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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF LEUCINE-ACETYLACETONE SCHIFF BASE AND ITS Mn(II), Ni(II) AND Cu(II) COMPLEXES

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ABSTRACT

A Schiff-base derived from leucine and acetylacetone, and its Cu(II), Ni(II) and Mn(II) complexes were synthesized and analyzed based on their melting point/decomposition temperature, solubility, molar conductance, magnetic and infrared analyses. Physical and analytical data suggested that the Schiff base acted as tridentate ligand towards metal ion via azomethine-N, deprotonated-O of carboxylic group and enolic-O group of acetylacetone. All the complexes have lower molar conductance values ($6.4-17.7\Omega^{-1}cm^2mol^{-1}$), indicating that they are non-electrolyte. The magnetic moment values showed that all the metal ions in the complexes are paramagnetic. The synthesized ligand and its respective metal complexes were screened for their antibacterial activity against Staphylococcus aureus (Gram-positive), Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumonia (Gram-negative) and antifungal activity against Candida albicans, Fusarium solani and Aspergillus fumigatus. The results of these studies revealed that the compound (ligand) and its metal complexes showed significant antibacterial and antifungal strength at high concentrations.

Keywords: Schiff base, acetylacetone, leucine, antimicrobial activity, Synthesis

INTRODUCTION

Schiff bases are among the most important class of organic compounds used as ligand in coordination chemistry. They are the condensation products of primary amine and carbonyl (aldehydes or ketone) and have been extensively studied and used as ligand in the past decades (Alan *et al.*, 2005), nonetheless to date they continue to attract the attention of different researchers in the field of Inorganic and Organic chemistry (Tariq, 2010) due to their ease of preparation and to a larger extent due to the availability of huge number of primary amine and carbonyls which have not been exhausted. Though at the early stage, research on the Schiff base complexes was focused mainly on the characteristics of the chelation of ligands with metal complexes (Gehad *et al.*, 2009). Now the focus has been shifted to the bioactivities of the Schiff bases and their metal complexes (Raman *et al.*, 2004) to explore their potential use as therapeutics. This is due to the remarkable biological properties shown by the Schiff bases such as antibacterial, antifungal, anti-tumor, antiviral and various other biological effects (Abu-Dief*et al.*, 2015).

A number of amino acids Schiff bases have been synthesized and reported (Misbah *et al.*, 2013), however, amino-acetylacetone Schiff base have only being studied to a lesser extent. Due to established role of amino acids in biochemistry, its Schiff bases and metal complexes present a huge potential in biological activities. Some reported amino acids Schiff bases and their complexes have been found to have remarkable biological activities including antibacterial, antifungal, antitumor (Abu-Dief*et al.*, 2015).

We here report the synthesis of leucine and acetylacetone Schiff base and its complexes with Mn (II), Ni (II), and Cu (II) ion. The work also explore the antimicrobial activities of the Schiff base and the respective metal complexes.

MATERIALS AND METHODS

All chemicals and solvents used in this study were of analytical grade and were used without further purification. All weighing were carried out on Mettler balance model H_3OAR, melting points/Decomposition temperature were determined using Galenkemp melting point apparatus. Molar conductance measurements were carried out in DMSO using Jenway conductivity meter 4010 model. Fourier Transform Infrared (FT-IR) spectra recorded on Cary 630 FT-IR spectrophotometer in the $650-4000cm^{-1}$. Biological activity studies were carried out at the Microbiology Department, Bayero University Kano, Nigeria.

Preparation of the Schiff base

Acetylacetone (0.12 g, 1.2 mmol), leucine (1.0 mmol) and potassium hydroxide (0.056 g, 1.0 mmol) were taken in methanol (50mL) and reflux for 6hours. The volume of the obtained brownish red solution was reduced on water bath, filtered and then washed with cold absolute ethanol. Anhydrous diethyl ether was then used to wash the product which was dried in air for 2 hrs (Zahraa *et al*, 2012).

Preparation of the Schiff base complexes

The metal complexes of the Schiff base were prepared by the addition of a hot solution (60ºC) of the appropriate metal (II) chloride (1mmol) in an ethanol (25mL) to the hot solution (60ºC) of the Schiff base (2 mmol) in the same solvent (25mL). The resulting mixture was stirred under reflux for 3hrs where upon the complexes precipitated. They were collected by filtration, washed with ethanol, diethyl ether and dried in an oven at 110ºC (Gehad*et al.*, 2006).

Determination of Metal to Ligand ratio

The number of ligand coordinated to the metal ion was determined using Job's method of continuous variation (Angelici, 1971).

3mmol aqueous solution of the ligand and the metal chlorides were prepared. The following ligand to metal ratio (ml); 0:16, 1:15, 3:13, 5:11, 7:9, 9:7, 11:5, 13:3, 15:1 were taken from the ligand solution and each of the metal chloride solutions respectively. A total volume of 16ml was maintained throughout the process and the mole fraction of the ligand was calculated in each mixture. The solutions of the metal chlorides were scanned (as blank) to find the wavelength of maximum absorption (λ_{max}) for that particular metal ion (Angelici, 1971). The machine was fixed at λ_{max} before taking the absorbance values. The absorbance values were extrapolated against mole fraction of the ligand and the number of coordinated ligand (coordination number) was determined using the relation below:

$n = x_i/(1-x_i)$

Where n= number of coordinated ligand, and

x_i= mole fraction at maximum absorbance.

Antibacterial Activity Test

The ligand and complexes were dissolved separately in DMSO to have three different concentrations (250µg, 500µg and 1000µg) per disc. They were placed on the surface of the culture media (nutrient agar) and incubated at 37°C for 24hrs. Then in vitro antibacterial activity against *Staphylococcus aureus* (Gram-positive), *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Klebsiellapneumoniae (*Gram-negative)of this ligand and its complexes were carried out by disc diffusion method. The diameter of zone of inhibition produced by the ligand and complexes respectively were compared with the standard (Yusha'u and Salisu, 2011).

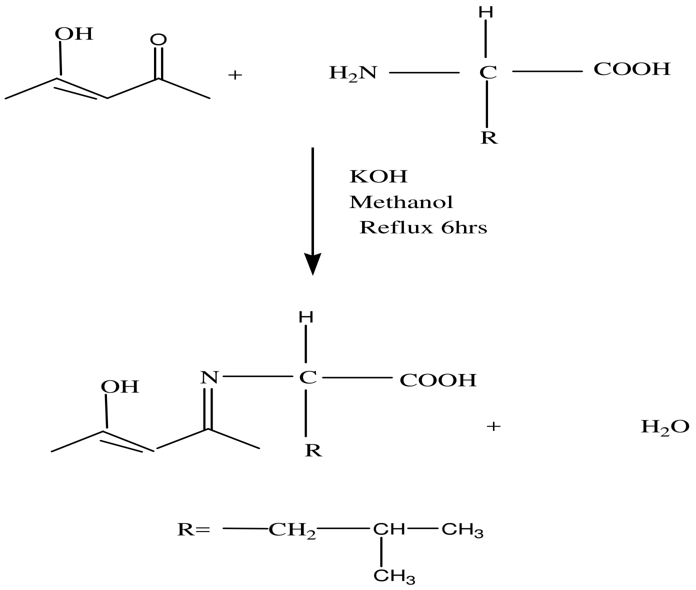
Antifungal Activity Test

The ligand and complexes were dissolved separately in DMSO to have three different concentrations (250µg, 500µg and 1000µg) per disc. They were placed on the surface of the culture media (sabouraud dextrose agar) and incubated at room temperature for 48hrs. Then in vitro antifungal activity against *Candida albicans, Fusariumsolani and Aspergillus fumigatus*of this ligand and complexes respectively were carried out by disc diffusion method. The diameter of zone of inhibition produced by the ligand and complexes respectively were compared with the standard (Hassan *et al.*, 2006).

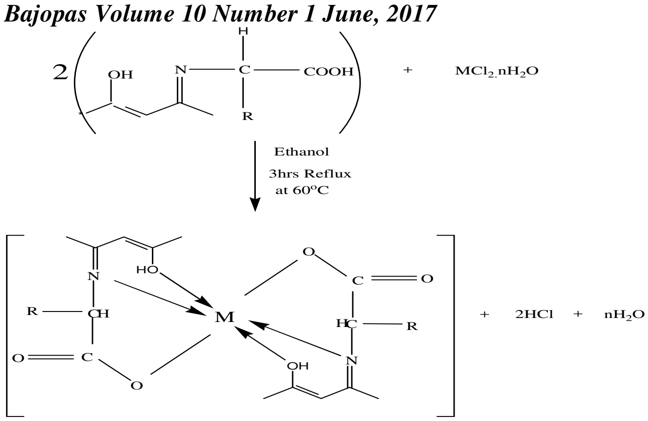
RESULTS AND DISCUSSION

Physicochemical studies

Condensation reaction between acetylacetone (ketone) and amino acid (Leucine) gave a brown powdery Schiff base (scheme 1), which was reacted with respective metal (II) chloride to give the corresponding metal (II) complexes (scheme 2). The ligand and the complexes were produced in high yield and of good stability. The ligand and complexes melted at 185°C and a range of 210 – 230°C respectively, this indicated that the complexes are more thermally stable than the ligand (Table 1).



Scheme 1: Schiff base formation.



Scheme 2: Schiff base-Metal complex formation.

Molar conductance measurements are carried out to establish the electrolytic status of complexes, and by extension to determine the ionization nature of the complexes which may indicate the presence or otherwise of ions outside coordination sphere. It was carried out in 10^{-3} molar DMSO solution at room temperature using a procedure reported by Geary, (1971). The value obtained (Table 1) were relatively low, within the range of6.4-17.7$\Omega^{-1}cm^2mol^{-1}$, implying that all the complexes were non electrolyte.

Table 1: Physical properties of Schiff bases and their metal (II) complexes

Compound	%Yield	Colour	M. P (°C)	Decom. Temp (°C)	Elect. Cond. ($\Omega^{-1}cm^{-1}$)	Molar Cond. ($\Omega^{-1}cm_2mol^{-1}$)	μeff (B.M)
$C_{11}H_{19}NO_3$	89.2	Brown	185	-			
$[Cu(C_{11}H_{18}NO_3)_2]$	71.72	Light blue	-	230	$6.4x10^{-6}$	6.4	2.12
$[Ni(C_{11}H_{18}NO_3)_2]$	73.71	Green	-	210	$11.2x10^{-6}$	11.2	3.05
$[Mn(C_{11}H_{18}NO_3)_2]$	80.58	Dark brown	-	220	$17.7x10^{-6}$	17.7	5.40

Table 1 present the room temperature magnetic susceptibility measurements results of the metal (II) complexes synthesized herein and the values indicated that all were paramagnetic in nature. The magnetic moment for Cu(II), Ni(II) and Mn(II) were 2.12, 3.05 and 5.40 respectively and are within range normally observed for octahedral complex of their respective ion as similar values were reported by Gehad*et al.*, (2006) and Nora, (2011).

The solubility test results of the Schiff base and the complexes are presented in Table 2. All were found to be completely soluble in some solvents, e.g methanol, ethanol, DMSO and water but insoluble in diethyl ether and n-hexane.

Table 2: Solubility test of Schiff bases and their metal (II) complexes

Solvent/Compound	$C_{11}H_{19}NO_3$	$[Cu(C_{11}H_{18}NO_3)_2]$	$[Ni(C_{11}H_{18}NO_3)_2]$	$[Mn(C_{11}H_{18}NO_3)_2]$
Water	S	S	S	S
Methanol	S	S	S	S
Ethanol	S	S	S	S
Diethylether	IS	IS	IS	IS
DMSO	S	S	S	S
n-hexane	IS	IS	IS	IS
DMF	IS	IS	IS	S
Chloroform	SS	S	SS	IS
Dichloromethane	IS	IS	IS	S
Acetonitrile	IS	SS	IS	SS
Acetone	IS	IS	IS	S
1,4-dioxane	SS	SS	SS	S
Nitrobenzene	SS	SS	SS	S

Key: S-Soluble SS- Slightly SolubleIS- Insoluble

IR spectral Studies

The bonding mode of ligand to metal in the complexes was studied by comparing the IR spectrum of free ligand with that of the complexes. The amine (NH_2) and carbonyl (CO) peak at 2957 and 1765cm^{-1} for the respective leucine and acetylacetone was found to be absent in their synthesized Schiff base however a new peak around 1657cm^{-1} assigned to the newly form azomethine bond (C=N) was observed, suggesting the formation of the Schiff base, similar result as reported by Byeong-Goo *et al*, (1996).

This band shifted in the complexes low frequencies1592-1618cm^{-1}as shown in Table 3. These relative shifts observed for v(C=N) bonds in the complexes showed the participation of azomethine nitrogen in coordination to the metal ions (Gehad*et al.*, 2006). Furthermore, the carboxylic acid (COOH) peak observed at 3458cm^{-1}in the Schiff base has shifted to 3243 – 3395cm^{-1} in the complexes suggesting also the coordination of carboxylate –O to the metal. Two absorption bands at 770-838cm^{-1} and 542-567cm^{-1}support the formation of M-N and M-O bonds. These data suggest that the azomethine –N, carboxylate –O and enolic –O groups were involved in the coordination with the metal (II) ions in the complexes.

Table 3: FTIR and Magnetic Susceptibity of metal (II) complexes

Solvent/Compound	**$C_{11}H_{19}NO_3$**	**$[Cu(C_{11}H_{18}NO_3)_2]$**	**$[Ni(C_{11}H_{18}NO_3)_2]$**	**$[Mn(C_{11}H_{18}NO_3)_2]$**
v(C=N)cm^{-1}	1657	1618	1618	1592
v(M-O)cm^{-1}	-	564	567	542
v(M-N)cm^{-1}	-	776	838	770
v(O-H)cm^{-1}	3458	3243	3245	3395
Xg(g^{-1})	-	3.85x10^{-6}	8.07x10^{-6}	25.5x10^{-6}
Xm(mol^{-1})	-	0.0019	0.0039	0.0122
μ_{eff}(B.M.)	-	2.12	3.05	5.40

The estimation of the ligand to metal ratio was carried out by Job's method of continuous variation. The plot of absorbance against mole fraction in each case at maximum absorbance corresponding to the ligand mole fraction suggest 1:2 metal-ligand ratio for all the complexes, indicating [M(L$_2$)] (M=Mn(II), Ni(II) and Cu(II), L= ligand) molecular formula, for the compounds.

Antimicrobial Studies

The antibacterial activity test for the Schiff base and the metal (II) complexes have been presented in Table 4. The diameter of the inhibition zones were measured and recorded for each treatment and it has been observed that the ligand (Schiff base) showed a weak activity against all the tested organisms at all concentrations. Cu (II) complexes have the highest activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumonia* while the highest activity against *Proteus mirabilis* was recorded by Ni (II) complex. Mn (II) complex was found to show least activity among the complexes towards the isolates, with activity in most cases similar to that of the free Schiff base, the only exception is on *Staphylococcus aureus.* In the entire tested organism, *Staphylococcus aureus* was found to be more prone to attack by the complexes as all recorded high activity. Even at low concentration, the activity was found to be higher than the control drug used in the test, suggesting potentially a good compound to be explored as antibacterial agent.

Table 4: Antibacterial activity of Schiff base and its metal (II) complexes

Test organism	**Compound**	**Zone of Inhibition(mm)/Concentration(µg/disc)**			**Control(mm) Ciproflaxacin (5µg/disc)**
		250	**500**	**1000**	
Escherichia coli	$C_{11}H_{19}NO_3$	07	07	08	25
	$[Cu(C_{11}H_{18}NO_3)_2]$	09	10	11	
	$[Ni(C_{11}H_{18}NO_3)_2]$	08	08	09	
	$[Mn(C_{11}H_{18}NO_3)_2]$	07	07	08	
Pseudomonas aeruginosa	$C_{11}H_{19}NO_3$	06	06	08	29
	$[Cu(C_{11}H_{18}NO_3)_2]$	12	13	14	
	$[Ni(C_{11}H_{18}NO_3)_2]$	06	08	10	
	$[Mn(C_{11}H_{18}NO_3)_2]$	06	06	10	
Staphylococcus aureus	$C_{11}H_{19}NO_3$	08	09	13	25
	$[Cu(C_{11}H_{18}NO_3)_2]$	42	48	52	
	$[Ni(C_{11}H_{18}NO_3)_2]$	36	46	48	
	$[Mn(C_{11}H_{18}NO_3)_2]$	22	35	50	
Proteus mirabilis	$C_{11}H_{19}NO_3$	06	08	10	25
	$[Cu(C_{11}H_{18}NO_3)_2]$	10	12	15	
	$[Ni(C_{11}H_{18}NO_3)_2]$	15	18	20	
	$[Mn(C_{11}H_{18}NO_3)_2]$	07	08	20	
Klebsiellapneumoniae	$C_{11}H_{19}NO_3$	06	06	06	06
	$[Cu(C_{11}H_{18}NO_3)_2]$	08	09	10	
	$[Ni(C_{11}H_{18}NO_3)_2]$	06	06	08	
	$[Mn(C_{11}H_{18}NO_3)_2]$	06	06	06	

Sensitivity of fungal isolates to the ligand and its respective metal (II) complexes (Table 5) indicated that Cu(II) and Ni(II) complexes exhibited high activity against all the isolates tested whileMn(II) complex maintain its trend of weak activity, similar to that of antibacterial assay. No significant difference was recorded between its activity and that of the free Schiff base.

Table 5: Antifungal activity of Schiff base and its metal (II) complexes

Test organism	**Compound**	**Zone of Inhibition(mm)/Concentration(μg/disc)**			**Control(mm) Mancozef (10mg/ml)**
		250	**500**	**1000**	
Candida albicans	$C_{11}H_{19}NO_3$	06	06	08	30
	$[Cu(C_{11}H_{18}NO_3)_2]$	10	15	18	
	$[Ni(C_{11}H_{18}NO_3)_2]$	06	08	08	
	$[Mn(C_{11}H_{18}NO_3)_2]$	06	08	08	
Fusariumsolani	$C_{11}H_{19}NO_3$	06	06	08	33
	$[Cu(C_{11}H_{18}NO_3)_2]$	08	10	12	
	$[Ni(C_{11}H_{18}NO_3)_2]$	08	10	12	
	$[Mn(C_{11}H_{18}NO_3)_2]$	06	08	10	
Aspergillus fumigatus	$C_{11}H_{19}NO_3$	06	06	08	25
	$[Cu(C_{11}H_{18}NO_3)_2]$	10	14	16	
	$[Ni(C_{11}H_{18}NO_3)_2]$	06	08	10	
	$[Mn(C_{11}H_{18}NO_3)_2]$	06	06	08	

In general, the antimicrobial data revealed that the activity of the ligand and its respective metal (II) complexes against the tested microorganisms increases with increase in concentrations. However, the compound activity in most of the organism were found to be lower than the standard antimicrobial used Ciproflaxacin and Mancozef for bacteria and fungi respectively, except activity on *Staphylococcus aureus* in which at low concentration the complexes were more active than the control.

CONCLUSION

Mn (II), Cu (II) and Ni (II) complexes with Schiff base derived from leucine and acetylacetone, acting as tridentate ligand was successfully synthesized. Conductivity studies revealed complexes neutrality and the magnetic susceptibility studies indicated that they are all paramagnetic. The IR and electronic studies confirmed that the metal (II) ions coordinated to azomethine-N, deprotonated-O of carboxylic group and enolic-O group of acetylacetone forming octahedral $[ML_2]$ type complexes. All its respective metal (II) complexes were evaluated in vitro against five bacteria (four Gram-negative, one Gram-positive) and three fungal strains. Both the ligand and its metal (II) complexes showed significant antibacterial and antifungal strength at high concentrations.

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