

Synthesis, Characterization and Evaluation of Antimicrobial Potency of Fe(II), Mn(II), Cu(II), Co(II) and Zn(II) ion complexes with Schiff Base, Derived from Salicylaldehyde and p.toluidine

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Abstract— Fe(II), Mn(II), Zn(II), Co(II) and Cu(II) complexes of Schiff base were synthesized and characterized by different physico-chemical techniques. The metal (II) complexes were characterized by solubility testing, melting point, molar conductance, UV-Vis and FTIR spectral studies. The high melting point of the complexes showed stability of the metal complexes. The values of molar conductance of the Schiff base, and all the metal (II) complexes are in the range of 3.0 – 36.6 S.cm² mol⁻¹. These values indicate that they are all non-electrolytes. The UV-Vis spectral studies have shown hypochromic shift in the metal (II) complexes of the Schiff base. The FT-IR spectral have shown that the Fe(II), Mn(II), Zn(II), Co(II) and Cu(II) ions coordinated to the Schiff base through the $\nu(\text{C}=\text{N})$ at 1618 cm⁻¹. The $\nu(\text{OH})$ of the Schiff base at 3469 cm⁻¹ disappeared in the metal(II) complexes showing the deprotonation and binding of the hydroxyl oxygen to the metal atom. The ability of these metal(II) complexes to inhibit the growth of disease causing organisms such as *Staphylococcus aureus* (Gram positive bacteria), *E.coli*, *Klesiella pneumonia*, *Salmonella typhi* (Gram negative bacteria) and *Aspergillus niger* and *Candida albicans* (Fungi isolates) were compared with the standard drugs (erythromycin and fungusol) respectively. The results of antimicrobial activity have revealed that most of the complexes are more potent against the isolated microbes as compared to the standard drugs.

Index Terms— Antimicrobial studies, hypochromic shift, metal complexes, Schiff base.

I. INTRODUCTION

Inorganic elements play crucial role in biological processes, and it is evident that many organic compounds used in medicine do not have a purely organic mode of action, some are activated or bio transformed by metal ions metabolism. Schiff bases are versatile C=N (Imine) containing compounds possessing broad spectrum of biological activity and incorporation of metals in form of complexes showed some degree of antibacterial, antifungal, antitumor and anti-inflammatory activity (Xavier and Srividhya, 2014). Schiff base are the compound containing azomethine group (C=N). They are condensation products of ketones (or) aldehydes (aldehyde and ketones) with primary amines and were first reported by Hugo Schiff in 1864. Schiff bases are

common ligands in coordination chemistry. The imine nitrogen is basic and exhibit acceptor properties. Schiff bases coordinate to metal ions via azomethine nitrogen (Ndahi and pindiga, 2012). Research has shown that Schiff bases and their metal complexes have very wild applications with regard to their anti-parasitic, anti-fungi, anti-bacteria and anti-cancer properties (Mishra *et al.*, 2012). This research was centered on Schiff base derived from p.toluidine and salicylaldehyde and their Fe(II), Mn(II), Zn(II), Co(II) and Cu(II) complexes.

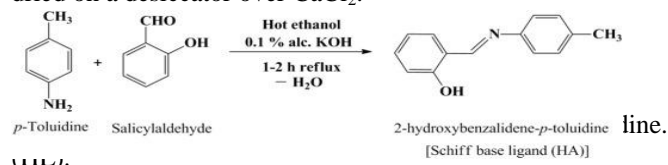
II. EXPERIMENTAL

MATERIALS AND METHODS

All chemicals used were of the analytical reagent grade (AR), and of highest purity available. They include p.toluidine, salicylaldehyde and metal (II) chlorides with exception of Zn(II) sulphate. The bacterial strains used are *Staphylococcus aureus* (Gram- positive), *E.coli*, *Klesiella pneumonia*, *Salmonella typhi* (Gram –negative) and Fungi isolates which are *Aspergillus niger* and *Candidas albicans*.

Synthesis of the Ligand (HL)

The Schiff base ligand was prepared using a modified literature method of (Aurora *et al.*, 2014). 2-hydroxybenzalidene.p.toluidine was prepared by condensation of p.toluidine and Salicylaldehyde with mole ratio of 1:1. Glacial acetic acid was added to the resulting mixture and it was refluxed for 2hrs. The bright yellow crystals formed was washed with ethanol and the product was dried on a desiccator over CaCl₂.



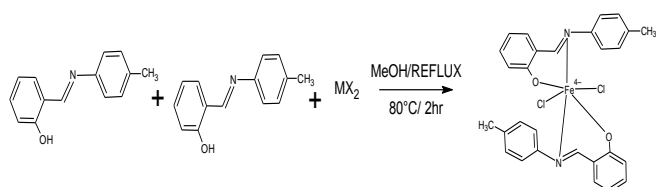
Synthesis of metal (II) complexes:

Synthesis of HL with Fe²⁺

The metal salt; FeCl₂.4 H₂O (2mmol, 0.398g) of hot methanolic solution was added to the methanolic solution of the Schiff base in the mole ratio of 1:2 (metal: ligand) respectively. The reaction mixture was refluxed for 2-3hrs cooled to an ambient temperature with the aid of an ice. The respective metal complexes separated were filtered, washed with ethanol and then dried.

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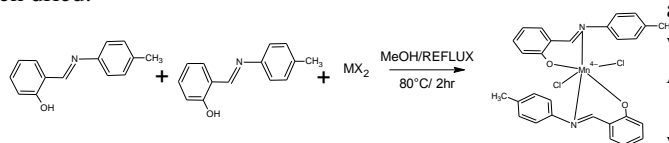
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Scheme 2: Synthesis of Schiff base with Fe(II) complex

Synthesis of HL with Mn²⁺

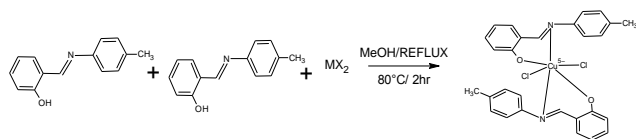
MnCl₂.4H₂O (2mmol, 0.396g) of hot methanolic solutions was added to the methanolic solution of the Schiff base in the mole ratio of 1:2 (metal: ligand) respectively. The reaction mixture was refluxed for 2-3hrs cooled to an ambient temperature with the aid of an ice. The respective metal complexes separated were filtered, washed with ethanol and then dried.



Scheme 3: Synthesis of Schiff base with Mn(II) complex.

Synthesis HL with Cu²⁺

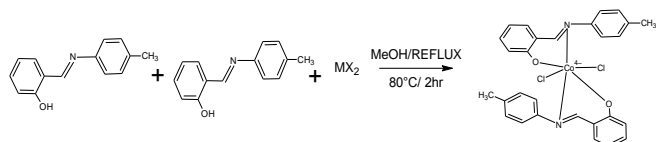
CuCl₂.4H₂O (2mmol, 0.414g) of hot methanolic solution was added to the methanolic solution of the Schiff base in the mole ratio of 1:2 (metal: ligand) respectively. The reaction mixture was refluxed for 2-3hrs cooled to an ambient temperature with the aid of an ice. The respective metal complexes separated were filtered, washed with ethanol and then dried.



Scheme 4: Synthesis of Schiff base with Cu(II) complex.

Synthesis of HL with Co²⁺

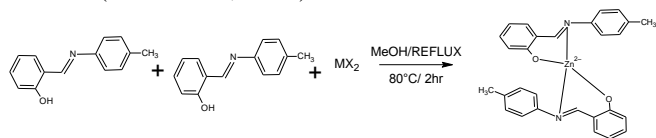
CoCl₂.6H₂O (2mmol, 0.476g) of hot methanolic solution was added to the methanolic solution of the Schiff base in the mole ratio of 1:2 (metal: ligand) respectively. The reaction mixture was refluxed for 2-3hrs cooled to an ambient temperature with the aid of an ice. The respective metal complexes separated were filtered, washed with ethanol and then dried.



Scheme 5: Synthesis of Schiff base with Co(II) complex.

Synthesis of HL with Zn²⁺

ZnSO₄.7H₂O (2mmol, 0.574g) of hot methanolic solutions was added to the methanolic solution of the Schiff base in the mole ratio of 1:2 (metal: ligand) respectively. The reaction mixture was refluxed for 2-3hrs cooled to an ambient temperature with the aid of an ice. The respective metal complexes separated were filtered, washed with ethanol and then dried. (Ndahi *et al.*, 2012).



Scheme 6: Synthesis of Schiff base with Zn(II) complex.

III. ANTIMICROBIAL STUDIES

The ligands and complexes were assayed for antimicrobial activity by the Kirby-Bauer antimicrobial disk diffusion procedure. Solutions of the complexes, ligands and pure erythromycin as well as fungisol were made in DMSO.

The culture media employed for the anti-microbial investigation were nutrient agar, for bacteria, and Saboraud's dextrose agar for fungi. 6.08g of Muller Hinton Agar powder was dissolved in 160ml of water and allow to set. The solution was sterilized using autoclave at 121⁰C for 15minutes. It was cooled to room temperature before transferring it to the plate, to gel for some time. The antibacterial activity of the ligand and its metal complexes were tested against *Staphylococcus aureus*, *Escherichia coli*, *Klebsilla pneumonia* and *Salmonella typhi*.

2mg/ml of the ligands and metal complexes in DMSO were prepared. The disc was impregnated with the complexes and finally introduced into the inoculum before incubation at 37⁰C for 24 Hours. The susceptibility test was determined by measuring the zone of inhibition (ZOI) and compared with erythromycin as a standard drug. Modified method by (Monica Cheesbrough, 2006). The antifungal activity of the ligand and its metal (II) complexes was tested against *Aspergillusniger* and *Candida albicans* species at 2mg/ml. The suspension of each microorganism was poured on the surface of solidified dextrose agar already poured into petri dishes. The impregnated disc were placed on the surface of the agar plate at 37⁰C for 48hrs. The activities were determined by measuring the diameter of zone of inhibition and compared with the standard drug, fungisol (miconazole Nitrate).

IV. RESULTS AND DISCUSSION

The Schiff base ligand was prepared by modified literature method of (Aurora *et al.*, 2014). It was a bright yellow crystal and the percentage yield was 82%. It has a conductivity of 3.0 showing that it's non -electrolyte. The Schiff base melting point was at 155⁰C showing the stability of the ligand. The metal (II) salts react with the Schiff base ligand in 1:2 molar ratio in alcoholic medium. The ligand and its metal complexes are stable and are non-electrolytes (Imran *et al.*, 2013). The complexes were characterized by Solubility, Conductivity, infrared and UV-Visible. The Schiff base and the metal (II) complexes were all soluble in both cold and hot ethanol, chloroform, diethyl ether, acetone and DMSO, not soluble in water with exception of Fe(HL)₂Cl₂ which was slightly soluble in cold ethanol. The Schiff base ligand and its metal (II) complexes were insoluble in water at both room temperature and elevated temperatures. The physical properties of the complexes are shown in Table I. All the complexes synthesized are coloured ranging from bright yellow, Dark grey, Golden yellow, Brown, Light brown and Light yellow. This is typical of transition metal complexes.

A. INFRARED SPECTRAL

The FT-IR spectral of the Schiff base and its metal (II) complexes are shown in Table 3. The Infrared spectrum of the free Schiff base ligand was compared with that of the

complexes to determine the coordination sites that may have involved in the chelation. Upon comparison, it was observed that the ligands were principally coordinated to the metal atom in the following ways:

- (i) It was found that $\nu(\text{C}=\text{N})$ stretching vibration from azomethine group in HL (Schiff base) at 1618 cm^{-1} shifted to higher ($1-30\text{ cm}^{-1}$) wave numbers in the complexes indicating the participation of azomethine nitrogen in coordination.
- (ii) A band appearing at 3469 cm^{-1} due to $\nu(\text{OH})$ in the Schiff base compound was not found in the spectra of metal complexes indicating deprotonation and binding of the hydroxyl oxygen to the metal atom.
- (iii) The appearance of new bands at $500-570\text{ cm}^{-1}$ shows the bonding of (M-N) and $460-495\text{ cm}^{-1}$ depict bonding between (M-O) of the ligands.
- (iv) The presence of coordinated water molecules in the complexes was determined by the appearance of bands at $3250-3450$ assignable to OH stretching.

B. Electronic Spectral

The electronic spectral of the ligand and its metal (II) complexes are shown in Table 4. The electronic spectral bands of the metal complexes studied in Dimethylsulphoxide indicated that the spectrum of the free ligand (HL) showed absorption band at the transition energy of 22220 cm^{-1} (450 nm). In the complexes, this transition energy has shifted to

28571 cm^{-1} (350 cm^{-1}) in $\text{Mn}(\text{HL})_2$, $\text{Co}(\text{HL})_2$, $\text{Cu}(\text{HL})_2$ and $\text{Zn}(\text{HL})_2$ with exception of $\text{Fe}(\text{HL})_2$ complex. The hypochromic shift observed was attributed to complexation of the ligand to the central metal (Ogunniran *et al.*, 2008).

C. Antimicrobial Screening

The antibacterial activity of HL and its synthesized complexes were investigated against three gram-negative strains, *E. coli*, *salmonella typhi*, and *K. pneumoniae*, as well as one gram-positive strain, *S. aureus*. Mn(II) complex had the largest zones of inhibition against *E. coli* and *K. pneumoniae*. Cu(II) complex produced the largest antibacterial activity against *salmonella typhi*, *S. aureus* and *E. coli*. Co(II) complex also showed higher inhibition zones for *salmonella typhi*. The Fe(II) complex also showed the highest antibacterial activity against *E. coli*. The order of antibacterial activity of the compounds was $\text{Mn (II)} > \text{Cu(II)} > \text{Co(II)} > \text{Zn(II)} > \text{Fe(II)} > \text{HL}$.

The result obtained on antifungal studies, showed that the Schiff base ligand (HL) had the highest zone of inhibition against *candida albicans*. Mn(II), Fe(II) and Zn(II) metal complexes of the Schiff base (HL) also showed a very higher zone of inhibition against *candida albicans*. Fe(II) complex of the Schiff base displayed an excellent zone of inhibition of 26.83 mm against *Aspergillus niger* and 26.48 mm against *Candida albicans* as compared to the fungusol (control) of 12.02 mm and 17.48 mm respectively.

Table I: The physical properties of the ligand and its metal (II) complexes

Compound	Molecular formula	Colour	Melting point($^{\circ}\text{C}$)	Yield (%)	Conductivity ($\text{S.cm}^2.\text{mol}^{-1}$)
HL	$\text{C}_{14}\text{H}_{13}\text{NO}$	Bright yellow	155	82	3.0
$\text{Fe}(\text{HL})_2\text{Cl}_2$	$[\text{Fe}(\text{C}_{14}\text{H}_{13}\text{NO})_2\text{Cl}_2]$	Dark grey	180	63	8.2
$\text{Mn}(\text{HL})_2\text{Cl}_2$	$[\text{Mn}(\text{C}_{14}\text{H}_{13}\text{NO})_2\text{Cl}_2]$	Golden yellow	160	73	13.5
$\text{Co}(\text{HL})_2\text{Cl}_2$	$[\text{Co}(\text{C}_{14}\text{H}_{13}\text{NO})_2\text{Cl}_2]$	Brown	160	67	2.7
$\text{Cu}(\text{HL})_2\text{Cl}_2$	$[\text{Cu}(\text{C}_{14}\text{H}_{13}\text{NO})_2\text{Cl}_2]$	Light brown	160	57	36.6
$\text{Zn}(\text{HL})\text{SO}_4$	$[\text{Zn}(\text{C}_{14}\text{H}_{13}\text{NO})_2\text{SO}_4]$	Light yellow.	170	78	19.2

HL = 2-hydroxybenzalidene-p-toluidine.

Table II. Solubility tests of the ligand and its metal (II) complexes

Compound	Distilled Water		Ethanol		Chloroform		Diethyl ether		Acetone		DMSO	
	C	H	C	H	C	H	C	H	C	H	C	H
HL	NS	NS	SS	S	S	S	S	S	S	S	S	S
$\text{Fe}(\text{HL})_2\text{Cl}_2$	SS	SS	SS	S	S	S	S	S	S	S	S	S
$\text{Mn}(\text{HL})_2\text{Cl}_2$	NS	SS	S	S	S	S	S	S	S	S	S	S
$\text{Co}(\text{HL})_2\text{Cl}_2$	NS	NS	SS	S	S	S	S	S	S	S	S	S
$\text{Cu}(\text{HL})_2\text{Cl}_2$	NS	SS	S	S	S	S	S	S	S	S	S	S
$\text{Zn}(\text{HL})\text{SO}_4$	NS	SS	S	S	S	S	S	S	S	S	S	S

S – Soluble, SS – slightly soluble, NS – Not Soluble, C – Room Temp. H – Elevated Temp. DMSO = Dimethylsulphoxide
HL = 2-hydroxybenzalidene-p-toluidine

Table III. FT-IR spectral of the ligand and the metal(II) complexes.

Compound	V(O-H)	V(C=N)	V(C=C)	V (M-O)	V(M-N)
HL	3469.79 w	1618.55 S	1457.95 S	-	-
Fe(HL) ₂ Cl ₂	3393.09 br	1639.16 S	1476.84 S	495 m	515 m
Mn(HL) ₂ Cl ₂	3363.50 br	1618.91 S	1457.73 S	460 m	530 m
Co(HL) ₂ Cl ₂	3436.67 br	1619.76 S	1498.01 S	485 m	508 m
Cu(HL) ₂ Cl ₂	3257.52 br	1638.62 S	1463.85 S	488 m	517 m
Zn(HL)SO ₄	3387.48 br	1637.72 S	1467.20 S	488 m	520 m

HL = 2-hydroxybenzalidene-p-toluidine

Table IV. Electronic spectral of the Ligands and metal (II) complexes

Compound	Abs	Wave number(cm ⁻¹)	ε _{max} (L.mol ⁻¹ .cm ⁻¹)	λ max (nm)
HL	0.787	22220	787	450
Fe(HL) ₂ Cl ₂	0.542	22220	542	450
Mn(HL) ₂ Cl ₂	1.251	28571	1251	350
Co(HL) ₂ Cl ₂	1.341	28571	1341	350
Cu(HL) ₂ Cl ₂	1.302	28571	1302	350
Zn(HL)SO ₄	1.242	28571	1242	350

Table V. Susceptibility Test of Ligands and its Metal (II) complexes against bacterial isolates

Compounds	<i>Staphylococcus</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Salmonella typhi</i>
	<i>aureus</i>		<i>pneumonia</i>	
HL	9.00	17.91	14.40	9.38
Mn(HL) ₂ Cl ₂	10.58	18.33	21.18	R
Fe(HL) ₂ Cl ₂	14.40	15.42	12.24	7.93
Cu(HL) ₂ Cl ₂	17.00	26.86	15.70	11.29
Zn(HL) ₂ SO ₄	9.20	11.44	15.87	10.40
Co(HL) ₂ Cl ₂	7.80	13.53	12.98	7.61
Erythromycin (Control)	21.28	19.98	26.55	23.28

Where R = Resistant (no zone of inhibition), less than 9 weak, 10 – 16 moderate and greater than 17 significant

Antibacterial test

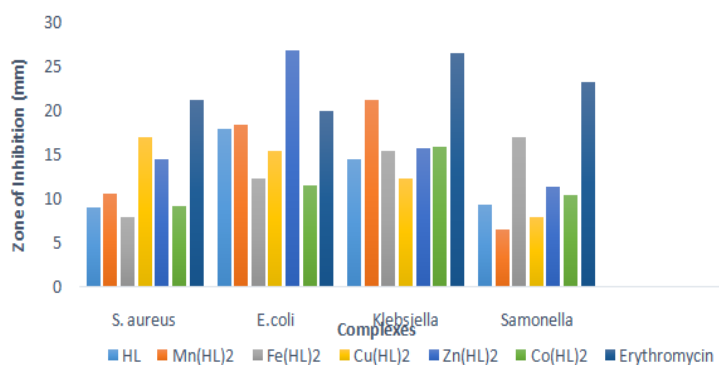


Fig 1: Graph of Antibacterial Test

Table VI. Susceptibility Test of Ligands and its Metal (II) complexes against fungi isolates

Compounds	<i>Candida Albicans</i>	<i>Aspergillus niger</i>
HL ¹	29.69	9.84
Mn(HL ¹) ₂ Cl ₂	23.12	9.85
Fe(HL ¹) ₂ Cl ₂	26.48	26.83
Cu(HL ¹) ₂ Cl ₂	14.13	20.32
Zn(HL ¹) ₂ SO ₄	24.87	9.61
Co(HL ¹) ₂ Cl ₂	16.57	9.86
Fungusol (Control)	17.48	12.02

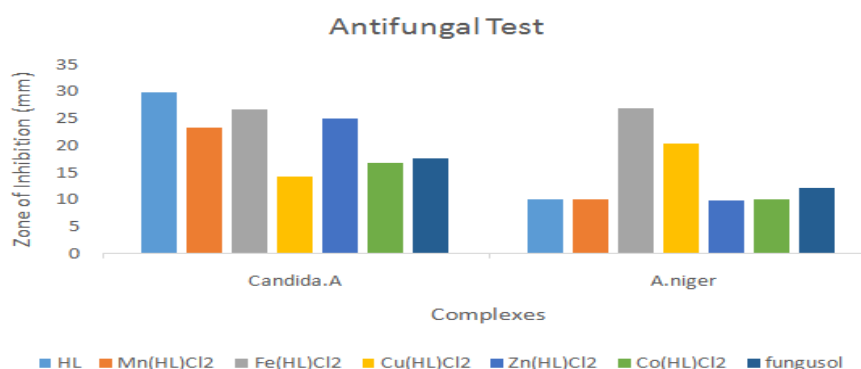


Fig 2: Graph of Antifungal Test

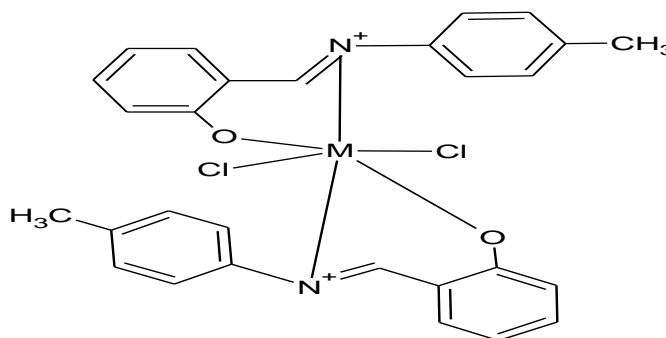


Fig 3: Proposed structure of Schiff base and metal (II) ion

V. CONCLUSION

The HL showed very good inhibition zone against *Candida albicans* as compared with other metal complexes. The complexes of HL was biologically more active than the parent ligand against *Escherichia coli*, *Klebsilla pneumonia*, *Salmonella typhi*, *Aspergillusniger* strains and the Mn(II) shows greatest potency This shows how the formation of metal complexes affect the biological activities of the parent organic molecules or ligand as a result of the ability of the metal ion to bind with organic molecules or the ligand which in turn increase the inhibitory potential of the chemotherapeutic agent.

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