Synthesis, Characterization and Evaluation of Antimicrobial Potency of Fe(II), Cu(II), Co(II) and Zn(II) ion complexes with Erythromycin and Schiff base.

*Nnamani Johnson Onuorah , Nasiru Pindiga Yahaya , Wilson.L. Danbature

E.mail: Johnson.nnamani@gmail.com

Abstract: The Schiff base ligand was first synthesized by the condensation reaction between salicylaldehyde and p.toluidine in molar ratio of 1:1. Fe(II), Zn(II), Co(II) and Cu(II) complexes of erythromycin and the Schiff base were synthesized in mole ratio of 1:1:1 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 mixed ligand (erythromycin and 2-hdroxybenzalidene.p.toluidine) and the metal complexes showed various shades of colours. The percentage yield of the ligand and the complexes are in the range of 56-72%. The melting point of the ligand is 200^0C while that of the complexes ranges from 220 -230^0C. The values of molar conductance of mixed ligand and all the metal (II) complexes are in the range of 16.3 – 32.2 S.cm^2 mol^{-1}. These values indicate that they are all non-electrolytes. The UV-Vis spectral studies have shown bathochromic shift in the metal (II) complexes of the ligand. The FT-IR spectral have shown that the Fe(II), Zn(II), Co(II) and Cu(II) ions coordinated to the erythromycin and 2-hydroxybenzalidene.p.toluidine through the v(C=N) and v(C=O) at 1645 cm^{-1} and 1538 cm^1 respectively. 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.

Keywords: Erythromycin, Schiff base, metal complexes, complexation, bathochromic shift, antimicrobial studies.

INTRODUCTION

Structurally, Schiff base (also known as imine or azomethine) is an analogue of a ketone or aldehyde in which the carbonyl group (C=O) has been replaced by an imine or azomethine group. Schiff bases ligands are essential in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions. Schiff base ligands of heterocyclic compounds and their transition metal complexes are of great interest as simple structural models of biological system due to the presence of heteroatoms

such as nitrogen, oxygen and sulphur groups (Mohammed *et al.*, 2016). The knowledge of the interactions between metal ions and antibiotics is of great importance because these reactions can influence the synthesis of metallo antibiotics depending on the idea of metal ion interaction with absorbed drugs. One of the ways of restoring the activity of organic drugs for which resistance has emerged is to modify the structure to contain a metal and some of these compounds are metal complexes. This is due to the fact that metal ions can interact with many different kinds of biomolecules including DNA, RNA, proteins and lipids making them unique and specific bioactive (Nancy *et al*., 2004).Antimicrobial resistance is fast becoming a global concern with scary increase in drug- resistant micro-organisms. The situation is critical in Sub-Sahara Africa as a result of the spread of resistance to the less expensive drugs widely used for treatment of diseases (Saha *et al*, 2009). A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and food borne diseases are becoming harder, and sometimes impossible, to treat as antibiotics become less effective (WHO, 2018). Antimicrobial resistance (AMR) causes an estimated 700,000 deaths annually worldwide, and every country is potentially affected (United States Pharmacopeia, 2015). If not properly addressed, the number of deaths could grow to 10 million by 2050. Antibiotics resistance leads to longer hospital stays, higher medical costs and increased mortality (WHO, 2018). The resistant micro-organisms also extends to both Gram negative organisms like *Salmonella typhi, Escherichia coli* and Gram positive *Bacillus Subtilis bacteria strains*. Even some fungal pathogens are also showing this drug resistance like *Aspergillus niger* (Saha *et al*, 2009).Antibiotic resistance is when bacteria get used to an antibiotic and no longer respond to it. This happens because doctors sometimes prescribe antibiotics to people who do not need them (Carol Der Sarkissian, 2017). Antimicrobial resistance is said to occur when bacteria undergoes transformation in such a manner that it can easily weaken or render the drug ineffective (Waziri *et al*, 2018). From time immemorial, there has been a continual battle between humans and the numerous of micro-organisms that cause infection and disease. These diseases have affected substantial portions of the human population, causing significant high mortality rate (Saha *et al*, 2009). At the middle of the 20th century, major advances in antibacterial drug development and other means of infection control helped turn the tide in favour of humans. To create a panacea for this alarming problem of pathogens resistance to antibiotics, the development of new metal based drugs which has been largely based on the ability of metals to increase inhibitory potentials of chemotherapeutic agents is a matter of urgency and cannot be over-emphasized. There is a continuous search for more potent and cheaper raw materials to feed the industry. That's why today's pharmaceutical industries are looking towards synthesizing the alternative compounds which act as drug. As a matter of fact, thousands of compounds have been prepared based on well-conceived ideals of improving their efficacy and have been subsequently screened but few of them have successfully passed the clinical tests (Paul and Giann, 2006).

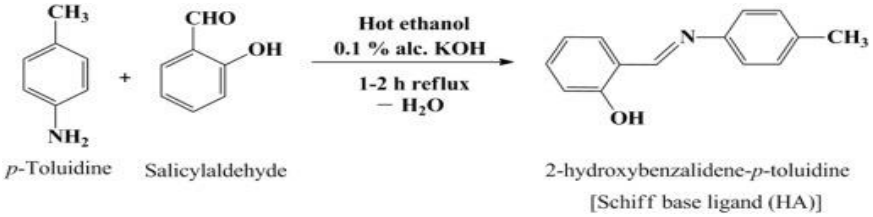
EXPERIMENTAL:

MATERIALS AND METHODS

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

Synthesis of the Schiff base

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 $CaCl_2$.



Scheme 1: Formation of 2-hydroxybenzalidene.p.toluidine.

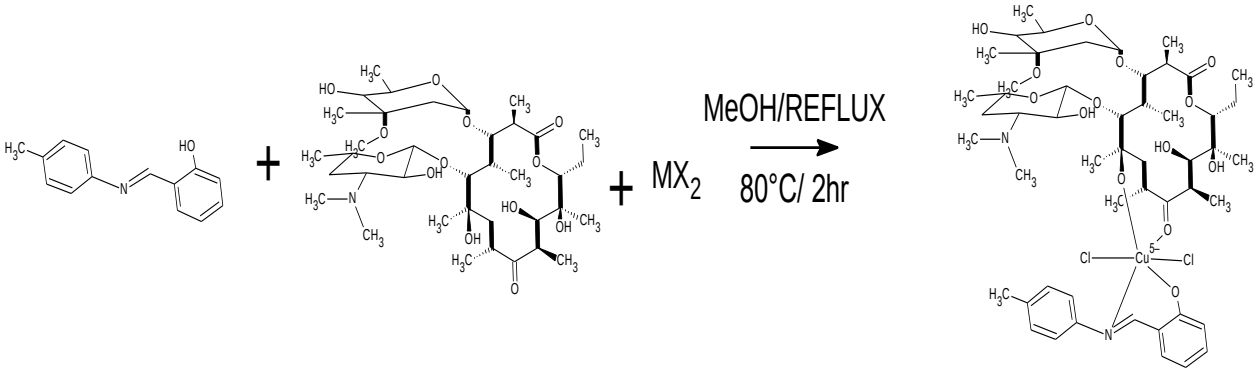
Synthesis of the Mixed of ligand

The mixed ligand was prepared using a modified literature method of (Ogunniran *et al*., 2008). It was prepared by mixing hot methanolic solution of erythromycin (0.002mol, 1.468g) and methanolic solution of 2-hydroxybenzalidene.p.toluidine (0.002mol, 0.422g) in the molar ratio of 1:1. The resultant mixture was refluxed for 3hrs on a hot magnetic stirrer at temperature of 80^0C and cooled to an ambient temperature with the aid of crushed ice. The solid precipitates were filtered, washed with ethanol and the product was dried on a desiccator over $CaCl_2$.

Synthesis of metal (II) complexes:

Synthesis of Fe^{2+} Complex with (erythromycin and 2-hydroxybenzalidene.p.toluidine)

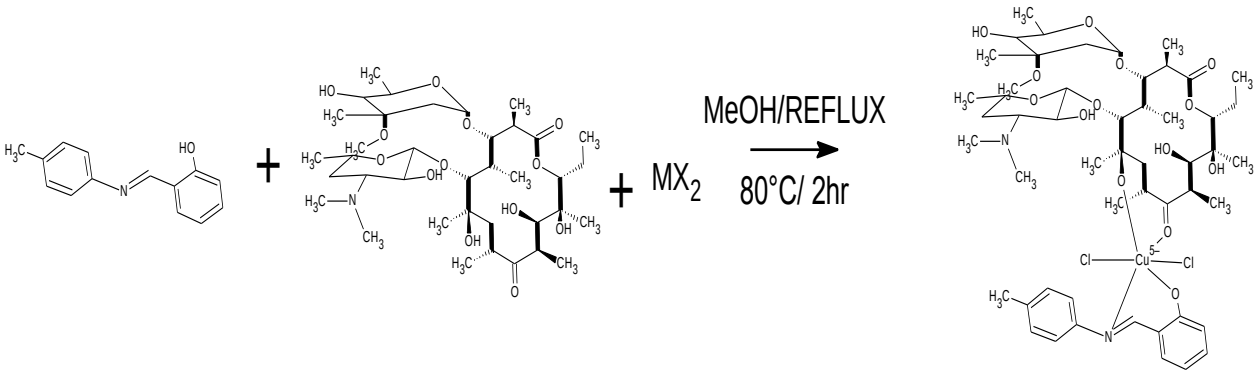
$FeCl_2.4H_2O$ (2mmol, 0.398g) of hot methanolic solution, solution of 2-hydroxybenzaliden-p-toluidine (2mmol, 0.422g) and solution of erythromycin (2mmol, 1.468g) were mixed in the ratio of 1:1:1 ($M:L_1:L_2$). The resultant mixture were refluxed for 2-3 hrs and cooled to room temperature. The precipitates were filtered, washed with ethanol and dried in a desiccator over CaCl



Scheme 2: Synthesis of erythromycin and 2-hydroxybenzalidene.p.toluidine with Fe(II) complex

Synthesis of Cu^{2+} Complex with (erythromycin and 2-hydroxybenzalidene.p.toluidine)

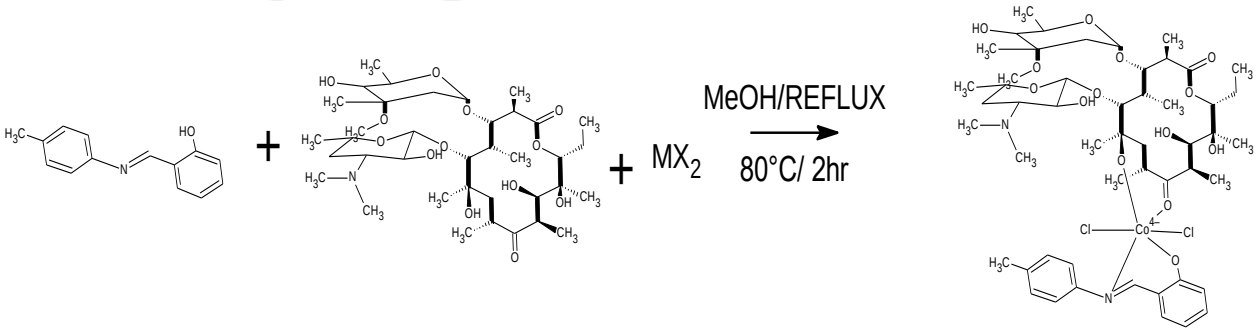
$CuCl_2.4H_2O$ (2mmol, 0.414g), (2mmol, 1.468g) of erythromycin and (2mmol, 0.422g) of 2-hydroxybenzaliden-p-toluidine hot methanolic solutions were mixed in the molar ratio of 1:1:1 ($M:L_1:L_2$). The resultant mixture were refluxed for 2-3 hrs and cooled to ambient temperature with the aid of crushed ice. The solid precipitates were filtered, washed with ethanol and dried.



Scheme 3: Synthesis of erythromycin and 2-hydroxybenzalidene.p.toluidine with CuII) complex

Synthesis of Co^{2+} Complex with erythromycin and 2-hydroxybenzalidenep.toluidine)

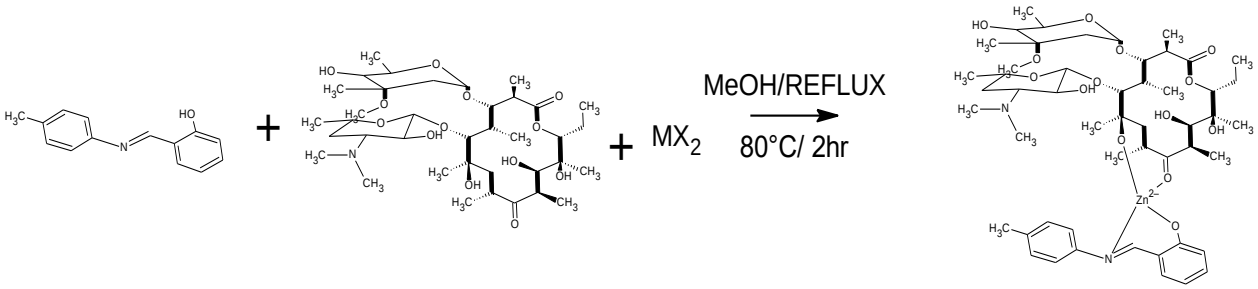
$CoCl_2.6H_2O$ (2mmol, 0.476g), (2mmol, 1.468g) of erythromycin and (2mmol, 0.422g) of 2-hydroxybenzaliden-p-toluidine of methanolic solutions were mixed in the molar ratio of 1:1:1 ($M:L_1:L_2$). The resultant mixture were refluxed for 2-3 hrs and cooled to ambient temperature with the aid of crushed ice. The solid precipitates were filtered, washed with ethanol and dried.



Scheme 4: Synthesis of erythromycin and 2-hydroxybenzalidene.p.toluidine with CoII) complex

Synthesis of Zn^{2+} Complex with (erythromycin and 2-hydroxybenzalidene.p.toluidine)

$ZnSO_4.7H_2O$ (2mmol, 0.574g), (2mmol, 1.468g) of erythromycin and (2mmol, 0.422g) of 2-hydroxybenzaliden-p-toluidine of hot methanolic solutions were mixed in the molar ratio of 1:1:1 ($M:L_1:L_2$). The resultant mixture were refluxed for 2-3 hrs and cooled to ambient temperature with the aid of crushed ice. The solid precipitates were filtered, washed with ethanol and dried.



Scheme 4: Synthesis of erythromycin and 2-hydroxybenzalidene.p.toluidine with ZnII) complex

Antimicrobial Studies

The ligand 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 fungusol 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 121^0C 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 37^0C 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 *Aspergillus niger 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 37^0C for 48hrs. The activities were determined by measuring the diameter of zone of inhibition and compared with the standard drug, fungusol (miconazole Nitrate) (Kumari *et al.*, 2003).

Results and Discussion.

The mixed ligand was prepared by modified literature method of (Ogunniran *et al.*, 2007). It was a light yellow crystal and the percentage yield was 68%. It has a conductivity of 101 S $.cm^2.mol^{-1}$. The mixed ligand melting point was at 200^0C showing the stability of the ligand. The metal (II) salts reacted with the erythromycin and 2-hydroxbenzalidene. p.toluidine in 1:1:1 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 mixed ligand and the metal (II) complexes were both insoluble in water at both room temperature and elevated temperature. The mixed ligand and the metal (II) complexes were both soluble in acetone, chloroform, ethanol and DMSO.(Yahaya *et al*., 2018). The physical properties of the complexes are shown in Table 1. The mixed ligand complexes of Fe(II), Cu(II), Co(II) and Zn(II) synthesized were dirty green, dark brown, light brown and yellow ochre respectively. This is typical of transition metal complexes.

Infrared Spectral

The FT-IR spectral of the mixed ligand and its metal (II) complexes are shown in Table 3. The Infrared spectral of the complexes were compared with that of free mixed ligand in order to ascertain the coordination or binding sites that may be involved in the chelation. Upon comparison, it was discovered that v(C=N) stretching vibration at 1645 cm^{1} shifted to lower (5-20 cm^{-1}) and the v(C=O) stretching at 1540 cm^{-1} shifted to higher (20- 70 cm^{-1}) wave numbers indicating participation in v(C=N) as well as the carbonyl v(C=O) in chelation. The new bands appearing at 478-490 and 549-550 cm^{-1} were assigned to metal – oxygen (M-O) and metal –nitrogen (M-N) stretching vibration respectively as reported by Fugu *et al*., 2013. These bands confirmed the coordination of the ligand to the metal ions through the N donor atom. Similarly, a band at 3060 – 3523 cm^{-1} in the complexes indicate the presence of water molecules (Suraj *et al*., 2012).

Electronic Spectral

The electronic spectral of the mixed 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 mixed ligand (HL) showed absorption band at the transition energy of 28571 cm^{-1} (350 nm). In the complexes, this transition energy has shifted to 22220 cm^{-1} (450 cm^{-1}) in $Co(HL)Cl_2$, $Cu(HL)Cl_2$, $Zn(HL)Cl_2$ and $Fe(HL)Cl_2$ complex. The bathochromic shift observed was attributed to complexation of the mixed ligand to the central metal (Ogunniran *et al*., 2008).

Antimicrobial Screening

The antibacterial activity of the 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* using erythromycin as the control drug. Mn(II) complex had shown good zones of inhibition against *E. coli* and *K. pneumoniae* more. Cu(II) and Co(II) complexes produced the largest antibacterial activity against *salmonella typhi.* The result obtained on antifungal studies, showed that the Cu(II), Mn(II) and Fe(II) of the mixed ligand depict good antifungal potency against *candida albicans* and *Aspergillus niger* when compared with the control drug.

Table 1: The physical properties of the mixed ligand and its metal (II) complexes

Compound	Molecular formula	Colour	Melting point(^{0}C)	Yield (%)	Conductivity ($S.cm^{2}.mol^{-1}$)
HL	$C_{51}H_{80}N_2O_{14}$	Light yellow	200	68	101.0
$Fe(HL)Cl_2$	$[Fe\ C_{51}H_{80}N_2O_{14}Cl_2]$	Dirty green	230	68	17.2
$Co(HL)Cl_2$	$[Co\ C_{51}H_{80}N_2O_{14}Cl_2]$	Light brown	220	72	17.2
$Cu(HL)Cl_2$	$[Cu(C_{51}H_{80}N_2O_{14}Cl_2]$	Dark brown	230	57	17.0
$Zn(HL)SO_4$	$[ZnC_{51}H_{80}N_2O_{14}]SO_4$	Yellow ochre	230	65	16.3

HL = (erythromycin) (2-hydroxybenzalidene.p.toluidine)

Table 2. Solubility tests of the mixed 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	S	S	S	S	S	S	S	S	S	S	S
$Fe(HL)Cl_2$	NS	SS	S	S	S	S	S	S	S	S	S	S
$Co(HL)Cl_2$	NS	NS	S	S	S	S	S	S	S	S	S	S
$Cu(HL)Cl_2$	NS	SS	S	S	S	S	S	S	S	S	S	S
$Zn(HL)SO_4$	NS	SS	SS	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 = (erythromycin) (2-hydroxybenzalidene.p.toluidine)

Table 3: FT-IR spectral of the mixed ligand and its metal (II) complex

Compound	**V(O-H)**	**V(C=N)**	**V(C=O)**	**V (M-O)**	**V(M-N)**
HL	3452.11 w	1643.66 S	1540.11 m	-	-
$Fe(HL)Cl_2$	3224.55 br	1644.59 S	1590.20 S	478 m	550 m
$Co(HL)Cl_2$	3060.87 br	1634.34 S	1570.73 S	478 m	549 m
$Cu(HL)Cl_2$	3230.45 br	1638.43 S	1509.25 S	484 m	548 m
$Zn(HL)SO_4$	3523.01 br	1645.37 S	1612.26 S	490 m	549 m

HL= (erythromycin) (2-hydroxybenzalidene.p.toluidine)

Table 3. Electronic spectral of the mixed Ligand and metal (II) complexes

Compound	Abs	Wave number(cm^{-1})	€max ($L.mol^{-1}.cm^{-1}$)	λ max (nm)
HL	1.526	28571	1526	350
$Fe(HL)Cl_2$	1.976	25000	1976	400
$Co(HL)Cl_2$	2.277	25000	2277	400
$Cu(HL)Cl_2$	2.370	25000	2370	400
$Zn(HL)SO_4$	2.179	25000	2179	400

HL= (erythromycin) (2-hydroxybenzalidene.p.toluidine)

Table 6. Susceptibility Test of Ligand and its Metal (II) complexes against bacterial isolates

Compounds	*Staphylococcus aureus*	*E. coli*	*Klebsiella pnemonia*	*Salmonella typhi*
$Fe(HL)Cl_2$	14.86	11.63	10.28	7.53
$Cu(HL)Cl_2$	14.40	15.42	15.64	18.56
$Zn(HL)SO_4$	7.33	15.64	18.45	10.06
$Co(HL)Cl_2$	10.53	16.71	13.72	16..37
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

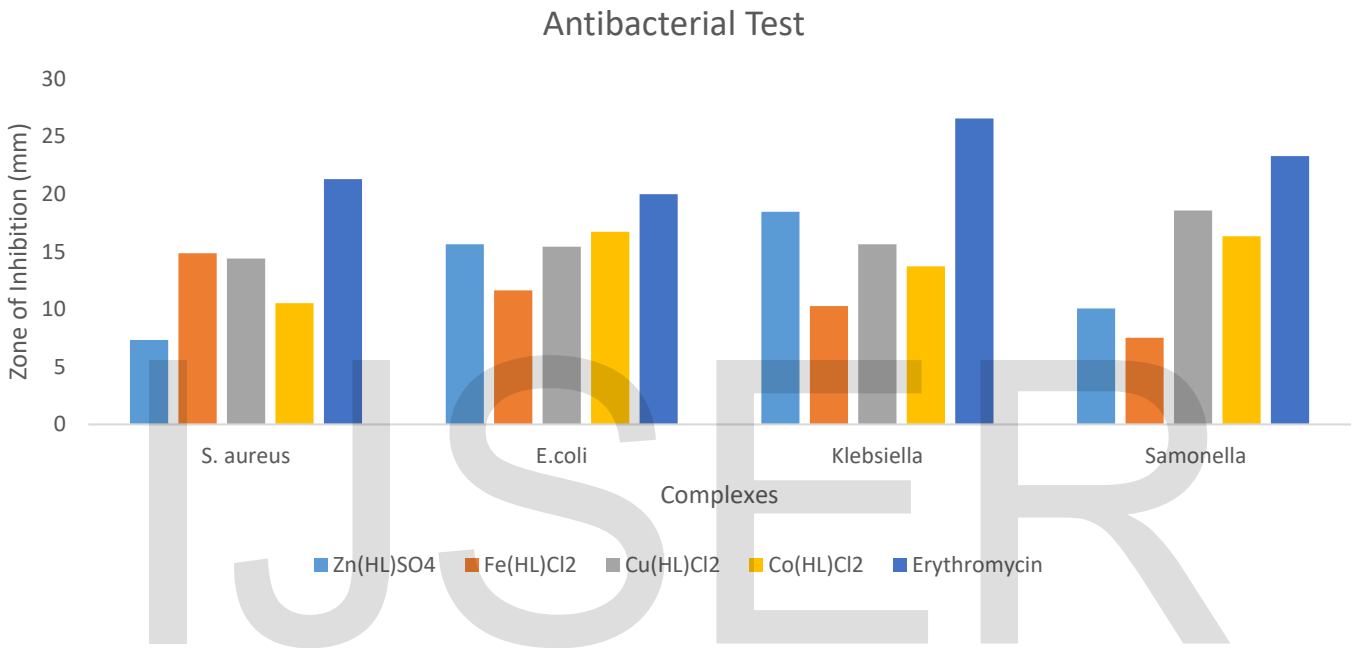


Figure 1: Graph of Antibacterial Activity.

Table 7. Susceptibility Test of mixed Ligand and its Metal (II) complexes against fungi isolates

Compounds	*Candida Albicans*	*Aspergillus niger*
$Fe(HL)Cl_2$	20.48	14.70
$Cu(HL)Cl_2$	19.56	12.42
$Zn(HL)SO_4$	13.64	10.71
$Co(HL)Cl_2$	14.40	9.82
Fungusol (Control)	17.48	12.02

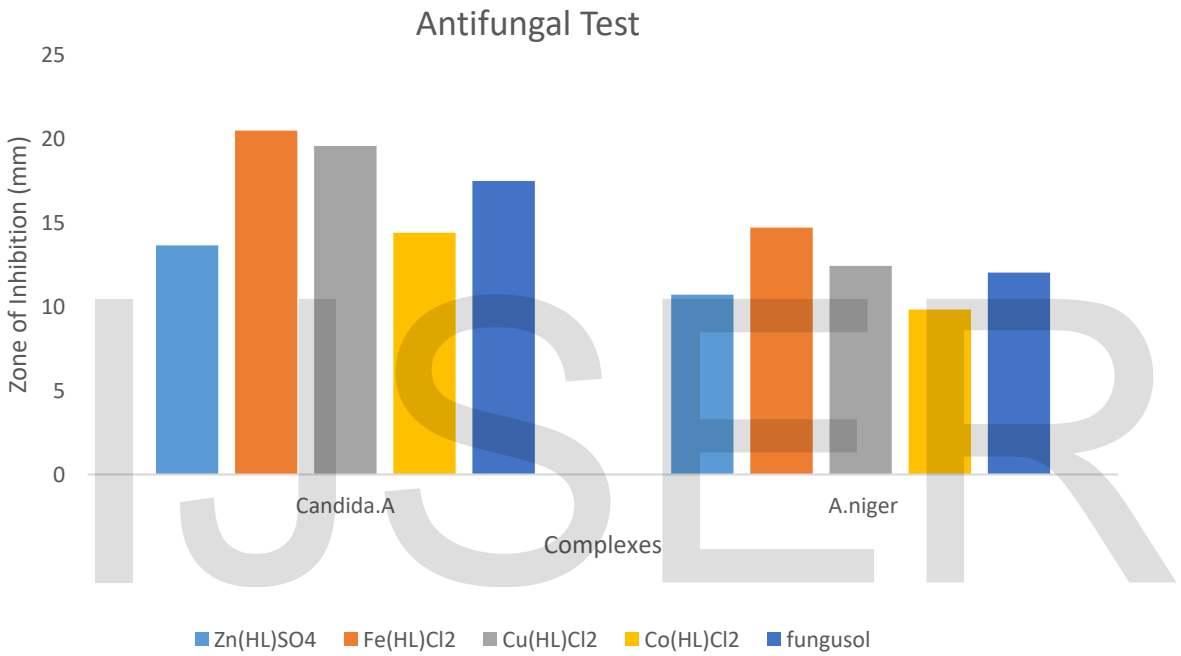
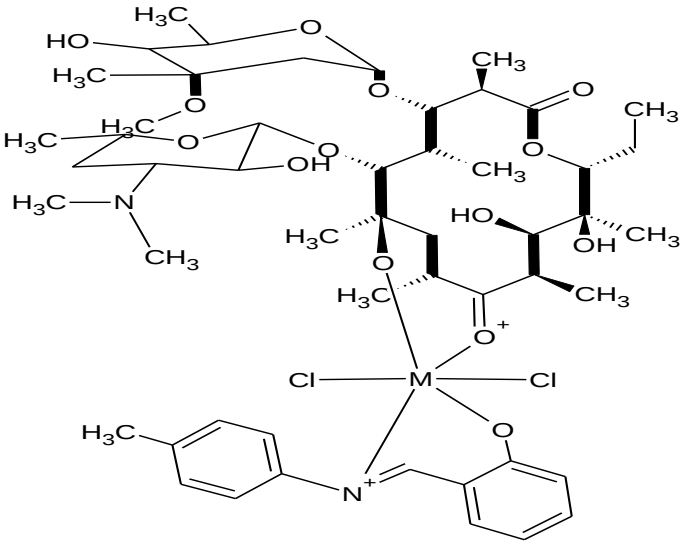


Figure 2: Graph of Antifungal Activity



M = [Cu(II), Zn(II), Fe(II), and Co(II)]

Figure 3: Proposed structure (erythromycin) (2-hydroxybenzalidene.p.toluidine) (HL) with Metal II ions.

Conclusion

This research work entailed the synthesis and spectroscopic characterization of series of iron (II), Copper (II), Zinc (II) and Cobalt (II) complexes with erythromycin and 2-hydroxybenzalidene.p.toluidine ligands. These complexes were characterized by using different physicochemical techniques. Based on the data obtained, the ligand coordinated to the metal ions through v(C=N) and v(C=O). The antimicrobial susceptibility test result showed increase activity for Fe(II), Co(II) and Cu(II) complexes. Decrease activity was observed against Zn(II) when compared with the control dugs. The former 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 agents.

References

Abdulkareem, A.A. (2017). Synthesis and Antibacterial Studies of Metal Complexes of Cu(II), Ni(II) and Co(II) with Tetradentate Ligand. *Journal of Biophysical Chemistry*, 8, 13-21.

Ajibade, P.A. and Zulu, N.H.(2010). Synthesis, Characterization and Antibacterial Activity of Metal Complexes of Phenylthiourea: The X-ray Single Crystal Structure of $[Zn(SC(NH_2)NHC_6H_5)_2(OOCCH_3)_2]$ C_2H_5OH,'' *Journal of Coordination Chemistry*. 63 (18), 3229- 3239.

Al Humayyd M.S.(1997). Pharmacokinetic interactions between erythromycin, Clarithromycin, roxithromycin and phenyltoin in the rat. Chemotherapy; 43 (2): 77 -85.

Ali, I., Wani, W.A. and Salem, K.(2013). Empirical Formula to Molecular Structures of Metal Complexes by Molar Conductance, *J. Ino. Nano-Metal chem.,* 43(9):1162-1170

Aurora, R., Mariana, C.C., Emilia, A., and Cezar, I.C. (2014). Transition metal (II) Complexes with Cefotaxime- Derived Schiff base: Synthesis, Characterization and Antimicrobial Studies, *Journal of Bioinorganic Chemistry* and Applications. 1-17.

Aurora, R., Stelian, F., Theodor, C., and Nicolae, S. (2009). Synthesis, Characterization, and Antibacterial activity of some transition metals with the Schiff base N-(2-furanymethylene)-3-aminodibenzofuran, 33, 775-783.

Chohan, Z., Arif, M.A., Muhammad, A, Akhtar, H. Zahid, Claudium, T. Supuran, (2006). Bioinorganic Chemistry and Applications, Article ID 83131, 1-13.

Chohan, Z.H. and Jaffery, M.F. (2010). Synthesis, Characterization and Biological Evaluation of Co(II), Cu(II), Ni(II) and Zn(II) complexes with Cephradine, Metal Based Drugs, *Int. J. Electrochem. Sci.*, 7(5): 256-269.

Connors, K.A. (2000). Complexes formation: In Alfonso R.G.(Ed) Remington: The science and practice of pharmacy,2 (20) 183-197.

Cotton, F.A., Geoffery, W., Cabs, A.M. (1999). Advanced Inorganic Chemistry, London: Blackwell sci., p.1355.

David Henderson, K. (2006). Managing methicillin-resistant staphylococci: A paradigm for preventing nosocomial transmission of resistant organisms. *American Journal of Infection Control*, 34, S46- 54.

Fugu, M.B., Ndahi, N.P., Paul, B.B. and Mustapha, A.N.(2013). Synthesis, Characterization and Antimicrobial Studies of some vanillin Schiff base metal (II) Complexes. *Journal of Chemical and Pharmaceutical Research*, (5): 22-28.

Golkar, Z., Bagazra, .O, Pace, D.G. (2014). Bacteriophage Therapy: A potential Solution for the Antibiotic Resistance Crisis. *Journal of Infectious Diseases*. 8(2) 129-136.

Imran, M., Iqbal, J., Mehmood, T. and latif, S.(2006). Synthesis, characterization and in vitro screening of amoxicillin and its complexes with Co(II), Cu(II), Zn(II) and Ni(II), *J. Bio. Sci.*, 5, 946-949.

Labro, M.T. (2004). Macrolides antibiotics: Current and Future uses. Expert. Opin. Pharmacother. (5): 541 -550.

Lopez-Gresa, M., Oritz, R., Perello, L., Latorre, J., Liu- Gonzalez, M., Garcia- Granda, S., Perez-priede, M., and Canton, E., (2002). Antibacterial Studies *Journal of Inorganic Biochemistry*, 92, 65.

Mahmoud, W.H., Mohammed, G.G., Dessoky, M. (2014). Synthesis, Characterization and in vitro Biological Activity of mixed Transition Metal Complexes of Lornoxicam with 1, 10-Phenanthroline. *Int. J. Electrochem. Sci.*, 9: 1415- 1438.

Matangi, S. Pragathi, J., Bathini U., and Kumari C.G (2012): Synthesis, Characterization and Antimicrobial Activity of transition Metal Complexes with Schiff base Derived from 3-ethoxy salicylaldehyde and 2-(2-aminophenyl) 1H-benzimidazole. *E-Journal of Chemistry* 9(4): 2516-2523.

Mohammed, G.G., Omar, M.M., Hindy, A.M., (2006). Metal complexes of Schiff bases: Preparation, Characterization and Biological activity, *Turk. J.Chem.*, (30), 361-382 (2006). Schiff, H. (1864). Communications from the university laboratory in Pisa: A new series of organic bases, *Jus. Liebig. Ann. Chem.*, 131(1): 118-119.

Monica, C. (2006). District Laboratory practice in Tropical Countries Part 2 Cambridge University press,New York, NY 1001-4211, USA.

Mounika, K., Anupama, B., Pragathi, J. and Gyanakumari, C. (2010). Synthesis, Characterization and Biological Activity of a Schiff base Derived from 3- Ethoxy Salicyladehyde and 2-Amino Benzoic acids and its transition Metal Complexes. *Journal of Science Research*. 3, 513-524.(2010)

Mustapha, A.N., Ndahi, N.P., Paul, B.B., Fugu, M.B. (2014). Synthesis, Characterization of Antimicrobial Studies of metal (II) complexes of ciprofloxacin. *Journal of chemical* and *Pharmaceutical Research* 6(4): 585-593.

Mustapha, M., Thorat, B.R., sadhir, S., Atram, R.G., Ramesh, Y.(2011). Synthesis of Novel Schiff bases and its transition metal complexes. *J. Chem.pharm. Res*., 3(4):5-9.

Najma, S., Saeed, M., and Rizwana, S. (2005). Erythromycin Synergism with essential and trace elements: *Pakistan Journal of pharmaceutical Sciences* (2): 35-39.

Nancy, M., Devendra, B.,Rakesh, S., Yogesh, C.and Ramseh, C.M., (2004). In vitro activity of antiamoebic drugs against clinical isolates of Entamoeba histolytical and Entamoeba dispar, Ann Clin Microbiol, Antimicrob. (3), 27.

Ndahi, N.P., Yahaya, N.P. and Sandabe, U.K. (2012). Synthesis, Characterization and Antibacterial Studies of Some Schiff base Complexes of Cobalt(II), Nickel (II) and Zinc(II). *Asian Journal of Biochemical and Pharmaceutical Research*. 1(2): 5-9

Ngoshe, A.M., Dahiru, H.B., Fugu, B.M. (2018). Synthesis, Characterization and Antimicobial Studies of metal (II) complexes of Amoxicillin. *Journal of chemical* and *Pharmaceutical Research* 1 (7): 30-40

Ogunniran, K.O., Ajanaku, K.O., James, O.O., Ajani, O.O, Asekoya, J.A., and Nwinyi, O.C. (2008). Synthesis, characterization, antimicrobial activity and toxicology study of some

metal complexes of mixed antibiotics. *African Journal of pure and Applied Chemistry*, 2(7): 069-074.

Ogunniran, K.O., Tella, A.C., Alensela, M. and Yakubu, M.T. (2007). Synthesis, Physical properties, Antimicrobial potentials of some antibiotics complexed with transition metals and their effects on alkaline phosphate activities of selected rat tissues , *African J.Biotech*, 10, 1202- 1208.

Olaniyi, A.A. (2005). Essential Medicinal Chemistry 3rd Ed. Ibadan: Hope publications 483 - 526.

Pallavi, G., Dinesh, K. and Sulekh, C. (2014). Schiff Base Ligands and their Transition Metal Complexes as Antimicrobial Agents, *Journal of Chemical, Biological and physical Science 3(4): 1946-1964.*

Paul, J.D. Giann, S. (2006). Metal- based Antitumour drugs in the past genomic era. Dalton trans., RSC. Dol: 1039/b601840h, p. 1929-1933.

Rajavel, R., Senthil, V.M., and Anitha, C. (2008). Synthesis, Physical Characterization and Biological Acitivity of Some Schiff Base Complexes, *E-Journal of Chemistry* 3(5):620-626. Kumar, S., Dhar, D.N., Saxena, P.N.(2009). Applications of metal complexes of Schiff bases A-Review, *J. Sci. Indus. Res.*, 68(3): 181-187.

Saha, D., Dhanasekaran, S., Chandraleka, A., Panneeselvam. (2009). Synthesis, Characterization and Antimicrobial Activity of Cobalt metal Complex Against Multi Drug Resistant Bacterial and Fungal pathogens: *facta universitatis series: Physics,Chemistry and Technology* 7(1): 73-80.

Spellberg, B., Gilbert, D.N. (2014). The future of Antibiotics and Resistance: A Tribute to a Career of leadership by John Bartlett. *Journal of Clinical Infectious Diseases*.59, 71-75.

Suman Malik and Bharti Nema. (2016). Antimicrobial Activities of Schiff bases: *International Journal of Theoritical and Applied Sciences* 8 (1): 28- 30.

Sundaram,A., Kandasamy, K., Sivagnama, P., Sutha, S., Jeyaprakash, D., Pasupathy (2017). Algal biomass energy carriers as fuels: An alternative green source. *Journal of the energy Institute* 90 (2): 300 – 315.

Suraj, B.A., Deshpande, M.N., Deshmukh, J.N. (2012). Synthesis and Characterization of transition metal complexes of Schiff base derived from Isatin and 2-Amino, 4-chlorobenzoic acid. *Rasayan J.Chem* 1 (5): 9-17

Warra, A.A. (2011).Transition Metal Complexes and their application in drugs and cosmetics – A Review ,*J. Chem. Pharm.Res.*, 3(4): 951- 958.

Waziri, I., Ndahi, N.P., Mala, G.A. and Fugu, M.B. (2014). Synthesis, Spectroscopic and biological studies of cobalt(II), Nickel (II) and Iron(III) mixed antibiotics metal complexes, *Der pharma chemical*, 6 (5):118-122.

World Health Organization. (2018). Antimicrobial Resistance, Global Report on Surveillance, WHO Library Cataloguing-in-Publication Data, 250 -257.

Wright, G.D. (2014). Something new: Revisiting Natural Products in Antibiotics Drug Discovery, *Canadian Journal of Microbiology*, 60(3):147-154,

Yahaya, N.P., Ndahi, N.P., loise Bako, Madugu, M.L., Zulqiflu and Yusuf Mamman.(2018). Synthesis and partial Characterization of Two Schiff Base ligand with (2 and 4-Nitroaniline) and their Transition Metal (II) (Co and Cu) complexes. Dutse *Journal of pure and applied Science 4(2), 25-30.*

Yahaya, N.P., Zulqiflu, A., Adamu, U.A., Hamidu, U. and Muhammed, Y. (2018). Synthesis, Characterization and Studies Antibacterial activity of Iron and Zinc metal complexes derived from Sulfamethoxazole. Dutse *Journal of pure and applied Science 4(2), 12-16*

Yousef, E., Barbara, M., Maryam, M., Moj, K. (2014). A Novel Cationic Cobalt (III) Schiff base Complex: Preparation, Crystal Structure, Hirshield Surface Analysis, Antimicrobial Activities and Molecular docking. *Journal of Microbial Pathogenesis*. (113), 160- 167.

Zahid, H.C., Asifa, M., Claudiu, T.S. (2001). Synthesis, Characterization and Antibacterial Properties of Transition Metal ion Complexes of Schiff base, *Journal of Metal Based Drugs* 8(3):137-143