SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES ON Mn(II), Ni(II) AND Co(II) COMPLEXES OF AMINO ACID-BASED LIGAND

ABSTRACT

Amino acid ligand derived from the condensation reaction of histidine and acetophenone and its Mn(II), Ni(II) and Co(II) complexes were characterized by solubility test, melting point/decomposition temperature, elemental analysis, UV-visible spectroscopy, molar conductance, FTIR spectroscopy and magnetic susceptibility. Elemental analysis indicated 1:2 metal:ligand ratio. Percentage yield of the ligand is 62.84% with a melting point of 188ºC. The percentage yields of the complexes were 89.98%, 57.64% and 81.82% for Mn(II), Ni(II) and Co(II) complexes respectively. The decomposition temperatures of the complexes were in the range of 208-222ºC, low molar conductance values of the complexes indicated their non-electrolytic nature. The values of the magnetic moments (5.9BM, 4.0BM and 4.3BM) showed that they are paramagnetic. The ligand and its complexes were tested against bacterial isolates; S. aureus & S. typhi and fungal isolates; C. albicans and A. flavus, using the disc diffusion method. The results indicated that the ligand and its metal(II) complexes exhibited activity at different concentrations against all the species tested.

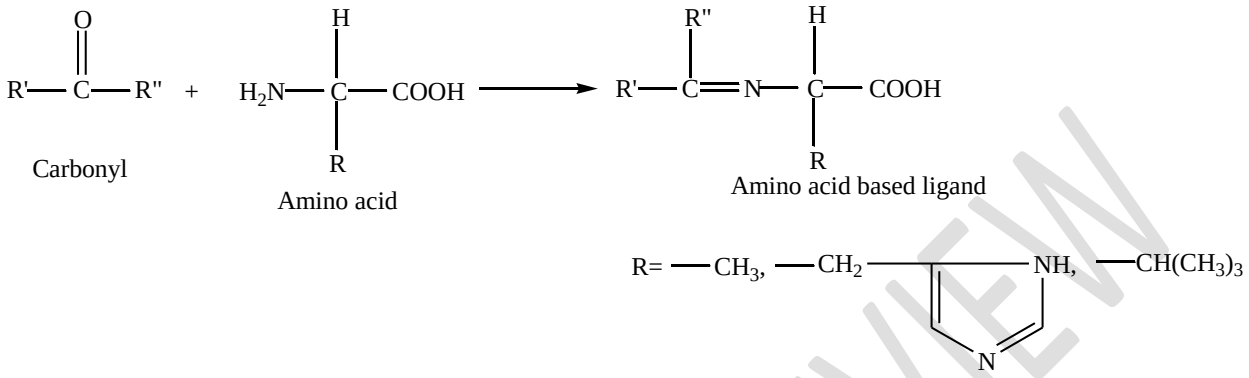
Keywords: Amino acid ligand, histidine, bacterial isolates, fungal isolates, antimicrobial activity.

INTRODUCTION

Amino acids are molecules containing an amine group, a carboxylic acid group and side chains, varying in different amino acids. Amino acids containing uncharged amino groups at physiological pH values undergo Schiff base formation which present another potential mechanism for metal complexes (Al-Jeborri *et al.,* 2014).

Much research has been conducted on the use of amino acids as ligands, and amino acids-based drugs have been found to be highly efficient due to their rigid structure. Coordination compounds of amino acids such as histidine (Lawal *et al.,* 2019; Nomiya *et al.,* 2000), arginine, glutamic acid (Legler *et al.,* 2001; See *et al.,* 1998), aspartic acid (Nomiya and Yokoyama, 2000; Hui *et al.,* 2007), phenylalanine (Aiyelabola *et al.,* 2012) and valine (chohan *et al.,* 2006) have been studied. The compounds were reported to demonstrate varying activities ranging from mild to significantly good.

Amino acid based ligands are ligands prepared from the condensation of an aldehyde or ketone with an amino acid under acid or base catalyst leading to the formation of an Imine (possessing the azomethine functional group, N=C)(Al-Salami *et al*., 2017).



Scheme 1: General preparation of amino acid based ligands

R' = CH_3, Ph R″= H or CH_3

Amino acid- based ligands have been utilized in various fields of science and engineering. They have been applied as antimicrobial agents against selected pathogens (Shaker *et al*., 2013; Abdel-Rahman *et al*., 2013), as anticancer agents (Li *et al*., 2013), as catalyst in oxidative addition reactions in organic chemistry and petrochemicals (Emara *et al*., 2014), and in hydrosilylation of ketones in the presence of catalyst (Liu *et al*., 2012)

There are large numbers of chemotherapeutic antimicrobials used in the treatment of various diseases. With the emergence of new strains of microbes resistant to such traditional antimicrobials, there is the need to intensify research in the development of newer drugs, to curb the menace of drug resistance. The search led to a new class of drugs called metallodrugs, where metals co-ordinate to an organic moiety (ligand). These drugs have created a niche for themselves in chemotherapy due to their high potency toward even the most persistent diseased condition such as cancer. This study reports the synthesis, characterization, and antimicrobial activity of the complexes of Mn(II), Co(II) and Ni(II) with acetophenone-histidine ligand against drug-resistant bacterial and fungal pathogens.

MATERIALS AND METHODS

All reagents and solvents used are of analytical grade and were used without any purification. Histidine and acetophenone were purchased from Sigma-Aldrich, Germany.

All glass wares were washed with detergent, rinsed with distilled water and dried in an oven at 110ºC before use. Weighing was carried out on an electric Mettler-Toledo balance model H30AR, melting point/decomposition temperatures were determined using Stuart SMP10 melting point apparatus. Molar conductance measurements were carried out in DMSO using Jenway conductivity meter model 4010T. Magnetic susceptibility measurements were conducted using magnetic susceptibility (Guoy) balance, model MK1. The infrared spectral analyses were recorded using FTIR **(**Agilent technology) instrument Model Cary 630, in the range of 4000 – 400cm^{-1}.Elemental microanalyses were carried out at OEA laboratories Devon, United Kingdom. Antibacterial and antifungal activity against selected isolates were conducted at the Microbiology Department of Bayero University Kano.

Preparation of the ligand

The histidine- acetophenone ligand was synthesized according to the modified method of Mir and Dildar (2016). 10mM of acetophenone (1.16cm^3) in 20cm^3 methanol with 10mM of histidine (1.55g) in 20cm^3water in the presence of 1cm^3 glacial acetic acid, was refluxed for four hours. The resultant crystals were filtered, washed with ethanol twice, then with diethylether, recrystallized and then dried in a desiccator to obtain an off white product (Mir and Dildar, 2016).

Synthesis of the metal(II) complexes

The metal (II) chlorides (0.0025mol) in 20cm^3were separately mixed with 0.005mol of the ligand in ethanol (20cm^3). The mixtures were refluxed with continuous stirring for 3 hours (Mir and Dildar, 2016). The products were cooled, filtered, washed with ethanol then with ether, recrystallized and dried in a desiccator.

Antibacterial Assay

The *in vitro* antibacterial test of the ligand and the metal(II) complexes against four bacterial isolates; *Staphylococcus aureus*, *Staphylococcus pyogens*, *Salmonella typhi* and *Escherichia coli* were studied by agar well diffusion method (Yushau and Sadisu, 2011). The suspension of each organism was smeared on the surface of solidified Muller-Hillon Agar (MHA) already poured into petridishes. The assay was evaluated using different concentration of the ligand and the metal(II) complexes. The stock solution was prepared by dissolving 0.008g of the ligand and metal(II) complexes in 1ml of DMSO which serves as solvent and thus yielded 80,000µg/ml. From the stook solution, three different concentrations (2000µg/ml, 1000µg/ml and 500µg/ml) were prepared through serial dilution, and placed on the nutrient agar before incubation at 37ºC for 24 hours (Bukar *et al.,* 2009). Antibacterial activities were determined by measuring (in mm) the diameter of zone of inhibition. The results were compared with standard drug, ciprofloxacin.

Antifungal Assay

The *in vitro* antifungal test of the ligand and its metal complexes were carried out on two fungal isolates; *Candida albicans* and *Aspergillus flavus* using agar well diffusion method (Yushau and Sadisu, 2011). The fungal suspension was smeared on the surface of solidified potato dextrose agar (PDA) already poured into petridishes. Three concentrations; 200µgml; 1000µg/ml and 500µg/ml) of the ligand and the metal complexes in DMSO were prepared through simple serial dilution and placed on the culture media. This was left to stand for 48 hours. Activities were determined by measuring (in mm) the diameter of zone of inhibition and the results were compared with standard drug, ketoconazole.

RESULTS AND DISCUSSION

Results

Results of the characterization, antibacterial and antifungal activity of the synthesized ligand and its metal(II) complexes are contained in the following tables.

Table 1: Physical Properties of ligand and its metal (II) complexes

Compound	Colour	Decomposition Temperature (ºC)	Melting point (ºC)	Percentage yield

Ligand (L)	Off White	-	188	62.84
$[MnL_2].2H_2O$	Brown	208		89.69
$[CoL_2].H_2O$	Grey	199		81.32
$[NiL_2].2H_2O$	Light blue	222		57.64

L = $C_{14}H_{15}N_3O_2$

Table 2: Solubility test on ligand and its metal(II) complexes

Solvent	**Ligand**	**$[MnL_2].2H_2O$**	**$[CoL_2].H_2O$**	**$[NiL_2].2H_2O$**
Water	SS	IS	IS	IS
Methanol	SS	IS	IS	IS
Ether	IS	S	S	S
DMSO	S	IS	IS	IS
Acetone	IS	S	S	S
n-hexane	IS	S	S	S

L = $C_{14}H_{15}N_3O_2$

Table 3: Conductivity measurement data of 0.003M metal complexes in Acetone

Compound	**Electrical conductivity ($\Omega^{-1}cm^{-1}$)×10^{-6}**	**Molar conductance ($\Omega^{-1}cm^2mol^{-1}$)**
$[MnL_2].2H_2O$	45.40	15.73
$[CoL_2]. H_2O$	27.00	9.0
$[NiL_2]. 2H_2O$	43.20	14.40

L = $C_{14}H_{15}N_3O_2$

Table 4: Magnetic properties of the metal(II) complexes

Compound	**Magnetic susceptibility (cm^3g^{-1})**	**Molar magnetic susceptibility**	**B.M**	**Property**

		(cm^3mol^{-1})		
$[MnL_2].2H_2O$	2.25×10^{-5}	1.48×10^{-2}	5.9	Paramagnetic
$[CoL_2].H_2O$	1.13×10^{-5}	7.72×10^{-3}	4.3	Paramagnetic
$[NiL_2].2H_2O$	1.024×10^{-6}	1.024×10^{-6}	4.0	Paramagnetic

L = $C_{14}H_{15}N_3O_2$

Table 5: FTIR spectral data for ligand and its metal(II) complexes

Compound	**ν(C=N)cm^{-1}**	**ν(M–N)cm^{-1}**	**ν(M–O)cm^{-1}**	**ν(OH)cm^{-1}**
Ligand	1633	–	–	3383
$[MnL_2].2H_2O$	1607	547	480	3402
$[CoL_2].H_2O$	1607	602	521	3408
$[NiL_2].2H_2O$	1577	573	561	3410

L = $C_{14}H_{15}N_3O_2$

Table 6: UV-visible spectral data for ligand and its metal(II) complexes

Compound	**λ(nm)**	**Transition**
Ligand	230	π –π*
	383	n –π*
$[MnL_2].2H_2O$	260	π –π*
	384	n –π*
$[CoL_2].H_2O$	231	π –π*
	383	n –π*
	497	
$[NiL_2].2H_2O$	231	π –π*
	383	n –π*

L = $C_{14}H_{15}N_3O_2$

Table 7: Microanalysis Data of the Schiff base and its metal(II) complexes

Compound	Observed (Calculated)		
	%C	%H	%N
Ligand	64.50 (65.35)	5.10 (5.87)	15.45 (16.33)
$[MnL_2].2H_2O$	53.90 (55.53)	4.15 (4.95)	13.15 (13.88)
$[CoL_2].H_2O$	54.70 (55.17)	4.03 (4.83)	13.90 (14.20)
$[NiL_2].2H_2O$	54.90 (55.20)	4.50 (4.96)	13.10 (13.80)

L = Ligand ($C_{14}H_{15}N_3O_2$)

Table 8: Antibacterial activity of the ligand and its metal (II) complexes against *S. aureus & S. Typhi*

Compounds	Zone of Inhibition (mm)				Standard Ciproflaxacin (500g) (mm)
	4000	2000	1000	500	
Ligand	18	14	10	06	28
$[MnL_2].2H_2O$	16	12	10	08	28
$[CoL_2].H_2O$	15	12	10	08	28
$[NiL_2].2H_2O$	21	18	14	11	28
Ligand	11	08	07	06	40
$[MnL_2].2H_2O$	14	12	10	07	40
$[CoL_2].H_2O$	15	13	10	08	40

$[NiL_2].2H_2O$	23	21	18	16	40

Table 9: Antifungal activity of the ligand and its metal (II) complexes

Isolates	Compounds	Zone of Inhibition (mm)			Standard Ketoconanole (200g)
		2000	1000	500	
A. flavus	Ligand	13	10	08	31
	$[MnL_2].2H_2O$	13	09	06	31
	$[CoL_2].H_2O$	11	09	06	31
	$[NiL_2].2H_2O$	15	12	10	31
C. albicans	Ligand	08	06	06	25
	$[MnL_2].2H_2O$	12	08	07	25
	$[CoL_2].H_2O$	12	10	07	25
	$[NiL_2].2H_2O$	12	10	06	25

Discussion

The ligand was synthesized by condensation reaction of acetophenone and histidine under reflux. The colour of the ligand was off-white which differed from the starting material, thus suggesting its formation. The melting point of the ligand was 188ºC, with percentage composition of 62.84%. The complexes of Mn(II), Co(II) and Ni(II) were synthesized and found to have different colours (Table 1), with percentages composition of 89.69%, 81.32% and 57.64% respectively. The decomposition temperatures of the complexes are relatively high, in the range of 208 – 222^{o}C, which is indicative of their stability, and could be due to formation of stable metal chelates. The low values of molar conductance observed, as presented in Table 2, are suggestive of the non-electrolytic nature of the complexes, which is in agreement with similar reports (Gufta *et al*., 2012)

The solubility of the ligand and its metal(II) complexes were carried out in water, methanol, ether, dimethylsulfoxide (DMSO), acetone and n-hexane. The ligand is slightly soluble in water, methanol (polar solvents) and DMSO, while the complexes were soluble in n-hexane, ether and acetone (Table 3).

Magnetic moment values (Table 4) of the complexes showed that the complexes of Mn(II), Co(II) and Ni(II)) are paramagnetic (Taghreed, 2016).The paramagnetic character increases with increased number of unpaired electrons. The observed magnetic moment for the complexes supported tetrahedral geometry.

The FTIR spectral data of the ligand and its complexes (Table 5) showed frequencies assignable to the ligand and its complexes due to interaction in the IR region. The IR spectra of the ligand showed a band in the region 1633cm^{-1} which is characteristic of the azomethine group (C=N) stretching frequency. In all the complexes there is a shift to higher (1607cm^{-1}) or lower (1577cm^{-1}) frequencies, indicating co-ordination of the azomethine nitrogen atom of the ligand to the metal. This is further supported by the appearance of new bands at 547, 602 and 573cm^{-1} due to ν(M – N) bond in the complexes. The bands at 480, 521 and 461cm^{-1}attributed to ν(M – O), support the involvement of the oxygen of the carboxylate (COO^{-}) group on the ligand in coordination with the metals (Yilmer, 2014). Both the ligand and the complexes showed the ν(OH) frequency between 3383-3410.

UV-visible spectra of the ligand showed two bands at 230 and 383 mm which are assigned to π - π* and n - π* transitions (Table 6) and attributed to the azomethine group and the benzene ring respectively. These bands were shifted in the Mn(II), Co(II) and Ni(II) complexes to, 260nm (π - π*): 384nm(n - π*), 236nm(π - π*): 383nm (n - π*): 497nm(CT) and 233nm(π - π*) : 383nm(n - π*) respectively.

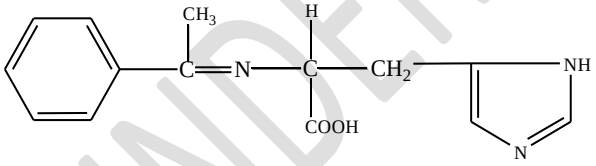
The percentage values of C, H and N observed (Table 7) from the microanalyses (Elemental analysis), are in close agreement with the calculated values and thus support metal: ligand ratio of 1:2.

The ligand and the metal complexes of Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) at concentrations of 2000µg/ml, 1000µg/ml and 500µg/ml were tested against two bacterial isolates; *S aureus* and *S. typhi* and two fungal isolates; *C. albicans* and *A. flavus.* For the evaluation of the antibacterial

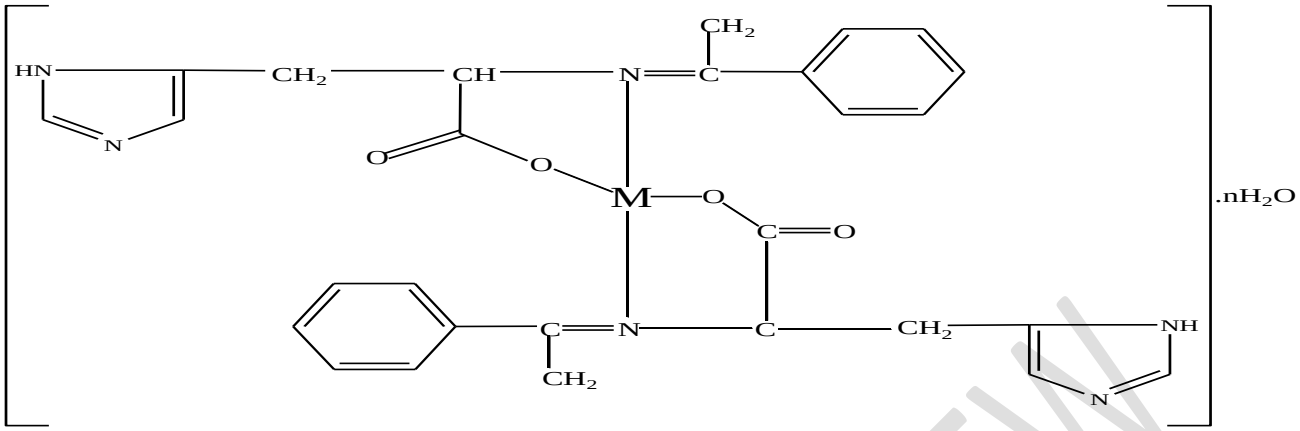
activity, ciprofloxacin was used as positive control and the diameter (in mm) of zone of inhibition was measured for each treatment (Table 8). Both the ligand and the complexes showed activity at high concentrations against all the isolates, but the activity was lower than that observed for the control, ciprofloxacin. The Ni(II) complex showed the highest activity at the highest concentration (2000g/ml) against *S. aureus* while, the Co(II) complex exhibited the highest activity at the same concentration. Generally, the activity is observed to increase with increased concentration of the samples, which is indicating the important role of concentration in the inhibition mechanism.

The antifungal activity of the ligand and its metal(II) complexes against the two fungal isolates: *C. albicans* and *A. flavus* was studied using 2000µg/ml, 1000µg/ml and 500µg/ml concentrations of the ligand and the complexes with ketoconazole as positive control. The ligand showed activity against *A. flavus* at all concentrations (even though the activity is lower than that observed for the control, ketoconazole), but it showed high activity against *C .albicans* at high concentrations. The highest activity was shown by the Ni(II) complex at all concentrations but, lower than the control. Generally, the activity increases with increased concentration of the ligand and complexes.

Based on the elemental analysis, FTIR spectroscopy, conductivity measurements, melting point/decomposition temperature determination, solubility test and literature review, the following structure is hereby proposed as the structure of the ligand and its metal (II) complexes.



Scheme 7: Proposed structure of the ligand



Scheme 8: Proposed structure of the metal (II) complexes

Where M = Mn(II), Co(II), Ni(II)

CONCLUSION

The ligand was synthesized by condensation of acetophenone and histidine. The complexes of Mn(II), Co(II), and Ni(II) were synthesized from the reaction of the ethanolic solution of the ligand and metal (II) chlorides. The conductivity data showed the complexes to be non-electrolytes. The decomposition temperatures of the complexes showed that they are stable. The ligand was slightly soluble in water, methanol, but very soluble in DMSO. The complexes, on the other hand, were soluble in acetone and n-hexane. The infra-red spectral data showed that the ligand is coordinated to the central metal ion in a bidentate way through the N atom of the azomethine (C=N), and through the O atom of the carboxylate (COO^-) group to form a tetrahedral geometry. The magnetic moment data indicated all the metal complexes to be paramagnetic. Elemental analysis data showed that the ligand: metal ratio is 2:1 in all the complexes. The ligand and complexes exhibited varying degrees of antimicrobial activities, though less than the control.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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