

Synthesis, Characterization and Antimicrobial Studies of Cinnamaldehydebenzylamine Schiff base Metal ion Complexes

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Abstract

The Schiff-base ligand cinnamaldehydebenzylamine was prepared via condensation reaction of Benzylamine (α ,Aminotoluene) and Trans-cinnamaldehyde (3-phenylpropenal) in the presence of alcohol and an acid reagent, acetic acid. Using the salts of Fe^{2+} , Ni^{2+} , Cu^{2+} of the transition elements and the salts of Ce^{4+} and Gd^{3+} of the Lanthanide series, the corresponding complexes were prepared in the metal-ligand (M - L) ratio of 1 : 2.. Using the salts of Fe^{2+} , Ni^{2+} , Cu^{2+} of the transition elements and the salts of Ce^{4+} and Gd^{3+} of the Lanthanide series, the corresponding complexes were prepared in the metal-ligand (M - L) ratio of 1 : 2. The prepared complexes were then characterized using the following physical techniques: elemental analysis, melting point determination, Ultra-Violet (UV) spectra analysis, X-Ray Diffraction analysis, Molar conductivity measurement, infra-red (FTIR) spectral analysis and mass spectral studies. They were properly screened for their antimicrobial activity against four bacteria namely, gram positive (*Bacillus subtilis*, *staphylococcus aureus*), gram negative (*Escherichia coli*, *salmonella typhi*) and two fungal strains (*Aspergillus niger* and *Candida albicans*) by measuring the diameter of zone of inhibition. Experimental results show that the biological activity of the synthesized Schiff base increases significantly when the transition metal ions such as Fe^{2+} , Ni^{2+} , Cu^{2+} were complexed than when the lanthanides such as Ce^{4+} and Gd^{3+} were introduced through co-ordination, with the Ni^{2+} complex having more than 80% antimicrobial activity on tested organisms especially on *E coli*.

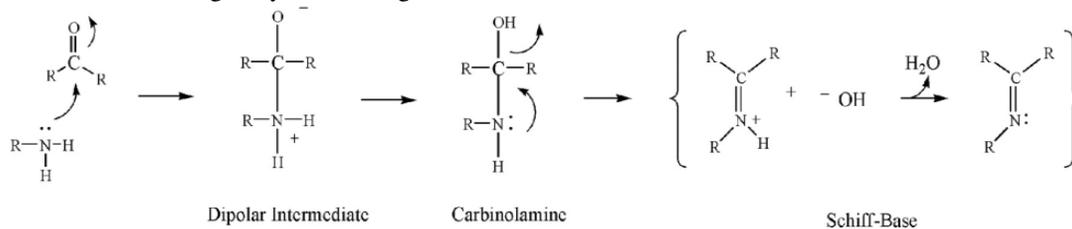
Keywords: Schiff base, metal complexes, antimicrobial activity, bacteria, fungi.

Introduction

Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported in 19th century by the German chemist Schiff Hugo Josef¹. The common structural feature of these compounds is the azomethine group with a general formula $RHC=N-R^1$, where R and R^1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. Several studies showed that the presence of a lone pair of electrons in an sp^2 hybridised orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance². Since metal ions have empty valence orbitals, they can act as Lewis acids (electron pair acceptors). Because ligands have unshared pair of electrons, they are able to function as Lewis base (electron pair donors). The bond between metal and ligand can therefore be pictured as resulting from the sharing of a pair of electrons that was originally on the ligand³. The

classical synthesis of a Schiff base is when any primary amine condenses with a carbonyl compound⁴, under specific conditions (for example, azeotropic distillation), according to the scheme-1.

Azomethine and sulfonamide functional groups are responsible for antimicrobial activity, which can be influenced depending on the type of substituent(s) present on the aromatic rings⁵. Transition metal complexes of such ligands are important enzyme models. The rapid development of these ligands resulted in an enhanced research activity in the field of coordination chemistry leading to very interesting conclusions that Schiff base ligands when coordinated with metal ions exhibits some, anti-bacterial, anti-fungal, anti-microbial, anti-convulsant, anti- HIV , anti-inflammatory and anti-tumour characteristics⁶.



Scheme-1

General Mechanism for A Schiff Base Ligand Formation

The azomethine linkage (-N=CH-) is a significant functional site that makes Schiff base ligands interesting candidates for biological activities as well as coordination or chelation with the metal ions. The interaction between metal ions and such biologically active ligands represents an important route in re-designing new metal-based anti-bacterial, anti-fungal and anti-cancer therapies against different kinds of bacteria, fungi and cancer-related viruses that become resistant to the use of conventional drugs⁷. Apart from the antimicrobial activity of Schiff bases, researchers have also find their use as an effective corrosion inhibitor which is based on their ability to spontaneously form a monolayer on the surface to be protected⁸. Schiff bases have been found to possess more inhibitory efficiency than their constituent carbonyls and amines⁹. This work is aimed at investigating whether less biologically active compounds such as Cinnamaldehydebenzylamine Schiff base ligand will become more active upon coordination with the ions of transition and inner transition (lanthanides) metals on gram positive, gram negative bacterial and fungal strains.

Material and Methods

Ligand preparation: The Schiff base ligand was synthesized by first dissolving liquid cinnamaldehyde (66.08 g, 64.1 cm³, 0.5 moldm⁻³) in 100 ml of ethanolic solution. This is followed by dissolving liquid Benzylamine (53.58 g, 54.88 cm³, 0.5 moldm⁻³) in 100ml of ethanol. These two solutions were mixed together and stirred for about 1 hr to give room for a proper reaction since the reaction is exothermic. This is followed by the addition of 2ml of glacial acetic acid since acetic acid provides optimum pH for Schiff base reaction. The resultant mixture was then poured into a 500 ml reflux equipment. This was then heated under continuous refluxing for 9- 12 h using a BHA heating apparatus at a steady temperature between 70 – 80 °C for condensation reaction to completely take place. The solid formed upon cooling was collected by filtration, washed several times with ethanol and recrystallized from hot ethanol. The product obtained was oven dried at 75 °C and weighed to give the desired pale yellow crystal compound as the Schiff base ligand. The ligand was made to undergo a complexation reaction with the metal ions in the ratio of 2:1 to give a coordinated compound.

Complex Preparation: To a hot stirred solution of the ligand of mass 44.2g in 100 cm³ of ethanol (2molar solution) was added to the solution of the metal salt for the complexation of each metal -ligand as follows: 29.081 g of Ni (NO₃)₂ in 100 cm³ of water (1molar solution), 27.801 g of FeSO₄ in 100 cm³ of water (1molar solution), 24.968 g of CuSO₄ in 100 cm³ of water (1molar solution), 6.3253 g of 2(NH₄)₂SO₄ Ce (SO₄)₂ in 10 cm³ of water(1 molar solution), 2.6361 g of GdCl₃. 6H₂O in 10 cm³ of water (1molar solution).

The solution of the metal was made in ethanol-water mixture. Then the ligand-metal salt mixture was heated with constant stirring using a magnetic heater for 3 minutes before being

transferred to a soxhlet extractor equipment for refluxing. The resultant mixture was allowed to reflux for 3 h and the solid formed during this reaction upon cooling was collected by first dissolving it in hot ethanol. This was allowed to cool, then recrystallization was employed to purify the complexes produced. The crystals obtained were thoroughly washed with hot ethanol-methanol mixture and were allowed to recrystallized to give a purified product.

Bio-Activity Test: Antimicrobial test was performed on two gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria, two gram-negative (*Escherichia coli*, *Salmonella Typhi*) bacteria and two fungal strains (*Aspergillus niger*, *Candida albicans*). The media used were prepared by dissolving separately 2g of the nutrient broth powder and 38g of Mueller Hinton agar powder in 250 ml and 1L of deionized water, respectively¹⁰. The two media were sterilized in an autoclave at 121°C for 15 minutes, cooled and then stored overnight in a refrigerator. Cultures of the micro-organism were prepared in sterile nutrient broth and incubated for 24 h at 37°C for the bacteria and 72 h at 27°C for the fungi. 0.1ml each of the overnight cultures in sterile test tube with caps were made up to 10ml with 9.9ml of sterile deionized water to give 1-100 or 10-2 dilution of the micro-organisms. The technique used for the study was agar-well diffusion.

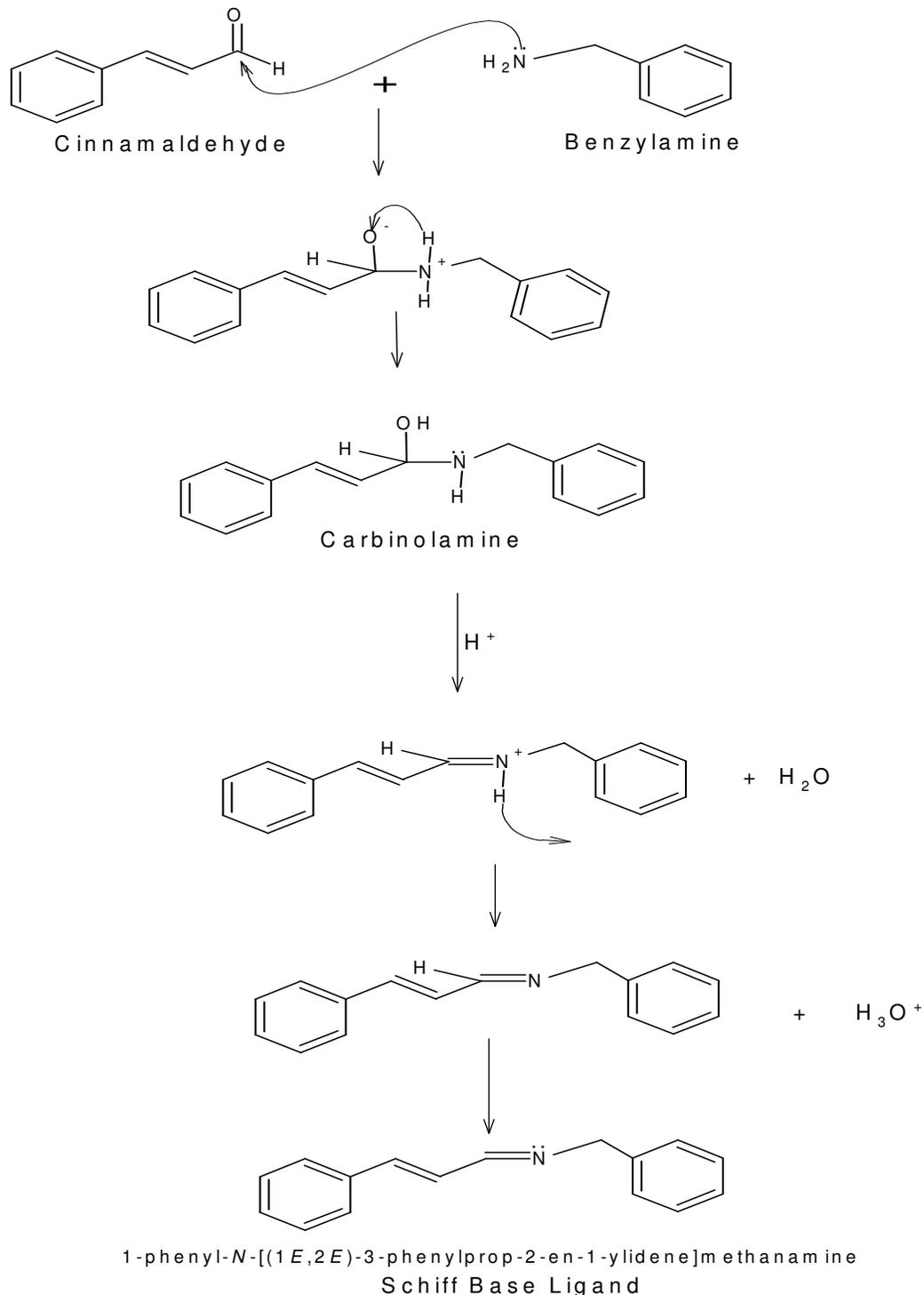
Solutions of concentration 10mg/ml of the compounds under study were made in dimethylsulphoxide (DMSO). DMSO was also used as a negative control. The positive controls for bacteria and fungi were disc of commercial antibiotics manufactured by Abtek Biological Ltd. and Fluconazole dissolved in DMSO.

The discs were carefully placed on the inoculated media with the aid of sterile forceps. The plates inoculated with bacteria were incubated at 37°C for 24 h while those inoculated with fungi were incubated at 27°C for 72h. Afterwards, the zone of inhibition of microbial growth that appeared around the walls of the compounds were examined and the diameters measured with centimetre rule and recorded in millimetres (mm). At the end of the incubation, the plates were observed for clear zone of inhibition produced by the ligand and complex. 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 Fe²⁺, Ni²⁺, Cu²⁺, Ce⁴⁺, and Gd³⁺ Schiff base complexes was evaluated *in-vitro* against ositive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) and fungi (*Candida albicans* and *Aspergillus niger*). Cinnamaldehydebenzylamine Schiff base ligand and Cinnamaldehydebenzylamine metal complexes of Fe²⁺, Ni²⁺, Cu²⁺, Ce⁴⁺ and Gd³⁺ were subjected to antimicrobial studies at a

concentration 100mg to compare the antimicrobial effects of the ligand and metal complexes at that given concentration. This is to verify whether at this concentration, the ligand or the complexes have equal antimicrobial effect on the organisms or the organisms resisted the drug at this concentration thereby increasing or decreasing the diameter of zone of inhibition.

Mechanism of Schiff base ligand synthesis



Scheme-2
Mechanism Of Schiff Base Ligand Under Investigation

Results and Discussion

Table 1 shows the selected Infrared Spectra of the Ligand and Complexes. The peaks 3027.38 and 3058.24 cm^{-1} were been assigned Aromatic C-H stretch. The vibration frequency 1495.85 has been assigned carbon aromatic C = C ring stretch. The peak at 1659.80 cm^{-1} has been assigned C = N which is the azomethine linkage (Imine) formed by the condensation reaction between cinnamaldehyde and Benzylamine. This band is an indication that a Schiff base ligand was formed.

For the metal complexes, the vibrational frequencies: 3059.20, 3064.99, 3068.85 and 3068.85 cm^{-1} have been attributed to the aromatic C – H stretch. The aromatic C = C ring stretch have been found in 1593.25, 1500.67, 1517.06, 1492.95 and 1593.20 cm^{-1} .

The C = N stretch in metal complexes were found in 1641.48, 1657.87, 1650.16, 1666.55 and 1635.72. This band was found in the Schiff base ligand at 1659.80 cm^{-1} . It can be seen that this band was shifted in the complexes either to higher or lower wave numbers. Therefore, this shift in metal complexes suggest the involvement of Azomethine nitrogen atom (C = N) Interacting with the metal ions thereby participating in the co-ordination process¹¹.

The bands 3547.21, 3435.34, 3232.8, 3238.59, 3392.9, 3486.45 and 3371.21 cm^{-1} have been assigned OH of water which implies that water molecule was involved in complexation by acting as a neutral ligand.

Furthermore, conclusive evidence of the co-ordination of the Schiff base ligand with metal ions was shown by the appearance of weak low frequency new bands at the peaks: 356.84, 384.81, 415.67, 467.75, 429.18, 357.81, 449.43 and 442.21 cm^{-1} which have been assigned Metal-Nitrogen bond¹².

Table 2 shows the selected vibrational frequencies of the Ligand and Complexes. The UV spectra of the ligand shows maximum absorption at 280, 300 and 320 nm. This absorption bands have been assigned intra-ligand charge transfer ($\pi - \pi^*$) in the Schiff base ligand. These transitions are from the chromophores (C = C, C = N) in the Schiff base ligand. The absorption bands 280, 315, 475 nm in the iron complex have been assigned intra ligand charge transition, ligand-metal charge transfer and E- T₂ respectively. In the Nickel complex, the absorption bands 270, 320 and 510nm have been assigned $\pi - \pi$, LMCT (in full) and A₂→T₂ respectively.

The copper complex has 300, 325 and 400nm as its absorption bands which have been assigned $\pi - \pi^*$, LMCT and E → T₂ respectively. The absorption band in cerium complex has 290 and 310nm have been assigned $\pi - \pi$ and LMCT while the absorption band in Gadolinium complex has 280 and 320nm which has been assigned $\pi - \pi^*$ and LMCT (Ligand- metal charge transfer).

The x-ray diffraction studies as given in table-3 reveals that copper complex has a monoclinic prismatic as its crystal system with the cell dimension as : a= 9.066, b= 6.286, c= 7.034, z= 2, beta= 114.857, volume= 391.13, density (calc)= 5.51 and Space group C 2/m. The cell dimation for Nickel complex is a: 8.92, b= 9.59, c= 6.84, z= 1, Alpha= 110; beta=111.983; gamma= 103.3; volume= 501.63; density (calc)= 3.22; while the Iron complex has its Cell division: a= 9.066, b= 6.286, c= 7.064, z= 2, beta= 114.857, v= 391.13, density (calc)= 5.51 with a crystal system as monoclinic – prismatic. Cerium complex has its Cell division: a = 14.49, b = 14.216, c = 7.88, z = 4, alpha = gamma = 90, beta = 117.1°, v = 1.444.99, density (calc) 2.63 and a Crystal system as monoclinic while the Schiff base has Crystal system: orthorhombic.

Table-1
Selected Infrared Spectra of the Ligand and Complexes (cm^{-1})

Compound	Aromatic C-H stretch	Aromatic C=C ring stretch	C=N stretch	OH	NO ₃ ⁻ Stretch	SO ₄ ²⁻ Stretch	M-N	
L	3027.38 3058.24	1495.85	1659.80	3432.44	-	-	-	-
NiL ₂	3059.20	1593.25	1641.48	3547.21	1346.36	-	356.84 384.81	-
FeL ₂	3064.99	1500.67	1657.87	3435.34	-	1360.02	415.67 467.75	-
CeL ₂	3068.85	1517.06	1650.16	3232.8 3238.59	-	643.28	429.18	-
CuL ₂	3068.85	1492.95	1666.55	3392.9 3486.45	-	1376.26	357.81 449.43	-
GdL ₂	3067.85	1575.20	1635.72	3371.21	-	-	442.21	-

L = Cinnamaldehyde benzylamine Schiff base ligand.

Table-2
Ultra-violet Spectra of the Ligand and Complexes

Compounds	Amax. Nm	$\epsilon^n = \frac{1}{\lambda} (\text{Lmol}^{-1} \text{cm}^{-1})$	Type of transition
L	280, 300, 320		$\pi - \pi$ (Intra- ligand transition)
FeL ₂	280	3.57×10^{-3}	$\pi - \pi$ (Intra- ligand transition) LMCT E \rightarrow T ₂
	315	3.17×10^{-3}	
	475	2.11×10^{-3}	
NiL ₂	270	3.70×10^{-3}	$\pi - \pi$ (Intra- ligand transition) LMCT A ₂ \rightarrow T ₂
	320	3.13×10^{-3}	
	510	1.96×10^{-3}	
CuL ₂	300	3.33×10^{-3}	$\pi - \pi^*$ (Intra- ligand transition) LMCT E- T ₂
	325	3.08×10^{-3}	
	400	2.50×10^{-3}	
CeL ₂	290	3.45×10^{-3}	$\pi - \pi^*$ LMCT
	310	3.25×10^{-3}	
GdL ₂	280	3.57×10^{-3}	$\pi - \pi^*$ LMCT
	320	3.13×10^{-3}	

Table-4
Solubility Test of ligand and metal complexes

Compounds	Colour	Water	Ethanol	Methanol	Chloroform	DMSO	DCM	DMF
Ligand	Yellow	Insoluble	Soluble when heated	Insoluble	Insoluble	Soluble	Sparingly soluble	Soluble
Fe Complex	Brown	Sparingly soluble	Sparingly soluble	Sparingly soluble	Insoluble	Soluble	Sparingly soluble	Soluble
Ni Complex	Brick Red	Sparingly soluble	Sparingly soluble	Sparingly soluble	Insoluble	Soluble	Sparingly soluble	Soluble
Cu complex	Yellow ochre	Sparingly soluble	Sparingly soluble	Sparingly soluble	Insoluble	Soluble	Sparingly soluble	Soluble
Ce complex	Gold	Insoluble	Sparingly soluble	Sparingly soluble	Insoluble	Soluble	Sparingly soluble	Soluble
Gd complex	Coffee brown	Insoluble	Sparingly soluble	Sparingly soluble	Insoluble	Soluble	Sparingly soluble	Soluble

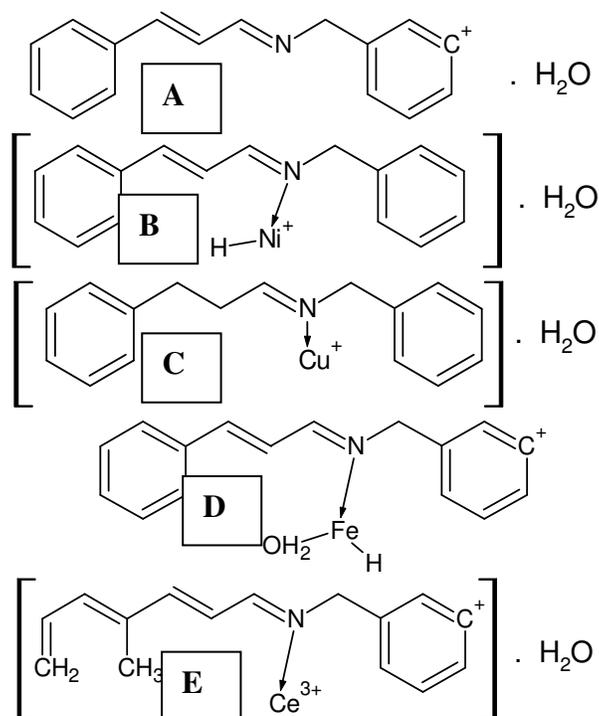
DCM =Dichloromethane, DMF =Dimethylformamide, DMSO=Dimethylsulphuroxide

Table-5 shows sensitivity of schiff base ligand and complexes on bacterial and fungal isolates at a concentration of 100milligrams (mg) with antimicrobial standards. Cinnamaldehydebenzylamine Schiff base ligand and Cinnamaldehydebenzylamine metal complexes of Fe²⁺, Ni²⁺, Cu²⁺, Ce⁴⁺ and Gd³⁺ were subjected to antimicrobial studies at a concentration 100mg to compare the antimicrobial effects of the ligand and metal complexes at that given concentration. This was to verify whether at this concentration, the ligand or the complexes have an equal antimicrobial effects on the organisms or whether the organisms resisted the drug at this concentration thereby decreasing the diameter of zone of inhibition.

The Schiff base ligand and the metal complexes under study do not have an equal antimicrobial effect on the organisms as there is a significant difference in the mode of inhibition as shown the table below. The increased toxicity of the metal complexes is due to the effect of the metal ion configuration and charge on the normal cell. A possible mode of toxicity has it that chelation reduces considerably the polarity of the metal ion mainly

because of partial sharing of its pi-electrons and delocalization over the whole chelate ring. The polarity of the metal ion reduces to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups¹³. Therefore, the large ring size of Cinnamaldehyde Benzylamine Schiff base complexes makes it more lipophilic and this increased lipophilicity enhances the penetration of the metal complexes into lipid membranes and block the metal binding sites in the enzymes of the organisms.

The azomethine linkage in the synthesized complexes and the metal ions contribute extensive biological activities due to increased liposolubility of the molecules in crossing cell membrane of the micro-organisms¹⁴, for instance, at the concentration of 100mg, the antimicrobial activity of the ligand for E.coli and S. Aureus recorded 6mm and 5mm respectively. The activity were increased when the ligand was complexed with metal ions such as Nickel to give an increased inhibition zone of 28mm and 18mm respectively for the cited organisms as illustrated in the figure-2.



A = Schiff base ligand, B=Nickel complex, C=Copper complex, D = Iron complex, E = Cerium complex.

Figure-1

Proposed structure for ligand and metal complexes formed

Table-5

Sensitivity of Schiff Base Ligand and complexes on Bacterial and Fungal Isolates at a concentration of 100milligrams (mg) with Antimicrobial standards

Compounds under investigation	Diameter of zone of inhibition at concentration of 100mg/l					
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
SB – Ligand	05	01	06	03	01	01
SB-Cu Complex	12	09	09	08	10	06
SB-Fe Complex	15	11	10	09	02	11
SB-Ni Complex	18	06	28	18	07	09
SB-Ce Complex	10	13	07	06	05	06
SB-Gd Complex	08	05	09	16	10	07
DMSO	03	04	03	03	04	04
Ciproflaxacin	25	26	30	27	-----	----
Ketoconazole	-----	-----	-----	-----	27	24

Zone Diameter Interpretation Key: 00 = Absence of measurable inhibition action, Below 5mm = Resistant (-), 5mm – 10mm = Considerably Active (+), 10mm – 15mm = Active (++) , 15mm – 20mm = Very Active (+++), 20mm – Above = Significant and Highly Active (++)

Table-6 shows a statistical comparison of the Effects of complexing the ligand under investigation with metal ions on the diameter of zone of inhibition as well as evaluating the antimicrobial strength of the metal complexes.

Conclusion

Schiff base metal complexes exhibit a wider scope of antimicrobial activity compared to its ligand because of their mesoinic nature, solubility and high sensitiveness towards the

biological behaviours. Comparison of the biological activity revealed that the biological activity of the synthesized Schiff base increases significantly when the transition metal ions such as Fe²⁺, Ni²⁺, Cu²⁺ and the lanthanides such as Ce⁴⁺ and Gd³⁺ were introduced through co-ordination. Introduction of metal ions into the Schiff base ligand produced a corresponding increase on the diameter of zone of inhibition, thereby resulting to an increase in antimicrobial activity of the complexes. Nickel complex shows a very high antimicrobial sensitivity to the

micro-organisms under study compared with the Schiff base complexes of Cu, Fe, Ce and Gd.

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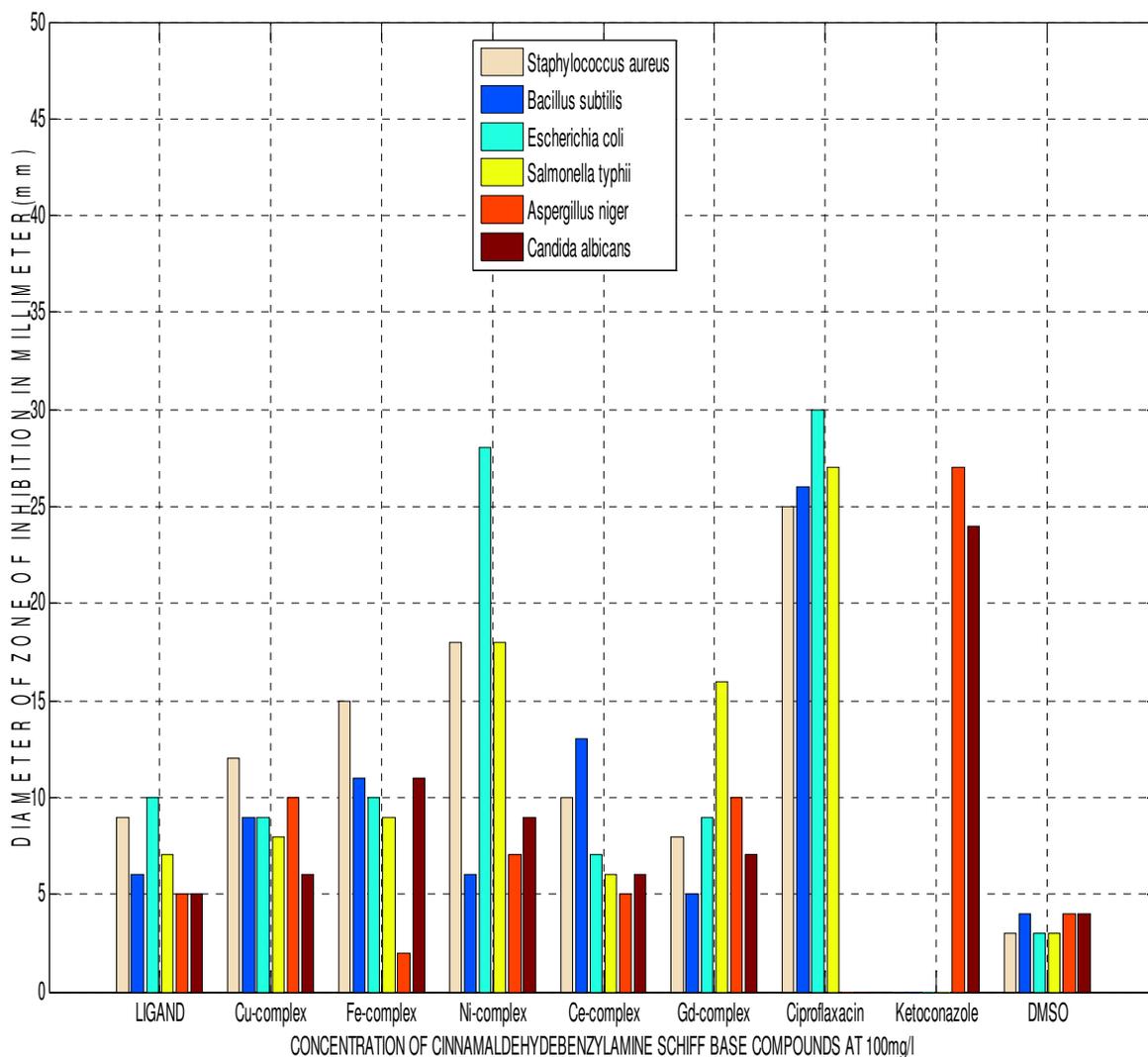


Figure-2

Multiple bar chart showing the bio-activity of the ligand and metal complexes at 100mg concentration

Table-6
The Effects of metal complexation on the Schiff base ligand

(I) Compounds under study	(J) Compounds under study	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SB-Ligand	SB-Cu Complex	-6.00*	2.416	.020	-10.98	-1.02
	SB-Fe Complex	-6.67*	2.416	.011	-11.64	-1.69
	SB-Ni Complex	-11.33*	2.416	.000	-16.31	-6.36
	SB-Ce Complex	-4.83	2.416	.056	-9.81	.14
	SB-Gd Complex	-6.17*	2.416	.017	-11.14	-1.19
SB-Cu Complex	SB-Ligand	6.00*	2.416	.020	1.02	10.98
	SB-Fe Complex	-.67	2.416	.785	-5.64	4.31
	SB-Ni Complex	-5.33*	2.416	.037	-10.31	-.36
	SB-Ce Complex	1.17	2.416	.633	-3.81	6.14
	SB-Gd Complex	-.17	2.416	.946	-5.14	4.81
SB-Fe Complex	SB-Ligand	6.67*	2.416	.011	1.69	11.64
	SB-Cu Complex	.67	2.416	.785	-4.31	5.64
	SB-Ni Complex	-4.67	2.416	.065	-9.64	.31
	SB-Ce Complex	1.83	2.416	.455	-3.14	6.81
	SB-Gd Complex	.50	2.416	.838	-4.48	5.48
SB-Ni Complex	SB-Ligand	11.33*	2.416	.000	6.36	16.31
	SB-Cu Complex	5.33*	2.416	.037	.36	10.31
	SB-Fe Complex	4.67	2.416	.065	-.31	9.64
	SB-Ce Complex	6.50*	2.416	.013	1.52	11.48
	SB-Gd Complex	5.17*	2.416	.042	.19	10.14
SB-Ce Complex	SB-Ligand	4.83	2.416	.056	-.14	9.81
	SB-Cu Complex	-1.17	2.416	.633	-6.14	3.81
	SB-Fe Complex	-1.83	2.416	.455	-6.81	3.14
	SB-Ni Complex	-6.50*	2.416	.013	-11.48	-1.52
	SB-Gd Complex	-1.33	2.416	.586	-6.31	3.64
SB-Gd Complex	SB-Ligand	6.17*	2.416	.017	1.19	11.14
	SB-Cu Complex	.17	2.416	.946	-4.81	5.14
	SB-Fe Complex	-.50	2.416	.838	-5.48	4.48
	SB-Ni Complex	-5.17*	2.416	.042	-10.14	-.19
	SB-Ce Complex	1.33	2.416	.586	-3.64	6.31

Based on observed means. The error term is Mean Square(Error) = 17.507. *. The mean difference is significant at the .05 level.

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