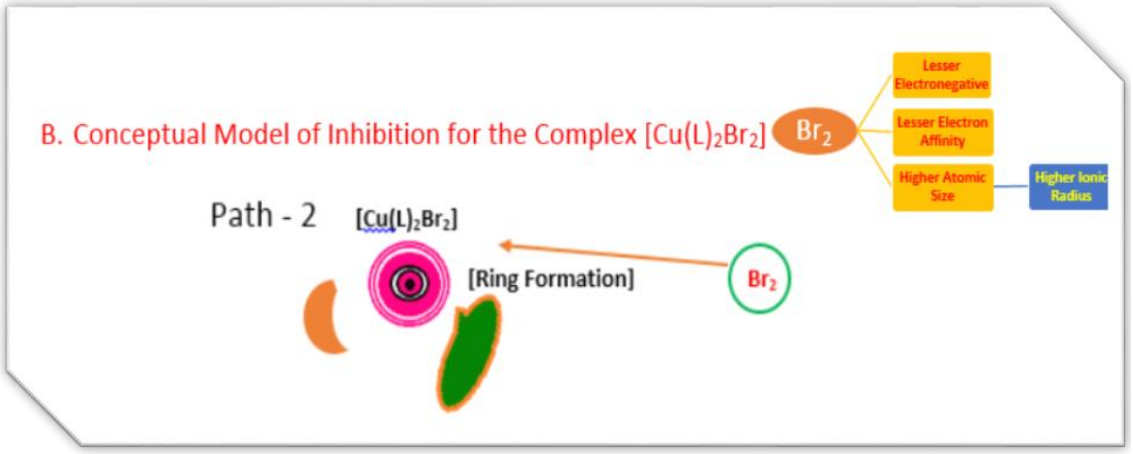

Fig 10: Scheme – 2 Conceptual Model of Inhibition of the Compound

Conclusion

Microbial studies revealed that the copper complexes showed important raised anti-bacterial activity than its corresponding free Schiff base ligand and the order of inhibition:

$[Cu(L)_2Cl_2] > [Cu(L)_2Br_2] >$ Antibiotic > SBL

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