Synthesis, characterization and antimicrobial evaluations of mixed ligand complexes of sulfamethoxazole and metronidazole with some transition metals (Co, Cu and Ni) in water methanol medium

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ABSTRACT

Sulfamethoxazole and metronidazole are antibiotics use for the treatment of various bacterial infections. Their use as ligand is very prominent in formation of metal complexes. The transition metal complexes are synthesized by reaction of Sulfamethoxazole and metronidazole with metals such Mn(II), Cu(II), Fe(II) and Ni(II). The synthesized metal complexes are tested as antibacterial and antifungal. The antimicrobial activity of the complexes displays good potency against some microorganism such as *Xanthomonas axonopodis, Streptococcus faecalia, Salmonella entrica, Claribacter michiganense, Xanthomonas phaseolin* for bacteria and *S.roofisii, M.phonoides, C.lindimuthianum* for the fungi, it is revealed that all copper complexes show stronger antibacterial activity than the free drugs. The spectroscopic properties of the complexes were investigated using UV/visible and FT-IR which show metal-charge from 3d to 3s transition in which the transition state shows that they are octahedral geometry and their coordination site respectively. Their percentage yield was moderately high and producible. The complexes synthesized have higher inhibitory activities than the free ligand. The drug resistance in microbes is resulting in the incompetence of available drugs to care for the infections. The thermal analysis shows that the complexes are stable.

Keyword*; Sulfamethoxazole, Metronidazole, Antimicrobial, Metal complexes and Spectroscopic study*

1.0 Introduction

Resistance to antimicrobial have become major challenges in our modern world which leads to extensive research on an innovative drug. The development of rapid diagnostics, vaccines and new antimicrobial agents, including alternative therapeutics (such as bacteriophages, monoclonal antibodies, virulence factor modulating products) need to be incentivized [1]. Many researchers are now focusing on the synthesis of a new metal based compound that are less toxic with high biological activity which involves coordinating ligands with metal ions that induce high antimicrobial activity [2],[3] . Increasing attention has been attracted by chemistry of metal complexes with heterocyclic compounds contain nitrogen, sulfur and oxygen as a ligand. The heterocyclic compounds exhibit antimicrobial, herbicidal and insecticidal [4]. Sulfamethoxazole (SMX), a sulfonamide drug is a structural analogue of p-aminobenzoic acid that inhibits the synthesis of intermediary dihydrofolic acid from its precursors [5]. Heterocyclic and their derivatives have an excellent broad spectrum of biological activities as reported by some literatures [6], [7]. A lot of literatures shows that many works has been using Sulfamethoxazole [8], [9]. The administration of sulfamethoxazole for the treatment of urinary infection is the main features that leads to the discovery of sulfamethoxazole complexes. It was able to interact with many biologically active metal ions and remained intact in human blood [10], [11]. Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is an antibacterial agent of a biological active molecule [12, [13]. Metronidazole has generally been used for the treatment of intra-abdominal infection, meningitis infection, urinary infections e.t.c. [14]. Copper (II) complexes of metronidazole and 1,10 phenanthroline show a great potent antimicrobial and antifungal by interacting with electron-donor and electron-withdrawing substituent of copper(II) complexes. [15], [16] is one of the latest research that used Metronidazole as a ligand. However, the use of both metronidazole and Sulfamethoxazole as a ligand has not been done individually. The aim of this research is to synthesized metal complexes using both Metronidazole and Sulfamethoxazole the broad spectrum of the antimicrobial of the metal complexes and characterization explained here.

2.0 Materials and Method

Metronidazole and Sulfamethoxazole was obtained as gifts from Ecomed Pharmaceuticals Industry, Ogun State Nigeria. Copper (II) chloride dehydrated salt ($CuCl_2.2H_2O$), Cobalt (II) chloride dehydrated salt ($CoCl_2.6H_2O$), ($C_{10}H_{11}N_3O_3S$), methanol (CH_3OH), sodium hydroxide (NaOH), ethanol (C_2H_5OH), n-hexane (C_6H_{14}), Chloroform (CCI_4), acetone (CH_3COCH_3), Dimethylsulphoxide, hydrochloric acid (HCl), Silver nitrate (8% $AgNO_3$), Barium chloride (5% BaCl), Hydrochloric acid, are obtained from Aldrich chemicals while the solvents are in analytical grade.

2.1 Physical Measurement and Characterization

Melting points of the complexes were determined using gallenkamp melting point apparatus with 300^0C capacity thermometer, Infrared spectra were recorded as KBr disc on a Perkin-Elmer FT-IR BX II spectrometer. The absorbance of the complexes was also determined using UV/Visible.

2.2 Synthesis of Metal Complexes of [M(SMX)(MTZ)X].nH$_2$O X=SO$_4$ or Cl$_2$

The mixed ligand complexes were synthesized using direct stirring method. 1.0g (0.005856mol) of metronidazole, 0.74g (0.002928mol) of sulfamethoxazole, and equimolar amount of the Metal (II) salt was mixed into 10ml of water-methanol each and stirred for 2-3 hours on the magnetic stirrer and sieved using filter paper with sorption pump, the residue was then put in the desiccation for 5days to dry and the residue was weighed and kept inside a sample bottle. The method was used for the synthesis of the metal Complexes with ratio proportion of the ligands and the same procedure was used for the preparation of Co (II), Ni(II) and Cu(II).

2.3 Antimicrobial Activity

The screening of the antimicrobial activity were standard pathogenic strains gotten from the culture collection unit of the Department of Microbiology, Ondo State University Teaching hospital, Akure, Nigeria, in which the tests were carried out at the Department of Crops and Protection, Federal University of Technology, Akure, Nigeria. The selected fungi of choice used for this experiment are *Phythophthora megacarrier Fisarium vasinfectum* and *Collectotrichum nigrium*. Poisoned food techniques of Shukia *et al.*, 2008 was employed for this investigation. 5ml of reconstituted compound containing 0.025g/ml concentration ere aseptically mixed with 20ml of sterile molten potato dextrose agar (P.D.A). autoclaved at 121^{o}C for 15 minutes. This was allowed to cool to about 45^{o}C before incorporated and pour phifer. 48hrs old pure culture of each of the isolates were inoculated at the center of the P.D.A plates with the aid of 4mm cork-borer and sterile inoculating needle. Mancozeb, a standard antifungal agent was used as a positive control at 0.25g/ml concentration. A negative control plates (NTR) without any treatment were also set up. All the set of plates were incubated at 27^{o}C for 72hrs. Mycelia growth were measured with the aid of Vernier calipers. Mycelia growth inhibition were calculated in percentage using the formular.

$$\frac{NTR - TR}{NTR} X \frac{100}{1}$$

Where NTR=Average diameter of fungal colony in negative control sets (plates without any treatment and TR= Average diameter of fungal colony in treated sets

2.4 The Antibacterial

The newly synthesized complexes is test against *Streptococcus faecalia, Xanthomonas axonopodis Salmonella entrica, Claribacter michiganense and Xanthomonas phaseoli.* The method used to evaluate the antimicrobial activity was 'Broth Dilution Method'. It is one of the non-automated in vitro susceptibility tests. Serial dilutions were prepared in primary and secondary screening [17]. The control tube containing no antibiotic is immediately sub-cultured by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The MIC (minimal inhibitory concentration) of the control organism is read to check the accuracy of the drug concentrations [18]. The lowest concentration inhibiting growth of the organism is recorded as the MIC.

3.0 Results and Discussion

Table 1. Physical properties of the complexes and Ligands

S/n	Complexes	Ratio	Melting point	Color	%Yield
1	$[Co(SMX)(MTZ)Cl_2].2H_2O$	1:1:1	220	Pale pink	22.39
2	$[Co(SMX)(MTZ)Cl_2].2H_2O$	1:2:1	220-225	Pale pink	38.02
3	$[Cu(SMX)(MTZ)Cl_2].2H_2O$	1:1:1	290	Purple	23.98
4	$[Cu(SMX)(MTZ)Cl_2].2H_2O$	1:2:1	290	Purple	33.03
5	$[Ni(SMX)(MTZ)Cl_2].2H_2O$	1:1:1	300	Green	5.21
6	$[Ni(SMX)(MTZ)Cl_2].2H_2O$	1:2:1	300	Green	23.63
7	SMX	-	169	White	-
8	MTZ	-	159-161	White	-

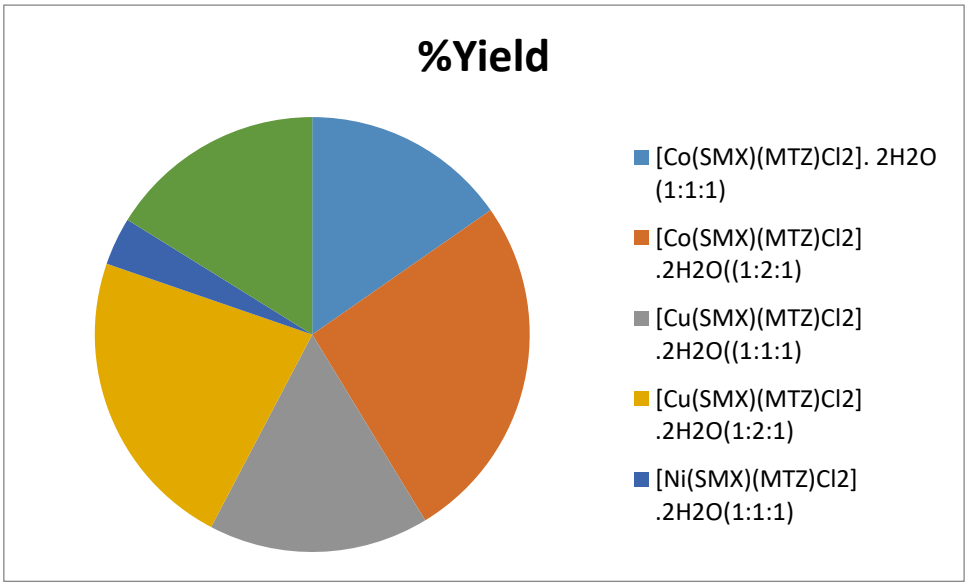

Figure 1: Chart showing the % yields of the metal complexes

Table 2: Solubility of the complexes

S/n	Complexes	Ratio	Methanol	Ethanol	Distilled water	Acetone	n-Hexane	Chloroform
1	$[Co(SMX)(MTZ)Cl_2].2H_2O$	1:1:1	IS	SS	IS	SS	SS	SS
2	$[Co(SMX)(MTZ)Cl_2].2H_2O$	1:2:1	IS	SS	IS	SS	SS	SS
3	$[Cu(SMX)(MTZ)Cl_2].2H_2O$	1:1:1	SS	IS	IS	IS	S	SS
4	$[Cu(SMX)(MTZ)Cl_2].2H_2O$	1:2:1	SS	SS	IS	IS	SS	IS

5	[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:1:1	SS	IS	IS	SS	IS	IS
6	[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	SS	SS	S	SS	IS	IS

IS=Insoluble, **SS**=slightly soluble S=Soluble

3.1 Antimicrobial Studies.

Table 3: Antibacterial activities of complexes

COMPLEX	RATIO	*Xanthomonas axonopodis*	*Streptococcus faecalia*	*Salmonella entrica*	*Claribacter michiganense*	*Xanthomonas phaseoli*
[Co(SMX)(MTZ)Cl_2]. 2H_2O	1:1:1	24	26	25	20	29
[Co(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	32	38	38	32	32
[Cu(SMX)(MTZ)Cl_2] .2H_2O	1:1:1	28	24	20	20	20
[Cu(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	33	38	30	30	30
[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:1:1	28	23	18	24	19
[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	23	28	28	28	23
Ligand(metronidazole)		0	18	18	20	24

Table 4: Antifungal activities of complexes

COMPLEX	RATIO	*S.roofisii*	*M.phonoides*	*C.lindimuthianum*
[Co(SMX)(MTZ)Cl_2]. 2H_2O	1:1:1	100	100	100
[Co(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	100	100	100
[Cu(SMX)(MTZ)Cl_2] .2H_2O	1:1:1	100	57.32	47.56
[Cu(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	100	82.93	100
[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:1:1	100	88.55	100
[Ni(SMX)(MTZ)Cl_2] .2H_2O	1:2:1	100	87.78	100
Control.Kocides		100	100	100

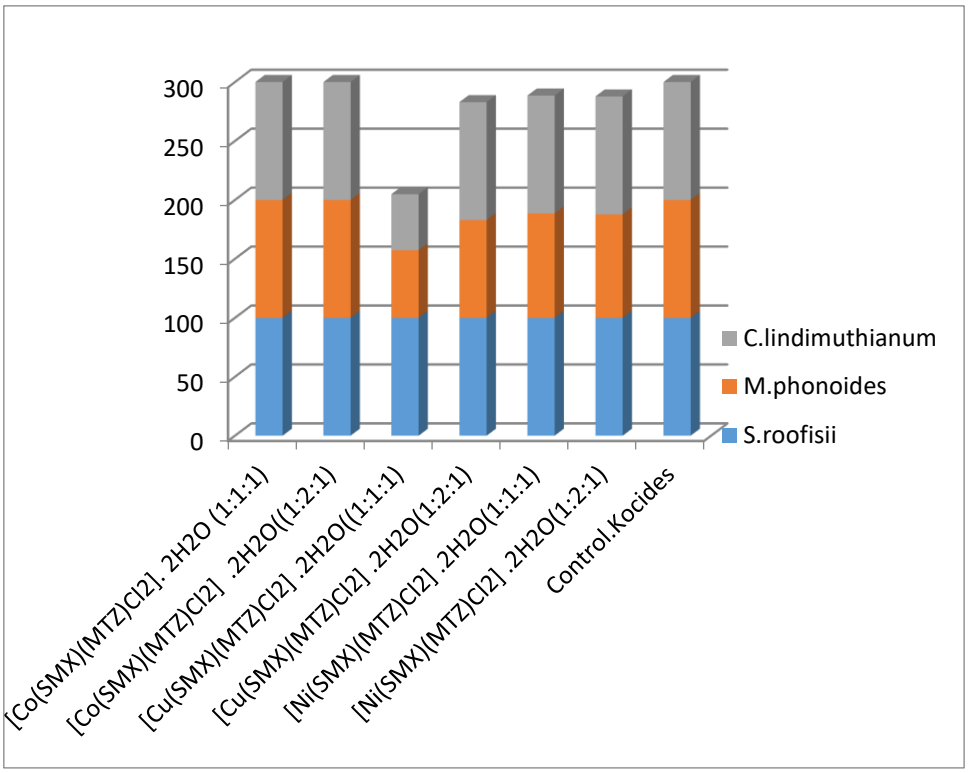

Figure 1 Chart showing the antifungal activities of the complexes

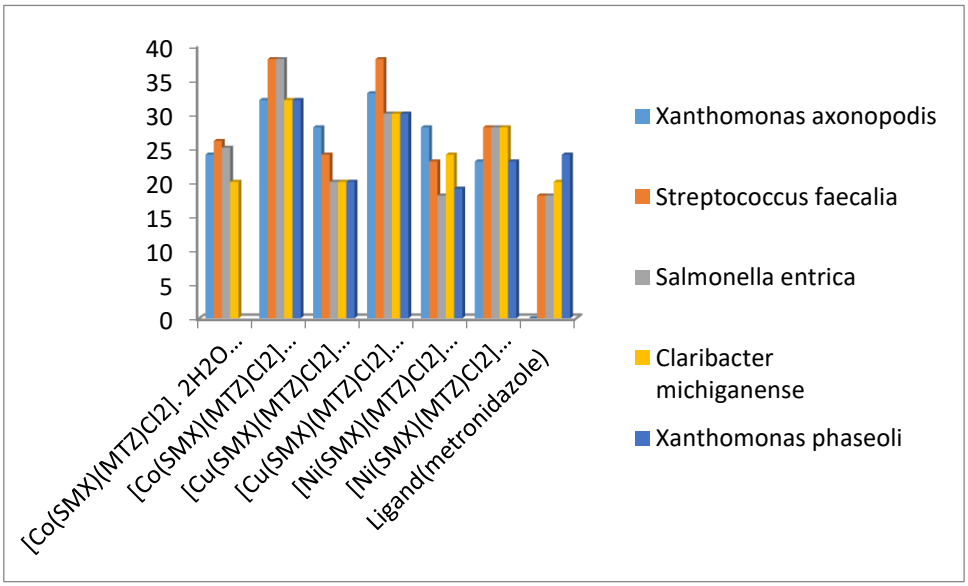

Figure 2: Chart showing the antifungal activities of the complexes

Antibacterial Activities of the compounds were screened against *Xanthomonas axonopodis, Streptococcus faecalia, Salmonella entrica, Claribacter michiganense, Xanthomonas phaseoli*, $[Co(SMX)(MTZ)Cl_2].2H_2O$ and $[Cu(SMX)(MTZ)Cl_2].2H_2O$ of ratio 1:2:1 show great potent against the bacteria. The increase in the antimicrobial is due to the chelation in which the polarity of the metal atoms is reduced favoring the permeation through lipid layers of the bacterial membrane. They are more potent than the ligands used. The Antifungal activity of the compounds were screened against: *S.roofisii, M.phonoides, C.lindimuthianum.*, the biological activity of metal complex increases on complexation. The complexes have greater inhibitory activities than the free ligand. The improvement of the activity of ligand

and complexation can be explained by overtone concept and chelation theory. Copper complexes of 1:1:1 Show less biological activity on *M. phonoides* and *C. lindimuthianum.*

3.2 Infrared Spectra

The table below show the assignment of peaks of the parent ligand (SMX) and (MTZ) and their respective metal complexes with the assistance of FT-IR, the bonding of the metals are investigated. As illustrated by the structure, SMX may act as a bidentate or tridentate because it is a positional ligand [19]. The $[Co(SMX)(MTZ)Cl_2].2H_2O$ of 1:1:1 shifted from 3710 of MTZ to 3836 indicated the coordination of the amino group nitrogen to the metal(II) without deprotonation [20]. The coordination of the compound for $[Co(SMX)(MTZ)Cl_2].2H_2O$ shows that it coordinates with H_2O which is absent for the remaining complexes. The band for asymmetric of sulfonyl group (1309-1385) shift toward higher frequencies while a shift toward lower frequency shows a small band. v(M-O), v(M-Cl) and v(M-N) were observed in the range and absent in the spectral of MTZ and SMX show the support for complexation of metal ion with O and N atom. [21], [22]. The SMX peak at 1613 is assigned to(C=N) stretching vibration, which showed large shift in the metal complexes spectral shows its participation in the coordination with the metal ions.

Table 5: Peaks of the Parent ligand and their respective metal complexes

COMPLEXES	Ratio	v(OH)	v(NH)	v(NH SULFAMET)	v(C=N)	v(S=O)	v(C=C)	v(M-N)	v(M-O)	v(M-Cl)
SMX			3465	3296	1613	1309	-	-	-	-
MTZ		3710		3226						
$[Co(SMX)(MTZ)Cl_2]$. $2H_2O$	1:1:1	3836	3230	-	-	1372	1438	558	423	380
$[Co(SMX)(MTZ)Cl_2]$.$2H_2O$	1:2:1	3922	-	-	1601	1250	1596	597	401	352
$[Cu(SMX)(MTZ)Cl_2]$.$2H_2O$	1:1:1	-	3562	3207	1773	1365	1491	522	404	358
$[Cu(SMX)(MTZ)Cl_2]$.$2H_2O$	1:2:1	-	3446	3213	1622	1368	1433	567	449	391
$[Ni(SMX)(MTZ)Cl_2]$.$2H_2O$	1:1:1	-	3443		1615	1263	1470	562	436	386
$[Ni(SMX)(MTZ)Cl_2]$.$2H_2O$	1:2:1	-	3454	3275	1616	1371	1536	556	419	354

3.3 Electronic Spectra

Table 6: Ultraviolet-visible analysis of the complexes and ligand.

Complexes	Ratio	Wavelength(nm)	Wavelength(cm^{-1})
SMX		267	**37453**
MTZ		330	**33003**
[Co(SMX)(MTZ)Cl_2]. $2H_2O$	1:1:1	307	32573
		296	33783
		251	39840
[Co(SMX)(MTZ)Cl_2] .$2H_2O$	1:2:1	296	33783
		250	40000
[Cu(SMX)(MTZ)Cl_2] .$2H_2O$	1:1:1	303	33003
		296	33733
		250	40000
[Cu(SMX)(MTZ)Cl_2] .$2H_2O$	1:2:1	327	30581
		307	32573
		296	33783
[Ni(SMX)(MTZ)Cl_2] .$2H_2O$	1:1:1	306	40160
		296	33783
		250	40000
[Ni(SMX)(MTZ)Cl_2] .$2H_2O$	1:2:1	307	32573
		296	33783
		249	40160

The Ultraviolent spectral of the ligand (SMX) and (MTZ) were characterized by $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively. There is a close absorption spectra of the ligand to the metal complexes [23]. The complexes transition consists of excitation of an electron from a non-bonding or bonding $\pi \rightarrow \pi^*$ to an unfilled molecule orbital. The UV-region around 240-280nm is a feature of a metal charge transfer, [24] and allocated to the d-orbital. The $d \rightarrow \pi^*$ of the donor ligand and inter ligands are moved to the d orbital of the meta $ln \rightarrow \pi$ transition [25]. The effective coordination of the ligands is established by the shift of the bands to higher wavelength in the metal complexes. d- d transition experiential in the metal ions which could be d_{xz} or $d_{yz} \rightarrow d_{x^2-y^2}$ electronic transition can be ascribed to the transition from ultraviolent region to visible region [26]. Their electronic spectral falls into the range of octahedral geometry.

4.0 Conclusion

Co(II), Cu(II) and Ni(II) Metal complexes were synthesized by reacting corresponding metal salt with mixed ligand [SMX]and [MTZ]. They were characterized by UV-visible and IR. The complexes showed great yield. The result showed that they are octahedral geometry which pose a very antimicrobial activity than their parent ligand**.**

5.0 Acknowledgement

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