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Research Article

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The effects of concentration on the bio-activity of novel 1-phenyl-N-[(1E,2E)-3-phenylprop-2-en-1-ylidene]methanamine schiff base complexes of Copper(II), Nickel(II), Iron(II), Cerium(III) and Gadolinium(IV)

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ABSTRACT

In this study, a novel 1-Phenyl-N-[(1E,2E)-3-Phenylprop-2-en-1-ylidene]Methanamine Schiff base ligand as well as its complexes with copper(II),nickel(II),Iron(II),cerium(III) and gadolinium(IV) metals were synthesized using trans-3-Phenyl-2-Propenal and α-AminoTulene as starting materials in acid medium which was followed by complexation reaction. Both the ligand and metal complexes were characterized by using spectroscopic techniques. The bio-activity of the metal complexes were assay on micro-organisms such as Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhii, Aspergillus niger and Candida albicans to ascertain its anti-microbial effects by varying the concentration of the metal complexes. Analysis of variance (ANOVA) was used to determine the effects of varying the concentration of the complexes on the microbial activity and on the measured zone of inhibition. Experimental results reveal that the first row transition metals under study: CU^{2+}, Fe^{2+}, Ni^{2+} are all significant at 5% level of significance while the lanthanides: Ce^{3+} and Gd^{4+} are not. An Increase in concentration of Cu^{2+}, Fe^{2+} and Ce^{3+} complexes produced a corresponding increase in antimicrobial activity. Varying the concentration of Ni complex has no significant effect as the complex destroys completely the organisms at all levels of concentration while Gd^{4+} complex show little antimicrobial effect on tested organisms at varying concentration.

Keywords: Schiff base, Metal complexes, Concentration, Antimicrobial activity, Bacteria, Fungi.

INTRODUCTION

Nowadays, considerable attention has been paid to the metal complexes due to the importance in understanding the effect of metal complexes in biological and industrial activities. Modification of the structure of the metal complexes by coordinating with different organic compounds has been carried out to enhance the potential use as drugs, oxidation catalysis, corrosion inhibiting materials that could probably solve the problem of rusting, agricultural research for insect-pest management, electrochemistry, and efficient reagents in trace analysis of some metal cations[1]. Therefore**,** any of a class of bases of the general formula RR′C=NR″ that are obtained typically by condensation of an aldehyde or ketone with a primary amine (as aniline) with elimination of water, that usually polymerize readily if made from aliphatic aldehydes, and that are used chiefly as intermediates in organic synthesis and in some cases as dyes refers to a Schiff base. A Schiff base , named after Hugo Schiff, is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not hydrogen[2]. Schiff bases in a broad sense have the general formula $R^1R^2C=NR^3$, where R is an organic side chain.

Transition metal complexes of such ligands are important enzyme model. The rapid development of these ligands resulted in an enhanced research activity in the field of coordination chemistry leading to very interesting conclusions. Many biologically important Schiff bases have been reported in the literature possessing, antibacterial,

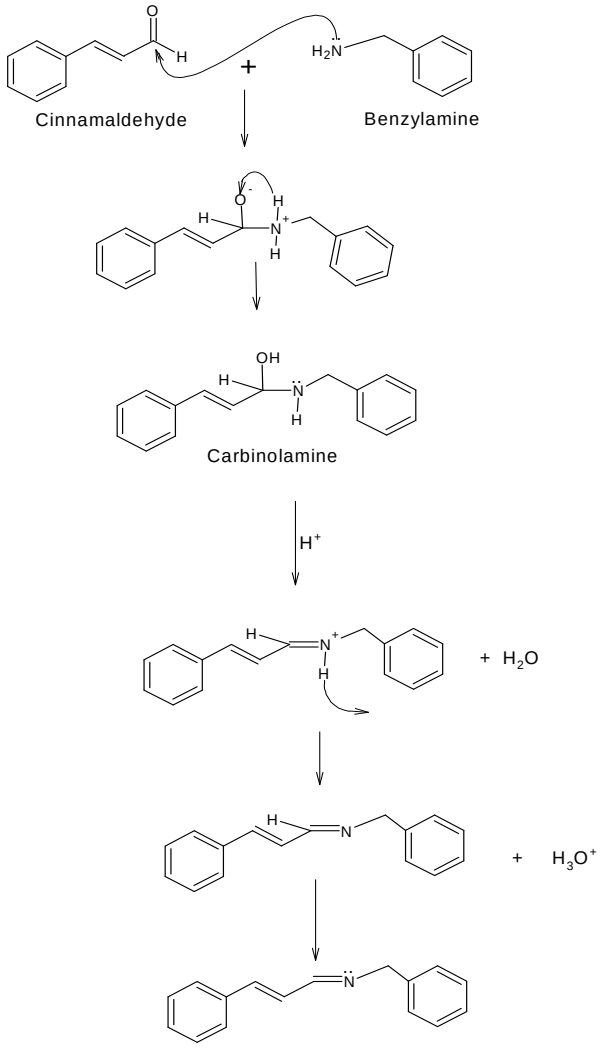
antifungal, antimicrobial, anticonvulsant, anti HIV, anti-inflammatory and anti-tumour activities[3]. According to Santosh Kumar [4] , the presence of azomethine and sulphonamide functional groups is responsible for antimicrobial activity which can be altered depending upon the type of substituent present on the aromatic rings. Therefore, this study is aimed at investigating the effect of changes in concentration of metal complexes on organisms as it affects their diameter of zone of inhibition with respect to its antimicrobial activity.

EXPERIMENTAL SECTION

The microwave assisted condensation of cinnamaldehyde and Benzylamine were carried out in a commercial LG domestic microwave oven, MS-2324B/MS-2324 RSN-850 watts as directed by H.J Yang [5].

Equimolar concentration of 3-phenypropenal (66.08g, 0.5M, 64.1cm^3) and alpha Amino Toluene (53.5g, 0.5m, 54.88cm^3) were mixed together at ambient temperature in an Erlenmeyer flask of volume 25ml. The mixture was subjected to microwave for an optimized time (4 minutes) on the "max high" setting. The crude product obtained was washed and recrystallized using a mixture of ethanol and dichloromethane to obtain a pale yellow amorphous solid as a ligand and shown in the scheme below.

Mechanism of Schiff base ligand synthesis



1-phenyl-*N*-[(1*E*,2*E*)-3-phenylprop-2-en-1-ylidene]methanamine

Schiff Base Ligand

The solution of the metal salts of $Fe^{2+,}$ $Cu^{2+,}$ $Ni^{2+,}$ Ce^{4+} and Gd^{3+} was made in ethanol-water mixture and was made to react with the ligand in the ratio of 1:2 respectively for complexation process to occur. Then the ligand-metal salt mixture was heated with constant stirring using a magnetic heater for 30 minutes before been transferred to a soxhlet extractor equipment for refluxing. The resultant mixture was allowed to reflux for 3 hours and the solid formed during this reaction or upon cooling was collected by first dissolving in DMSO. This is allowed to cool, then recrystallized.

The crystals obtained were thoroughly washed with hot ethanol-methanol mixture and are allowed to recrystallized to give purified products.

Antimicrobial test was performed on four bacteria namely: gram-positive (*Bacillus Subtilis, Staphylocous aureus),* gram-negative *(Escherichia coli, salmonella Typhii)* and two fungal strains namely: (*Aspergillus niger*, *Candida albicans*) as given by Ochie J and Kolhatkar [6].

The media used were prepared by dissolving separately 2g of the nutrient broth powder and 38g of the Mueller Hinton agar powder in 250ml and 1litre of deinonized water, respectively. The two media were sterilized in an autoclave at 121^0c for 15 minutes and then stored overnight in a refrigerator after cooling. Cultures of the micro-organism were prepared in sterile nutrient broth and incubated for 24hrs at 37^0c for the bacteria and for 72hrs at 27^0c for the fungi. 0.1ml of each of the overnight cultures in sterile test tube with caps were made up to 10ml with 9.9ml of sterile deinonized water to give 1-100 or 10-2 dilution of the micro-organisms. The technique used for the study was agar-well diffusion.

Solutions of varying concentration of 1000,100, 10 and 0.1mg/ml of the compounds under study were made in Dimethylsulphoxide (DMSO). DMSO was also used as a negative control. The positive controls for bacteria and fungi were disc of commercial antibiotics manufactured by Abtek biological limited and fluconazole dissolved in DMSO. The discs were carefully placed on the inoculated media with the aid of sterile forceps. The plates inoculated with bacteria at 37^0c for 24 hours, and those inoculated with fungi were incubated at 27^0c for 72hrs.

Afterwards, the zone of inhibition of microbial growth that appeared around the walls of the compounds were examined and the diameters measured and recorded in millimetres (mm). Therefore, by subtracting the diameter of inhibition zone resulting with DMSO from that obtained in each case, the antimicrobial activities of the complexes under study were finally calculated and compared with antimicrobial standards such as ciprofloxacin for bacteria and ketoconazole for fungal strains. Antimicrobial activity of Fe^{2+}, Ni^{2+}, Cu^{2+}, Ce^{4+}, and Gd^{+3} Schiff base complexes was evaluated in Vitro against gram-positive bacteria. *Staphylococcus aureus and Bacillus Subtilis,* gram-negative bacteria such as: *Escherichia coli and Salmonella Typhii* and fungi such as: *Candida albicans and aspergillus niger.*

RESULTS AND DISCUSSION

The four pathogenic bacteria, viz *Escherichia Coli, Staphylococus aureus, Salmonella Typhii, Bacillus Substilis* and two fungal Isolates used are *candida albicans and Aspergillus niger* were all isolated, characterized and identified from the Department of Microbiology, College of Sciences, University of Port Harcourt, Nigeria. At the end of the incubation, the plates were observed for clear or zone of inhibition produced by the complex. Variations in concentration of the Schiff Base complexes under investigation, its antimicrobial effect and the zone of inhibition on pathogenic bacteria and fungal isolates were also measured.

From Fig 1, the concentration of copper complex is significant at 5% level of significance. The observations differ significantly based on the variations of concentration of copper complex in milligrams. This implies that the concentration of copper complex under study did not produce same effect on the diameter of zone of inhibition. Generally, the different levels of concentration of copper complex produced different effects on the diameter of zone of inhibition in millimetre (mm). For instance, at the concentration of 10mg, the diameter of zone of inhibition of *staphyllococus aureus, Bacillas subtilis, Escherichia coli, salmonella typhii, Aspergillus niger and candida albicans* recorded 08mm, 07mm, 08mm, 06mm, 07mm and 04mm while at 1000mg the inhibition zone increases to 16mm, 12mm, 10mm, 11mm, 14mm and 08mm respectively.

On the other hand, considering the effect of organisms under study on the diameter of zone of inhibition, it was revealed that the quantities of the organisms that were killed with respect to the varying concentration of copper complex did not differ significantly. Since the organisms are not significant at 5% level of significance, this simply implies that each level of concentration of copper complex kills the organisms equally. For example, when 10mg of copper complex was applied on *staphyloccus aureus*, the extent of inhibition observed is not significantly different from the extent it had on other organisms.

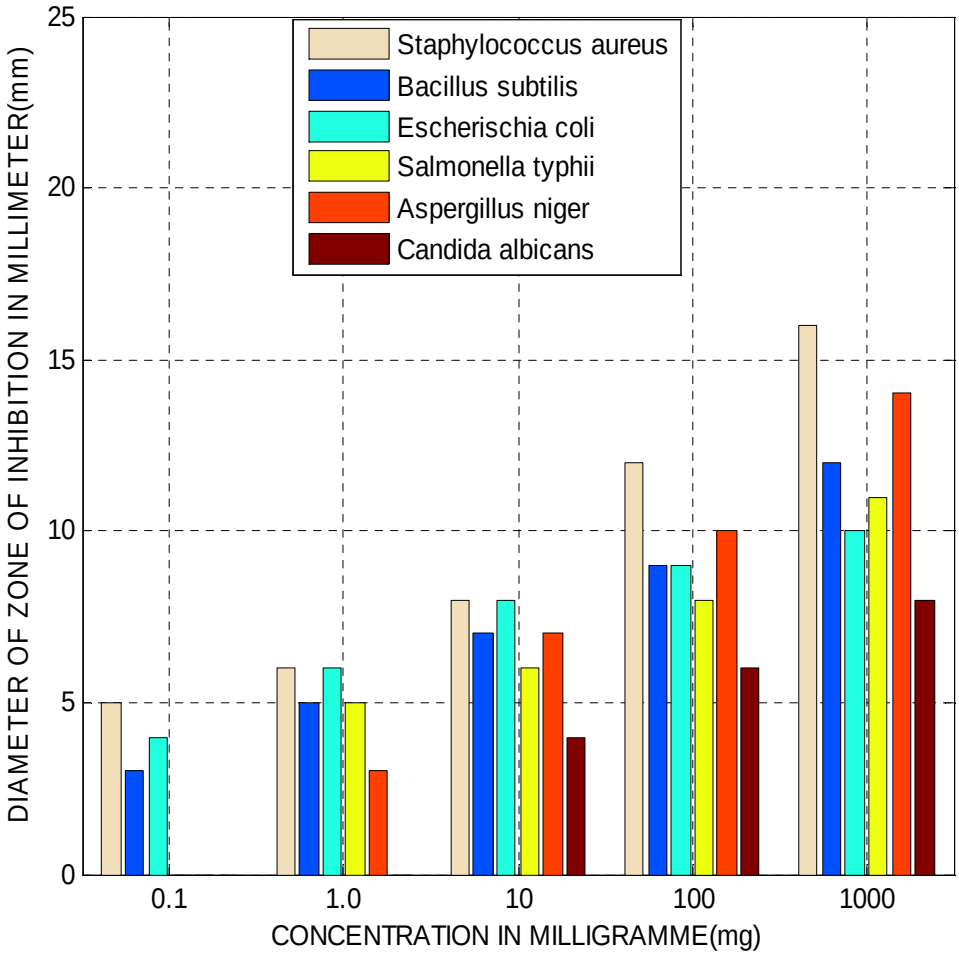


Fig 1: Antimicrobial sensitivity of Copper (II) cinnamaldehydebenzylamine complex on bacterial and fungal isolates

From the analysis of variance carried out, it was observed in figure 2 that the concentration of iron complex is significant at 5% level of significance, pointing that the different level of concentration of iron complex also produced different effects on the diameter of zone of inhibition. For example, at different concentrations of iron complex; 1000mg, 100mg and 0.1mg, the diameter of zone of inhibition on *salmonella typhi* gave 14mm, 09mm and 03mm while it is 13mm, 11mm and 04mm for *candida albicans* respectively showing that the antimicrobial activity of iron (II) complex is directly proportional to the varying concentration. It was also discovered that the organisms are not significant at 5% level of significant, implying that the iron (II) cinnamaldehydebenzylamine exhibited a cidal effect at equal levels of concentration to the micro-organisms under investigation.

Varying the concentration of Nickel (II) complex produced no significant effect on the diameter of zone of inhibition stipulating that the different levels of concentration terminates or destroys the organisms at equal rate. That signifies that any given concentration of Ni(II) complex has a tremendous cidal effect on the micro-organisms in such a manner that increasing the concentration of Ni(II) has no significance or creates no additional cidal effect on the organisms under investigation.

Primarily speaking, it implies that any little quantity of Ni-complex for instance, 10mg have the same cidal effect on organisms, the same way 100mg of Ni-complex will have as shown in fig3

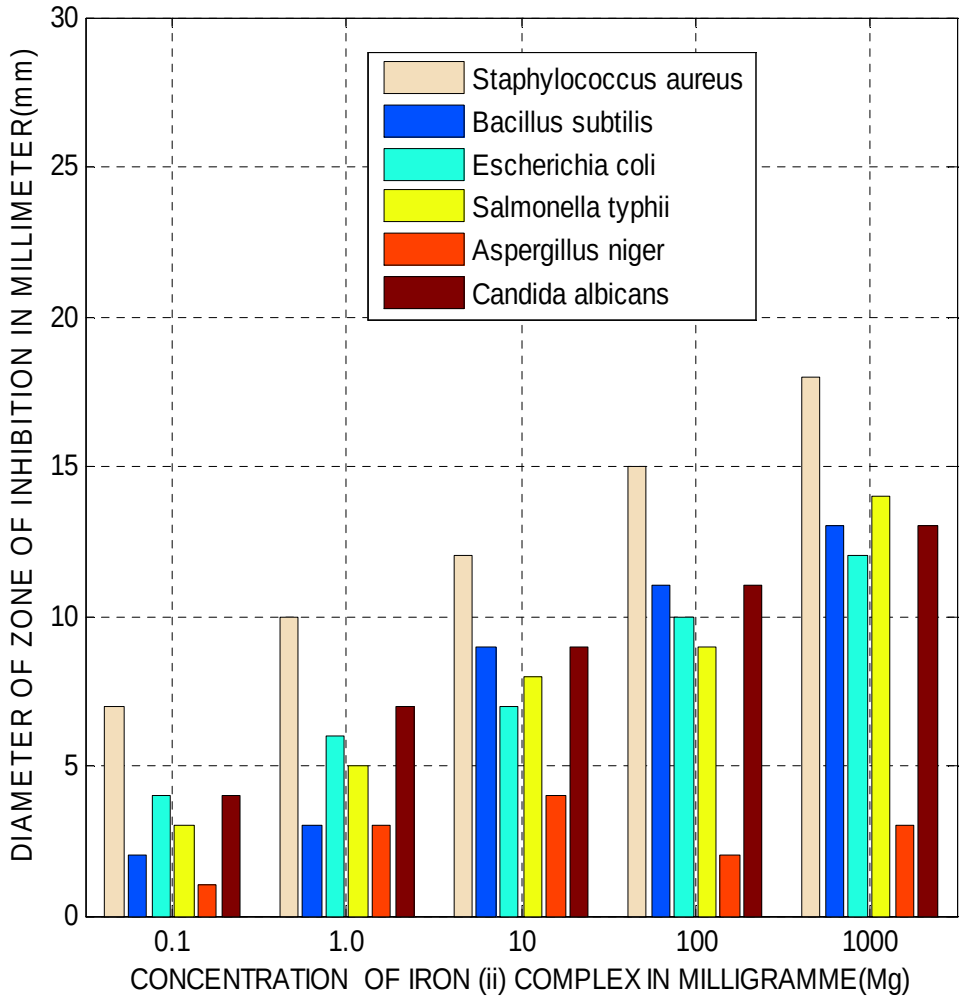


Fig 2; Multiple bar chart showing the anti-microbial sensitivity of Iron(ii) cinnamidealdehydebenzylamine Schiff base complex

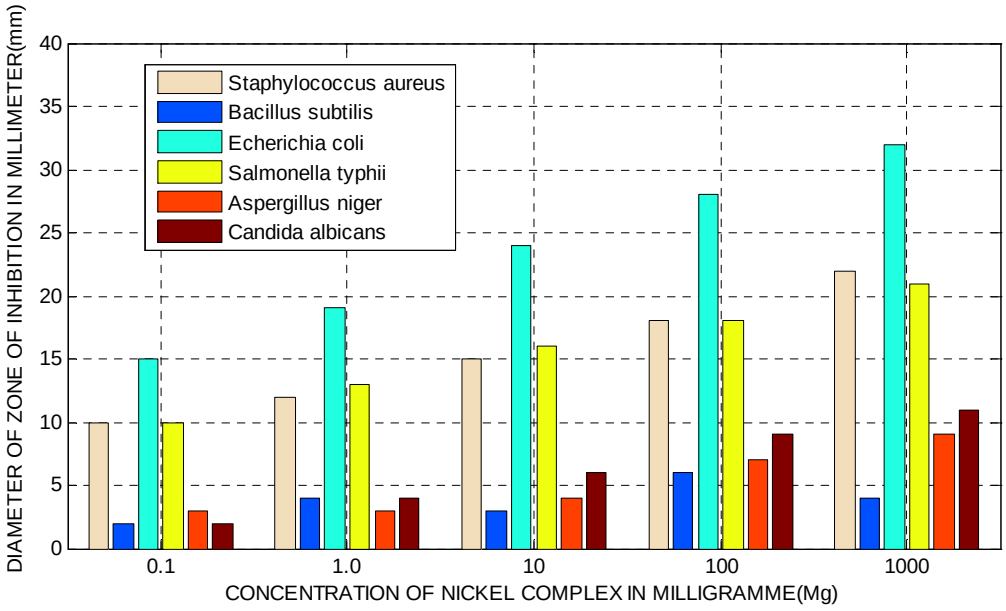


Fig 3; Multiple bar chart showing the anti-microbial activity of nickel(II)cinnamaldehydebenzylamine schiff base complex

Figure 4 illustrates that the antimicrobial sensitivity of cerium (IV) complex reveals that varying concentration of Cerium-complex plays a vital role on the test organisms which has been made visible through their diameter of zone of inhibition. The result of the analysis made us to understand that increasing the concentration of Cerium complex has a corresponding increasing effect on the diameter of zone of inhibition measured. This means that the higher the concentration of cerium complex, the higher its toxic effect on the micro-organisms under study. For example, at a concentration of 100mg, the diameter of zone of inhibition *of Staph aureues, Bacillus, E. coli, salmonella typhi* and *candida albicans* measured 10mm, 13mm, 07mm, 06mm and 04mm while at increased concentration of 1000mg, there is a corresponding increased in the diameter of zone of inhibition as 12mm, 15mm, 10mm, 07mm and 06mm respectively, though, not significant at 5% level of significance.

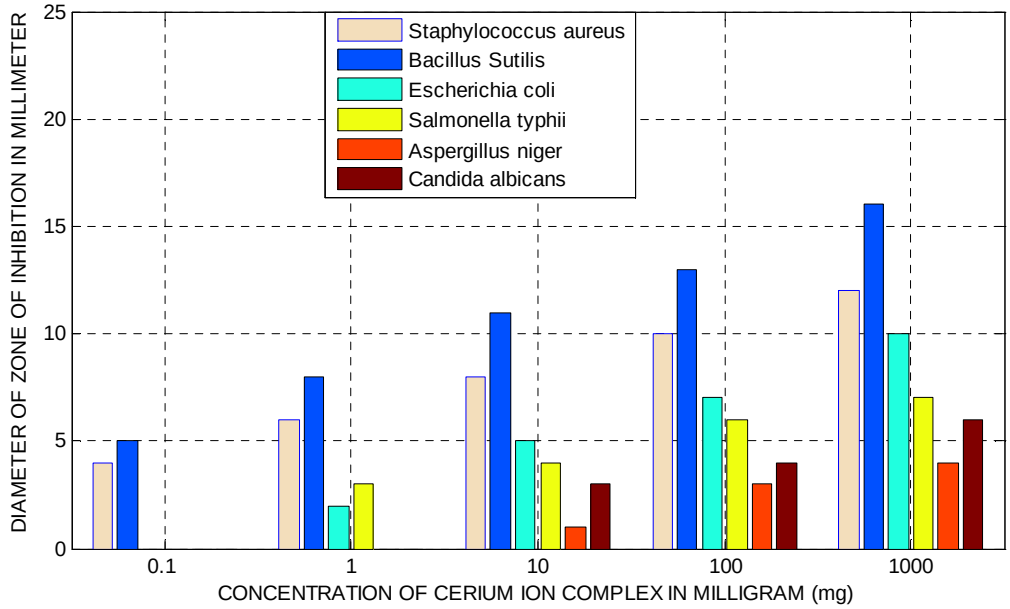


Fig. 4: Multiple bar chart showing the anti-microbial activity of cerium(iv) cinnamaldehydebenzylamine Schiff base complex

The different levels of concentration of Gd-complex produced no significant effect on the diameter of zone of inhibition slowing that vary the concentration of Gd-complex do not yield a corresponding antimicrobial effect on the test-organisms and shown in Figure5.

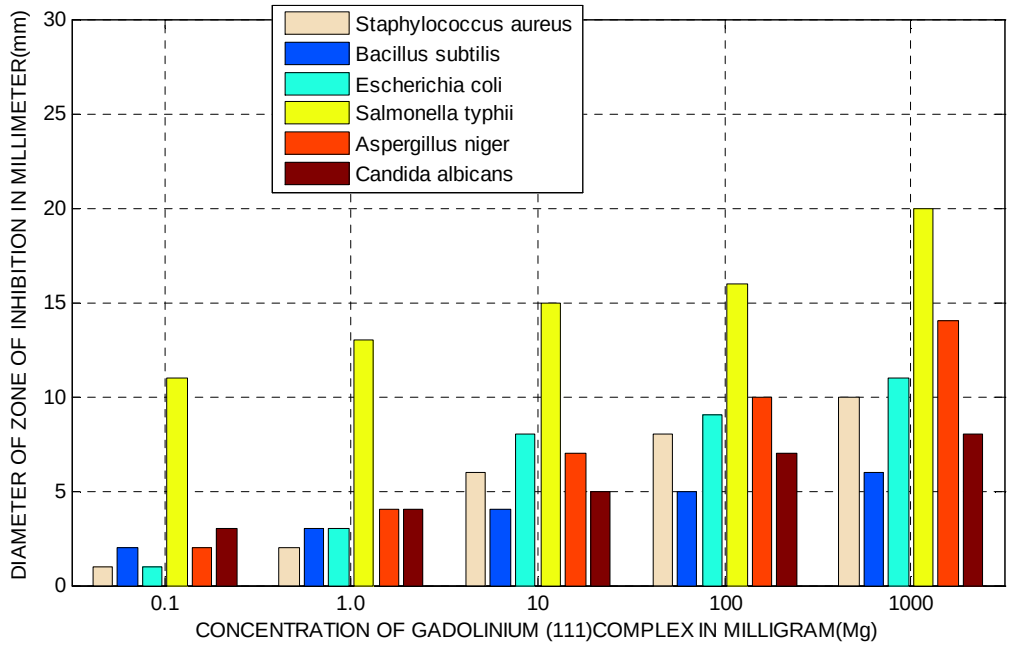


Fig 5; Multiple bar chart showing the anti-microbial activity of Gadolinium(iii) cinnamaldehydebenzylamine Schiff base complex

CONCLUSION

All the tested compounds showed a considerable biological activity against different types gram- positive , gram – negative bacterial and fungal strains under study. The first row transition metals under study: CU^{2+}, Fe^{2+}, Ni^{2+} are all significant at 5% level of significance while the lanthanides: Ce^{3+} and Gd^{4+} are not. An Increase in concentration of Cu^{2+}, Fe^{2+} and Ce^{3+} complexes produced a corresponding increase on the diameter of zone of inhibition, thereby resulting to an increase in antimicrobial activity of the complexes. Varying the concentrations of Ni^{2+} complex produced no significant effect on the diameter of zone of inhibition showing that the complex terminates the organisms at equal rate while Gd^{4+} complex show little antimicrobial activity at varying concentrations.

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REFERENCES

[1] Z Cimerman, N Galic, B Bosner, *Anal chem*., **1997** Acta 343, 145.
[2] IUPAC, *Compendium of Chemical Terminology*, 2nd ed. (**1997**) (the "Gold Book") . Online corrected version: (2006-) "Schiff base".
[3] SK Sridhar, A Ramesh , *Indian Drugs*, **2001** , 38-40.
[4] K Santosh, MS Niranjan, KC Chaluvaraju, CM Jamakhandi, D Kadadevar, *Journal of current pharmaceutical Research*, **2010,** 1, 39—42.
[5] HJ Yang, WH Sun, ZL Li, MA Zhi, *Chinese Chemical Letters*. **(2002)**, 13(1), 3 – 6.
[6] J. Ochei ,A. Kolhatkar : Medical Laboratory Science, Theory and Practice; Tata McGraw-Hill publishing company limited New Delhi**,2008** , 801-811.
[7] KJ Victory , KU Sherin and MK Muraleedharan Nair, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* ,*RJPBCS*, **2010** ,1(2),324.
[8] F Basolo RC Johnson, 'coordination chemistry; The chemistry of metal complexes, **1968,** Benjamin,8.