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Synthesis, Characterization and Evaluation of Antimicrobial Potency of Fe(II), Mn(II), Cu(II), Co(II) and Zn(II) ion Complexes with Erythromycin and p.toluidine

Nnamani Johnson Onuorah, Nasiru Pindiga Yahaya, Wilson.L. Danbature, Muhammad Mukhtar Sani

***Abstract*— Fe(II), Mn(II), Zn(II), Co(II) and Cu(II) complexes of erythromycin and p.toluidine 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 HL ligand (erythromycin.p.toluidine) and the metal complexes showed various shades of colours ranging from cream, dark green, yellow ochre, grey ash, light green and pale green. The percentage yield of the mixed ligand and the complexes are in the range of 72-79%. The melting point of the ligand is 1900C while that of the complexes ranges from 200 -2300C. The values of molar conductance of erythromycin.p.toluidine and all the metal (II) complexes are in the range of 13.2 – 24 S.cm2 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), Mn(II), Zn(II), Co(II) and Cu(II) ions coordinated to the erythromycin.p.toluidine through the ν(C=N) and ν(C=O) at 1650 cm-1 and 1537cm1 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.**

***Index Terms*— Antimicrobial studies, Bathochromic shift, Complexation, Erythromycin, p.toluidine.**

I. INTRODUCTION

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 foodborne 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 *Salmonellatyphi*, *Escherichia coli* and Gram positive *Bacillus Subtilisbacteria 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 20thcentury, 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).

II. EXPERIMENTAL:

A. MATERIALS AND METHODS

All chemicals used were of the analytical reagent grade (AR), and of highest purity available. They include

Nnamani Johnson Onuorah, Gombe State University, Nigeria.
Nasiru Pindiga Yahaya, Gombe State University, Nigeria.
Wilson.L. Danbature,Department of Chemistry, Gombe State University, Nigeria
Muhammad Mukhtar Sani, Gombe State University, Nigeria.

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 Ligand (HL)

The mixed ligand was prepared using a modified literature method of (Ogunniran *et al*., 2007).Erythromycin.p.toluidine was prepared by mixing hot methanolic solution of erythromycin (0.002mol, 1.468g) and methanolic solution of p.toluidine (0.004mol, 0.429g) in the molar ratio of 1:2. 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 light cream 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 HL (erythromycin and .p.toluidine)

A hot methanolic solution of $FeCl_2.4H_2O$ (0.002mol, 0.398g), methanolic solution of P.toluidine (0.004mol, 0.429g) and methanolic solution of erythromycin (0.002mol, 1.468g) were mixed in the molar ratio of 1:2:1 (M: L:L) .The resultant mixture were refluxed for 2-3hrs and cooled to ambient temperature with the aid of an ice. The solid precipitates were filtered, washed with ethanol and dried.

Scheme 1: Synthesis of erythromycin.p.toluidine with Fe(II) complex

Synthesis of Mn^{2+} Complex with HL (Erythromycin and P.toluidine)

$MnCl_2.4H_2O$ (0.002mol, 0.396g) of hot methanolic solution was added to the methanolic solution of P.toluidine (0.004mol, 0.429g) and methanolic solution of erythromycin (0.002mol, 1.468g) were mixed in the molar ratio of 1:2:1 (M: L:L) .The resultant mixture were refluxed for 2-3hrs and cooled to ambient temperature with the aid of an ice. The solid precipitates were filtered, washed with ethanol and dried.

Scheme 2: Synthesis of erythromycin.p.toluidine with Mn(II) complex.

Synthesis of Cu^{2+} Complex with HL (Erythromycin and P.toluidine)

A hot methanolic solution of $CuCl_2.4H_2O$ (0.002mol, 0.414g), solution of P.toluidine of (0.004mol, 0.429g) and methanolic solution of erythromycin (0.002mol, 1.468g) were mixed in the molar ratio of 1:2:1 (M: L:L) .The resultant mixture were refluxed for 2-3hrs and cooled to ambient temperature with the aid of an ice. The solid precipitates were filtered, washed with ethanol and dried.

Scheme 3: Synthesis of erythromycin.p.toluidine with CuII) complex

Synthesis of Co^{2+} Complex with HL (Erythromycin and P.toluidine)

$CoCl_2.6H_2O$ (0.002mol, 0.476g) of hot methanolic solution was added to the methanolic solution of P.toluidine (0.004mol, 0.429g) and methanolic solution of erythromycin (0.002mol, 1.468g) were mixed in the molar ratio of 1:2:1 (M: L:L) .The resultant mixture were refluxed for 2-3hrs and cooled to ambient temperature with the aid of an ice. The solid precipitates were filtered, washed with ethanol and dried.

Scheme 4: Synthesis of erythromycin.p.toluidine with Co(II) complex

Synthesis of Zn^{2+} Complex with HL (Erythromycin and P.toluidine)

$ZnSO_4.7H_2O$ (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.

Scheme 5: Synthesis of erythromycin.p.toluidine with Zn(II) complex

III. 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 *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 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).

IV. Results and Discussion.

The mixed erythromycin.p.toluidine ligand was prepared by modified literature method of (Ogunniran *et al.*, 2007). It was a light cream crystal and the percentage yield was 75%. It has a conductivity of 24.0 showing that it's non –electrolyte. The erythromycin.p.toluidine melting point was at 190^0C showing the stability of the ligand. The metal (II) salts reacted with the erythromycin and p.toluidine in 1:1:2 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 erythromycin.p.toluidine ligand and the metal (II) complexes were both soluble in acetone and DMSO. The ligand is soluble in ethanol, chloroform and diethyl ether at room temperature but very soluble in them at elevated temperature. The Fe(II) and Co(II) complexes are slightly soluble in ethanol at both room temperature and elevated temperature (Yahaya *et al*., 2018).The physical properties of the complexes are shown in **Table 1**. All the complexes synthesized are coloured ranging from cream, dark green, yellow ochre, grey ash, light green and pale green. This is typical of transition metal complexes.

A. Infrared Spectral

The FT-IR spectral of the erythromycin.p.toluidine 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 1650 cm^1 shifted to lower (5-15 cm^{-1}) and the v(C=O) stretching at 1573 cm^{-1} shifted to higher (30- 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 490-494 and 540-599 cm^{-1} were assigned to metal – oxygen (M-O) and metal –nitrogen (M-N) respectively.

V. 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 $Mn(HL)Cl_2$, $Co(HL)Cl_2$, $Cu(HL)Cl_2$, $Zn(HL)Cl_2$ and $Fe(HL)Cl_2$ complex. The bathochromic shift observed was attributed to complexation of the ligand to the central metal (Ogunniran *et al*., 2008).

VI. Antimicrobial Screening

The antibacterial activity of the synthesized complexes were investigatedagainst 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 the largest zonesof inhibition against *E. coli* and *K. pneumoniae* more than the control drug.Cu(II) complex produced the largestantibacterial activity against *salmonella typhi, S. aureus and E.coli.* Co(II) complex also showed higher inhibition zones for *salmonella typhi*. The Zn(II) complex also showed a very goodt antibacterial activity against *E.coli.* more than the control drug. Most of the complexes were more potent against these pathogens as compared to the parent drug. 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.* Cu(II) and Mn(II) complexes of the mixed ligand displayed an excellent zone of inhibition of 23.78 mm and 24.88 mm respectively against *Candida albicans* as compared to the fungusol (control) 17.48 mm. Also Fe(II) and Mn(II) complexes of the mixed ligand showed higher zone of inhibition of 12.87 mm and 14.70 mm respectively against *Aspergillus niger*.

Table I: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	$C_{51}H_{85}N_3O_{13}$	Cream	190	75	24.0
$Fe(HL)Cl_2$	[Fe $C_{51}H_{85}N_3O_{13}Cl_2$]	Dark green	230	79	17.2
$Mn(HL)Cl_2$	[Mn $C_{51}H_{85}N_3O_{13}Cl_2$]	Yellow ochre	200	76	20
$Co(HL)Cl_2$	[Co($C_{51}H_{85}N_3O_{13}Cl_2$]	Grey ash	220	72	15.3
$Cu(HL)Cl_2$	[Cu($C_{51}H_{85}N_3O_{13}Cl_2$]	Light green	230	75	13.2
$Zn(HL)SO_4$	[Zn($C_{51}H_{85}N_3O_{13}SO_4$]	Pale green.	220	72	16.4

HL = erythromycin-p-toluidine

Table II. 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	SS .S		S	S	S	S	SS	S	S	S	S	S
$Fe(HL)Cl_2$	NS	SS	SS	SS	SSS		SS	SS	S	S	S	S
$Mn(HL)Cl_2$	SS	S	S	S	S	S	S	S	S	S	S	S
$Co(HL)Cl_2$	NS	NS	NS	NS	SS	SS	S	S	S	S	S	S
$Cu(HL)Cl_2$	NS	SS	NS	SS	S	S	S	S	S	S	S	S
$Zn(HL)SO_4$	NS	SS	NS	SS	S	S	S	S	S	S	S	S

S – Soluble, SS – slightly soluble, NS – Not Soluble, C – Room Temp. H – Elevated Temp. DMSO = Dimethylsulphoxide

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

Compound	V(O-H)	V(C=N)	V(C=C)	V (M-O)	V(M-N)
				-	-
HL	3203.50 w	1650.09 S	1537.83 m		
$Fe(HL)Cl_2$	3224.55 br	1643.13 S	1580.11 S	492 m	550 m
$Mn(HL)Cl_2$	3348.05 br	1635.30 S	1560.75 S	494 m	540 m
$Co(HL_2Cl_2$	3376.09 br	1644.20 S	1579.73 S	491 m	550 m
$Cu(HL)Cl_2$	3418.08 br	1636.00 S	1513.56 S	494 m	599 m
$Zn(HL)SO_4$	3437.85 br	1645.57 S	1610.26 S	490 m	548 m

HL = erythromycin-p-toluidine

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

Compound	Abs	Wave number(cm^{-1})	€max (L.mol^{-1}.cm^{-1})	λ max (nm)
HL	1.194	28571	1194	350
$Fe(HL)Cl_2$	1.913	22220	542	450
$Mn(HL)Cl_2$	1.247	22220	1247	450
$Co(HL)Cl_2$	1.263	22220	1263	450
$Cu(HL)_2Cl_2$	2.626	22220	2626	450
$Zn(HL)SO_4$	1.252	22220	1252	450

HL = erythromycin-p-toluidine

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

Compounds	*Staphylococcus aureus*	*E. coli*	*Klebsiella pnemonia*	*Salmonella typhi*
$Mn(HL)Cl_2$	15.75	28.78	27.76	12.69
$Fe(HL)Cl_2$	7.77	12.73	11.06	13.24
$Cu(HL)Cl_2$	12.29	20.24	15.08	26.37
$Zn(HL)SO_4$	7.67	20.60	20.16	16.57
$Co(HL)Cl_2$	10.93	20.51	12.41	25.18
Erythromycin (Control)	21.28	19.98	26.55	23.28

Less than 9 weak, 10 – 16 moderate and greater than 17 significant

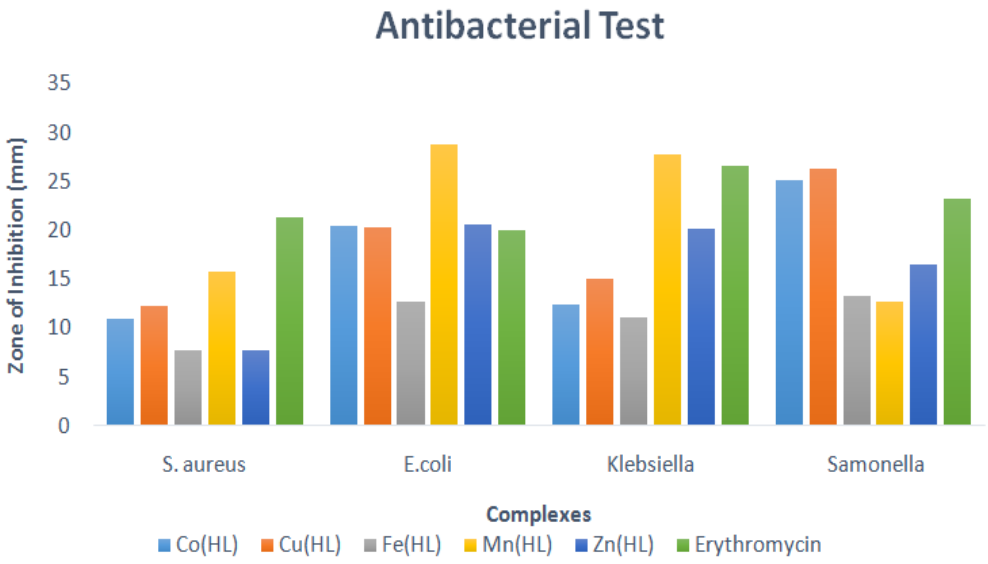


Fig 1: Graph of Antibacterial Test

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

Compounds	*Candida Albicans*	*Aspergillus niger*
$Mn(HL)Cl_2$	24.88	17.29
$Fe(HL)Cl_2$	16.79	12.89
$Cu(HL)Cl_2$	23.78	8.12
$Zn(HL)SO_4$	17.86	9.51
$Co(HL)Cl_2$	9.68	9.90
Fungusol (Control)	17.48	12.02

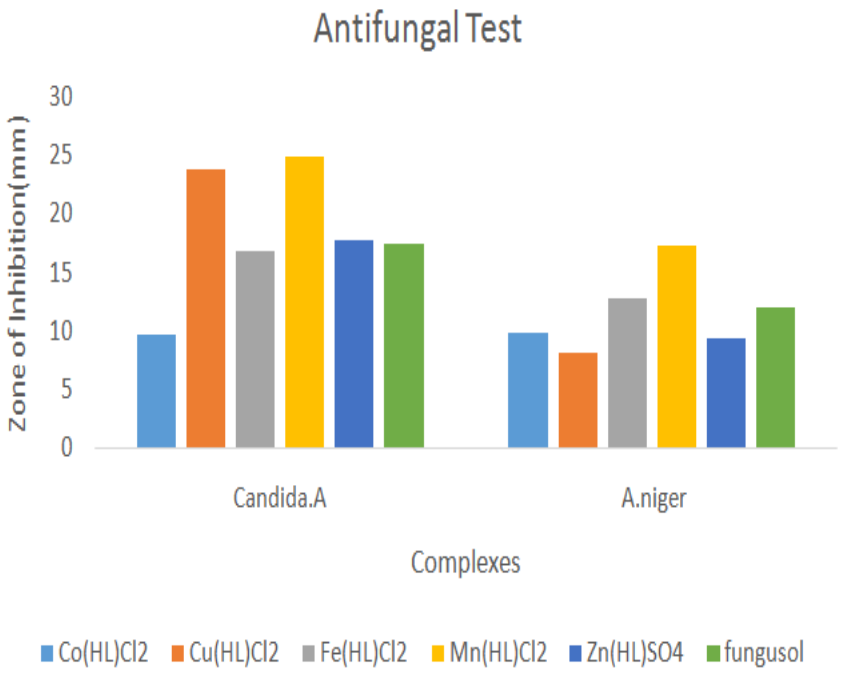
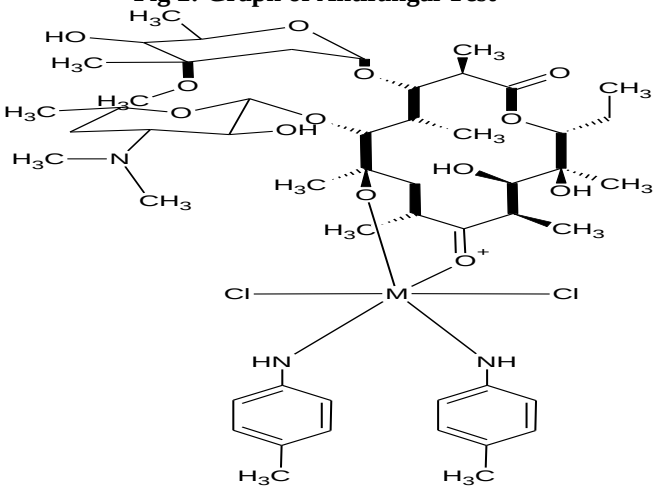


Fig 2: Graph of Antifungal Test



M = [Cu(II), Fe(II), Mn(II) , Co(II) and $ZnSO_4$]

Fig 3: Proposed structure of Erythromycin.p.toluidine (HL) with Metal II ion

VII. CONCLUSION

This research work entailed the synthesis and spectroscopic characterization of series of iron (II), Manganese (II), Copper (II), Zinc (II) and Cobalt (II) complexes with erythromycin and 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 Mn(II), Co(II), Zn(II) and Cu(II) complexes. Decrease activity was observed against Fe(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.

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