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STRUCTURAL, SPECTRAL AND ANTIMICROBIAL STUDIES OF COPPER(II) COMPLEX OF PYRROLIDIN-2-YLIDENE-2,(4-CHLOROPHENYL) SEMICARBAZONE

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ABSTRACT

Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone and its copper (II) complex were synthesized with aim to elucidating their structures and determining their antimicrobial activities. Melting point, magnetic susceptibility, conductivity measurement, XRD, GC-MS, UV-Vis., IR. Spectroscopic studies were used to characterize and propose structures of the synthesized compounds. Copper (II) complex showed effective magnetic moment of 1.83BM at room temperature. The ligand and its copper (II) complex are trigonal crystals with space groups of $p3_121$ and $p3_112$ and respective cell volumes of 130.907 and 701.505Å^3. GC-MS results revealed respective m/z values of 256.2 and 429.1 for the ligand and the complex. The ligand and its copper (II) were tested in vitro antibacterial activities against *S. aureus*, *E. coli*, *K. pneumonia*, *B. subtilis* and for antifungal activities againt *A. flavus* and *C. albicans.* Among organisms tested, copper (II) complex of the ligand proved to be a more potent antibacterial agent against *E. coli*, *K. pneumonia* and *B. subtilis*. The complex exhibited better antimicrobial activity than the free ligand.

KEYWORDS: Phenylsemicarbazone, pyrrolidone moiety, Metal complexes, Anti-bacterial activities, Anti-fungal activities.

1. INTRODUCTION

Co-ordination reduces the polarity of the metal ion by partial sharing of its positive charge with donor groups and possibly enhancing the pi-electron delocalization within the chelating ring (Wissner *et al*., 2000). This process thus increases the lipophilic nature of semicarbazone metal complexes, which in turn favours penetration through the membrane wall (Sulekh *et al*., 2003; Agarwal *et al.*, 2005). The co-ordination chemistry of semicarbazones appears to be very interesting from the point of view of both the number of metals forming the complexes with them and the diversity of the ligand system which include the macrocycle (Lima *et al.* 1999; Zahid *et al*., 2005). According to Tudor *et al*., (2007), the geometry of semicarbazone metal complexes are influenced by the nature of the ligand which determines a variation of charge density at the co-ordination site and by the nature of metal salt used in their preparation. The significance of semicarbazone metal complexes apart from their diverse chemical and structural characteristics, stem not only from their potential, but also from their proven applications as biologically active molecules with wide spectrum of activities (Leovac *et al*., 2005; Marina *et al*., 2007; Tudor *et al*., 2007). Many semicarbazones and thiosemicarbazones form stable coloured metal complexes, some of which have been proposed as analytical reagents (Martin *et al*., 2007). Semicarbazones which can also be regarded as urea derivatives, have gained considerable importance (Offiong and Martelli, 1994) in recent years in the design of enzyme inhibitors (Molavi, 2003), as replacement for the amide (–CO–NH–) bond in peptidomimetics (Omar *et al*., 2001) and as sources of self complementary bidirectional hydrogen bonding motif in supramolecular chemistry (Offiong and Martelli, 2006). Semicarbazones which contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Semicarbazones of aliphatic aldehydes are relatively unstable and readily polymerizable while those of aromatic aldehydes having effective conjugation are more stable (Bahl and Bahl, 2010). In general, aldehydes react faster than ketones in condensation reactions, leading to the formation of semicarbazones as the reaction centre of aldehyde are sterically less hindered than that of ketone (West *et al*., 1991). Furthermore, the extra carbon of ketone donates

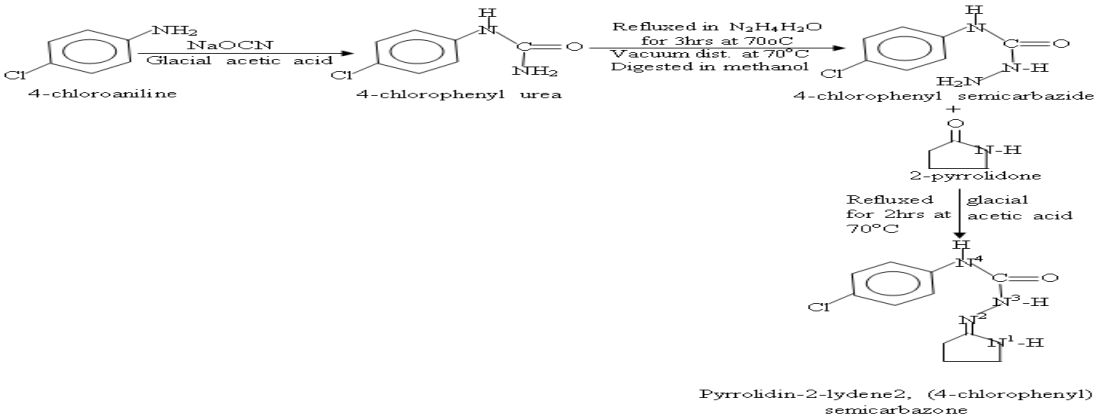
electron density to the azomethine carbon and thus, makes the ketone less electrophilic compared to aldehyde. Schiff bases are generally bidentate, tridentate, tetradentate or polydentate ligands capable of forming very stable complexes with transition metals (Abraham and Mobil, 2008). They can only act as coordinating ligands if they bear a functional group, usually the hydroxyl, sufficiently near the site of condensation in such a way that a five or six member ring can be formed when reacting with ametal ion. The electrophilic carbon atoms of aldehydes and ketones can be targets of nucleophilic attack by amines. The end result of this reaction is a compound in which the C=O double bond is replaced by a C=N double bond. This type of compound is known as an imine, or Schiff base (Cocco *et al*., 2002). Some thiosemicarbazone ligands have been synthesized by using equimolar quantities of each anisaldehyde, 4-chlorobenzaldehye, 4-fluorobenzaldehyde and vanillin in ethanol with an ethanolic solution of 4-phenylthiosemicarbazide or 4-nitrophenylthiosemicarbazide (Belicchi *et al*., 1992). This work is aimed to achieve the synthesis, structural and antimicrobial studies of 4-chlorophenylsemicarbazone with pyrrolidone moiety and its copper (II) complex.

2. Experimental

All reagents used in this work are of analytical grade and were obtained from directly from Aldrich Chemical Co. Inc. The reagents were used as starting materials for synthesis of more complex compounds without further purification. Metal salts were obtained from E. Merk and distilled water that was purchased from Chifok Scientific Co. (Nig.). Culture broths, Muller Hinton Agar, yeast, mould were obtained from Microbiology Department of Nnamdi Azikiwe University, Awka, Nigeria. Melting points were determined using capillary tube, Molar conductivity was measured in deionized water at 25^{o}Cusing a WTW conductivity meter. The infra red (FTIR) spectra were recorded using FTIR.8300 Shimadzu Spectrophotometer using CsI disc in the frequency range of 4000-400cm^{-1}. The ultra violet-visible spectra of the ligand and complexes will be recorded by using ultrospec. 2100 pro. ultra violet Spectrophotometer in the range of 200-800nm.Mass Spectra were recorded using gas chromatography-mass spectrophotometer (QP2010 plus, shimadzu), crystallinity was measured using x-ray diffractometer (PANalytical X' pert PRO MPD), Magnetic susceptibility was measured using Sherwood scientific magnetic moment balance (MK1 model) and weights were measured using digital balance (Mettler Toledo PL 203).

2.1 Synthesis of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L)

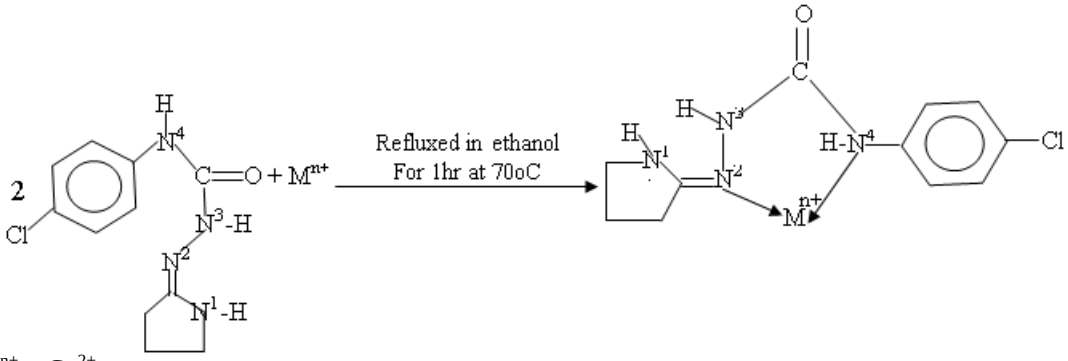
4-chloroanaline (0.1M, 1.2757g) was dissolved in 10cm^3 of glacial acetic acid and diluted to 100cm^3 with distilled water. Equimolar quantity (0.1M, 0.33g) of sodium cyanate in 50cm^3 of warm water will be added in the previous solution with stirring. The reaction mixture was allowed to stand for 30minutes, and 4-chlorophenylurea crystals formed were filtered, washed, dried and recrystallised from boiling water. 60g of 4-chlorophenylurea in 100ml of hydrazine hydrate refluxed for 3hours. Vacuum distilated at 70^{o}C, the product remaining in the flask wass digested with 250ml methanol and refluxed for 45minutes, cooled room temperature and filter. The filtrate obtained was cooled in ice bath to obtain 4-chlorophenylsemicarbazide which was further purified by recrystallization from ethanol. A solution of the 4-chlorophenylsemicarbazide (0.1M, 2.22g) and equimolar quantity of pyrrolidne (0.1M) in 100cm^3 of ethanol will be refluxed at 70^{o}C for 2hours in the presence of glacial acetic acid (1cm^3). The product obtained after cooling was filtered and recrystallized from 95% ethanol to give pure Pyrrolidin-2-ylidene-2,(4-chlorophenyl) semicarbazone (L) ligand. Yield: 51%, M.p.: 198^{o}C, $C_{11}H_{13}ON_4Cl$(L), Anal. Found: C, 51.50; H, 5.07; O, 6.24; N.21.85; Cl, 13.85%. Calc. C, 52.23; H, 5.15; O, 6.34; N, 22.18; Cl, 14.06% IR (KBr, ν/cm^{-}): ν(C=O) 1650; ν(C=N) 1540; ν(N4-H) 3413; ν(N1-H) 3310; ν(C-N) 1248; ν(C-Cl) 817. UV-Vis.(DMSO) λ_{max}/cm^{-} 36,765, 31,447. XRD: cell dimension (Å) 4.91, 4.91, 5.43. interfacial angles (◦)90, 90, 120. Volume of unit cell (Å^3), 130.907. GC-MS (m/z): cal., 252.5; found, 256.3



Scheme1: Synthesis of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone(L).

2.2 Synthesis of Copper(II) Complex of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone

50cm^{3}of 0.25M ethanolic solution of Copper (II) trioxonitrate (V) hexahydrate [$Cu(NO_3)_2.6H_2O$] was added to 50cm^{3}of 0.5M ethanolic solution of pyrrolidin-2-ylidene-2,(4-cholrophenyl)semicabarzone and heated under reflux at 70^{o}C for 1hour and cooled in ice. The product was washed with distilled water and recrystallized from 95% ethanol. Yield: 47%, M.p.: 258^{o}C, $CuL(H_2O)_3NO_3$: Anal. Found: C, 30.76; H, 4.43; O, 26.10; N, 16.31; Cl, 8.27; Cu, 14.13%. calc. C, 30.52; H, 4.39; O, 25.90; N, 16.18; Cl, 8.21, Cu, 14.80% IR (KBr, v/cm$^-$): v(C=O) 1649; v(C=N) 1541; v(N^4-H) 3410; v(N^1-H) 3309; v(C-N) 1248; v(C-Cl) 817,v(Cu-N),441; v(Cu-O), 411. UV-Vis.(DMSO) λ_{max}/cm$^-$: 37,879. XRD: cell dimension (Å) 5.19, 4.51, 29.97. interfacial angles (◦)90, 90, 120. Volume of unit cell (Å^3), 701.505. conductivity (µs/cm), 200; effective magnetic moment, 1.83. GC-MS (m/z): cal., 429.1; found, 432.5.



Where $M^{n+} = Cu^{2+}$

Scheme 2: Synthesis of Copper(II) Complex of Synthesis of pyrrolidin-2-ylidene-2,(4-chlo rophenyl) semicarbazone (L)

2.3 Antimicrobial Activities of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L) and its Cu(II) Complex

Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone and its Cu(II) complex were tested invitro growth the inhibitory activities two gram positive bacteria (*A. Aureus* and *B. Subtilis*), two gram negative (*E. coli* and *K. Pneumonae)* bacteria and two fungi (*C. albicans* and *A. flavus*) by standard disc diffusion technique using 100% DMSO as control. Effectiveness of antimicrobial agent was based on the diameter of inhibition. Antimcrobial agents were considered effective when the diameter of inhibited zone is 9mm and above, but ineffective or negative when the diameter of inhibited zone is less than 9mm as was adopted from Joseph *et al*., (2006).

3. RESULTS AND DISCUSSION

The reaction of 4-cholorophenylsemicarbazide with 2-pyrrolidone in the presence of glacial acetic acid yielded the ligand (L); Pyrrolidin-2-ylidene-2,(4-chlorophenyl) semicarbazone (Scheme 1). The reaction of the ligand (L); Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarb-azonewith copper (II) trioxonitrate (V) hexahydrate [$Cu(NO_3)_2.6H_2O$], in 2:1 ligand to metal ratio yielded the corresponding copper (II) complex of the ligand, $CuL(H_2O)_3NO_3$. The ligand and its copper (II) complex were isolated as air stable microcrystalline solids in good percentage yield of 51 and 47% respectively (Table 1).While the white colour of the ligand is seen to result from less conjugated nature of the compound, dark green colour of the complex is attributed to the presence of an unpaired electron in the d-orbital (d^9) of copper. Solubility test of the ligand and complex was done to determine suitable solvents that could be utilized for recrystallization and spectroscopic measurement. The solubility test revealed that both the ligand and its Cu(II) complex were soluble in ethanol, methanol, chloroform and DMSO at room temperature. While the ligand was insoluble in water and diethylether, it showed partial solubility in n-hexane. This supports the neutral nature of the ligand. Cu(II) complex of the ligand was partially soluble in water but insoluble in diethylether and n-hexane. This justifies the polar nature of the complex, while the observed increase in solubility of the complex in water could be attributed to chelation which increases the lipophilic nature of semicarbozone metal complexes (Wissner *et al*., 2000). The high melting points of the ligand and complexes suggest that the synthesized compounds are air and moisture stable (Table 1). Observed increase in the melting point of the ligand from 198 to 258^{o}C upon complexation with Cu(II) ion is indicative of metal-ligand bond formation. Cu(II) complex of the ligand was a monometallic centred compound. This was deduced from the results of percentage elemental analysis which are in good agreement with the assigned formulations. The theoretically calculated percentage values of elements in the ligand and in the complex were in close agreement with experimental values obtained from the results of gas chromatography-mass spectroscopic analysis (Table 1).

Table 1: Physicochemical Properties of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarba- zone (L) and its Copper (II) Complex.

% Element found (% calculated)											
S/N	**Compounds**	**Colours**	**Melting Points (°C)**	**Yield (%)**	**Molar mass g/mol**	**%C**	**%H**	**%O**	**%N**	**%CL**	**%M**
1.	$C_{11}H_{13}ON_4Cl(L)$	White	198	51	256.3 (252.5)	51.50 (52.23)	5.07 (5.15)	6.24 (6.34)	21.85 (22.18)	13.85 (14.06)	- -
2.	$CuL(H_2O)_3NO_3$	White	260	41	429.1 (432.5)	30.76 (30.52)	4.43 (4.39)	26.10 (25.90)	16.31 (16.18)	8.27 (8.21)	14.13 (14.80)

The infra red spectra of the ligand were almost the same in the regions of 3413cm^{-1} -1540cm^{-1} to those of the corresponding metal complex (fig.1 and fig.2). This is suggests that the molecular functional groups in both the ligand and the complex are identical. In Cu(II) complex, the most feasible coordination option was bonding through N^4-H and C=N^2. This is due to stability of five membered chelates over seven membered chelates (Bitu *et al*., 2019). This was suggested by variations in the absorption frequencies in the complex (N^4-H at 3410cm-1, C=N at 1541cm^{-1}) compared to those of the ligand (N^4-H at 3413cm^{-1}, C=N at 1540cm^{-1}). However, the band at 3410cm^{-1} for V(N^1-H) and the band at 1649cm^{-1} for V(C=O) were lower in complex as compared to their absorption frequencies in the free ligand suggesting repulsion, enlongation, lengthening or weakening of N^1-H and C=O bonds within the complex as result of chelation. The changes in the absorption frequencies of these functional groups could be seen to have resulted from the electrostatic nature of ligand-metal ion interaction (Ali *et al*., 2019), while new absorption bands observed in the complex in the far infra red regions; 441 and 411cm^{-1} could be attributed the emergence of v(M-N) and v(M-O) bonds as result of complexation (fig.2).

Table 2: Selected IR Absorption bands of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicar- bazone (L) and itsCopper(II) Complex

S/N	Compounds	(N^1-H)	(N^4-H)	(C=O)	(C=N)	(M-N)	(M-O)
1.	$C_{11}H_{13}ON_4Cl(L)$	3413	3310	1650	1540	-	-
2.	$CuL(H_2O)_3NO_3$	3410	3309	1649	1541	441	411

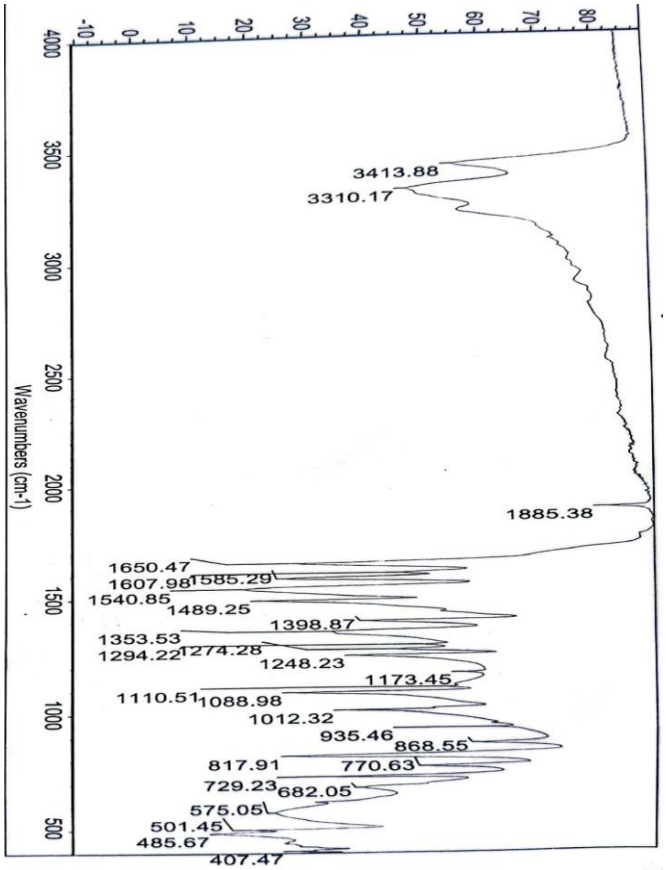


Fig. 1: Infra Red Spectrum of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L).

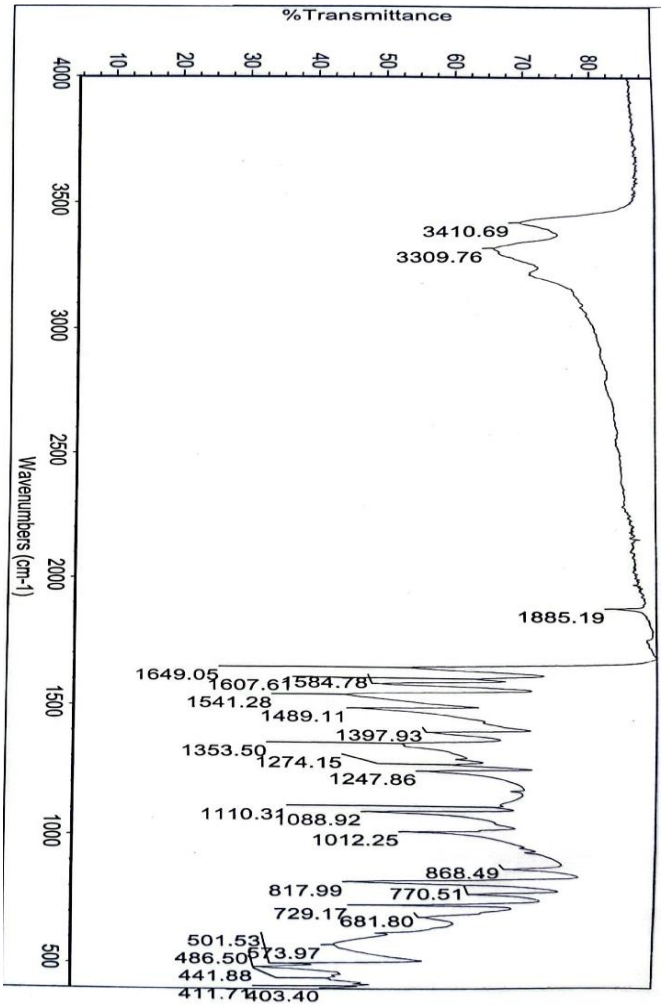


Fig. 2: Copper (II) Complex of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L).

Spectra of the ligand showed peaks at 36,765cm^{-1} (272nm) and 31,447cm^{-1} (318nm) assignable to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively (fig.3). The former transition band arose from the excitation of pi-elections to the next higher energy level which is attributed to the presence of double bonds of the benzene ring that are in conjugation with lone pair of electrons of the anilinic nitrogen group, while the later transition band occurring at 31,447cm (318nm) revealed the excitation of non-bonding electrons to pi antibonding orbitals of the imine ($C=N^2$) functional group. While the peak at 37,879cm^{-1} represents $^2E_g \rightarrow {}^2T_{2g}$ transition occurring in the low lying 3d-orbital of Cu^{2+} ion. The observed variation in the transitions occurring in the free ligand and those of its metal complexes is indicative of metal-ligand bond formation resulting from complexation (Wissner *et al*., 2000).

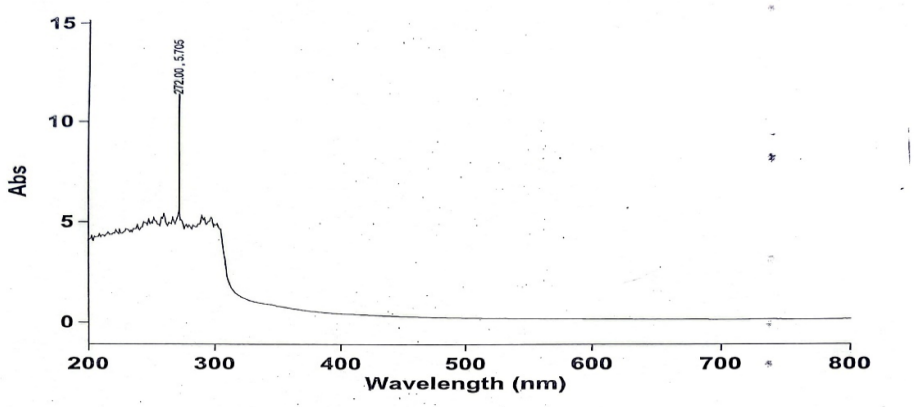


Fig. 3: UV-VIS. Spectrum of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L).

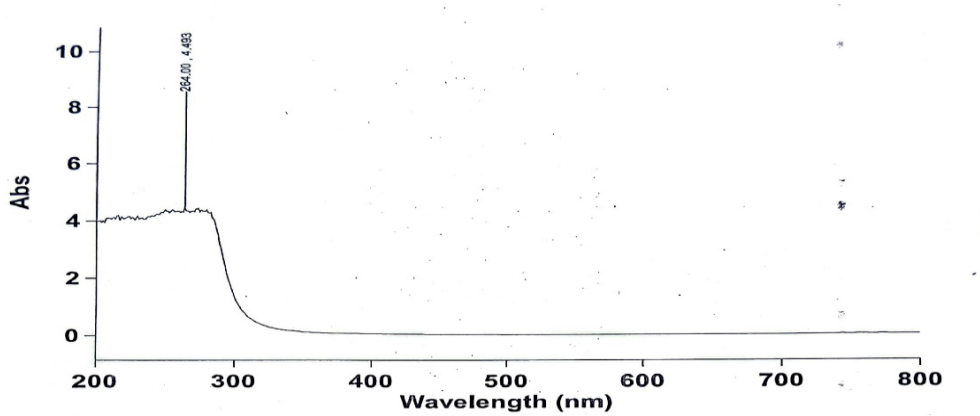


Fig. 4: UV-VIS of Spectrum of Copper (II) Complex of pyrrolidin-2-ylidene-2,(4-chloroph- enyl)semicarbazone (L).

High molar conductivity value of the complex confirmed chelation, presence of chloride ions (Cl^{-}), electrolytic nature of the complex and the possible coordination of the chloride atom to the metal center (Płowaś *et al*., 2014; Vandana, 2014). The effective magnetic moment of Cu(II) complex of the ligand measured at room temperature was 1.83BM, which suggests one unpaired electron in the $^{2}e_{g}$ orbital and further confirms the octahedral geometry of the six-coordinated Cu(II) ion in the complex. This is in agreement with expected magnetic moment value range (1.75-2.20BM) (Reddy *et al*., 2008) for Cu(II) ion in octahedral environment.

Table 3: Electrical Data of Pyrrohdin-2-hydene-2-, (4-chlorophenyl) Semicarbonzone and Its Cupper (II) Complex.

S/N	Compounds	Conductivity µS/CM	Magnetic Moment (BM)	Transition Bands (CM^{-1})	Assigned Transitions	Geometry
1.	$C_{11}H_{13}ON_4Cl(L)$	-	-	36,765, 31,447	□ → □ *, n→ □ *	
2.	$CuL(H_2O)_3NO_3$	220	1.83	37,879	$^{2}E_g \rightarrow {}^{2}T_{2g}$)	Octahedral

Gas chromatogram of the ligand showed peaks at m/z 429.2, 355.1, 284.3 and 252.5, with the peak at m/z 429.2 resulting from the interaction between the already formed ligand with 2-pyrrolidone dimer present in the reaction medium. This interaction was necessitated by high tendency of intermolecular hydrogen bond formation between the ligand and coupled 2-pyrrolidone molecules (Yekeler, 2001). Upon fragmentation, the two pyrrole rings that made up the 2-pyrrolidone dimer were lost gradually in two step mechanisms. At first, one pyrrole ring was detached leaving behind a cation with m/z 355.1, in the second step, another pyrrole ring fragmented out resulting to a cation with m/z 284.3. The fragment with m/z 284.3 then lost an hydroxyl ion and an atom of oxygen to give the ligand peak m/z 256.3. All the observed fragments possessed m/z values that are in agreement with the calculated values confirming the ligand formation (Fig.5).

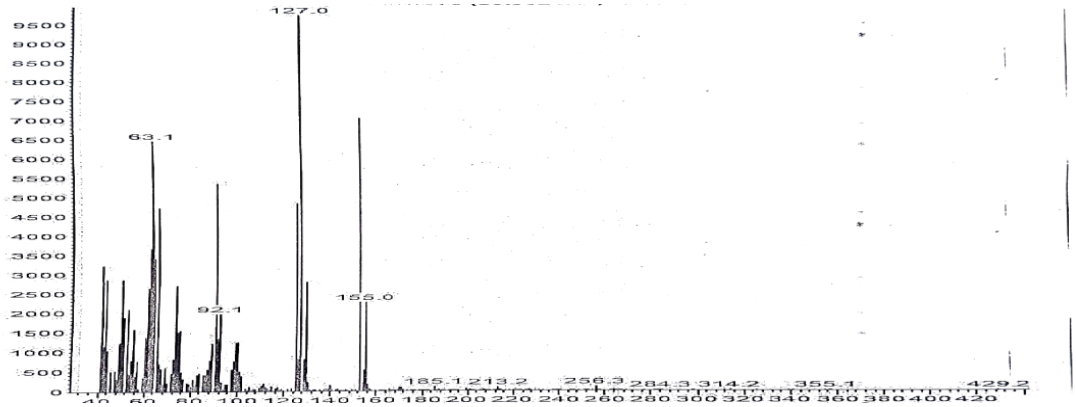


Fig. 5: GC-MS Spectrum of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L).

But for the Cu(II) complex of the ligand, $CuL(H_2O)_3NO_3$, the observed $M^{+\cdot}$ was m/z 429.1and the calculated molecular mass was 432.5gmol^{-1}, this showed total agreement between the observed and the calculated molecular masses for the complex (fig.6).

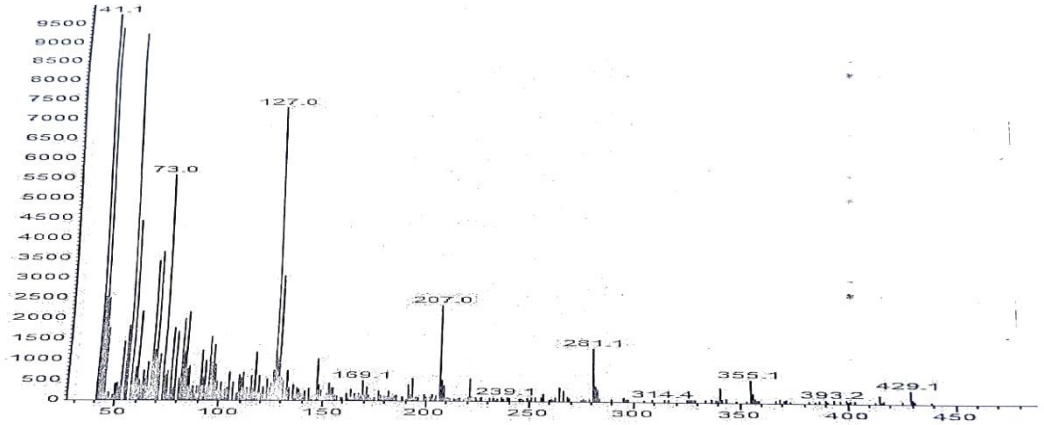


Fig. 6: GC-MS Spectrum of Cu(II) Complex of pyrrolidin-2-ylidene-2,(4-chlorophenyl) semi carbazone (L).

Sharp peaks obtained from the single crystal x-ray analyses of the compounds confirmed that both the ligand and its Cu(II) complex exist as pure crystals. The spectra obtained in this work were interpreted by matching with standard libraries and similar materials in the literature. The ligand was identified to be of $p3_121$ symmetry and in trigonal crystal system while Cu(II) complexes of the ligand possessed a symmetry of $p3_112$ symmetry and also trigonal in shape.

Table 4: X-ray Diffractometry Data of pyrrolidin-2-ylidene-2,(4-chlorophenyl)semica- rbazone (L) and its Copper(II) complex.

Parameters	**$C_{11}H_{13}ON_4Cl$(L)**	**$CuL(H_2O)_3\ NO_3$**
Empirical formula	$C_{11}H_{13}N_4OCl$	$CuC_{11}H_{19}O_7N_5Cl$
Formula weight (g/mol)	252.5	432.5
Temperature (K)	298	298
Wave length (Mo Kα)(Å)	0.71073	0.71073
Crystal system	Trigonal	Trigonal
Space group	$P3_121$	$P3_112$
Lattice constant		
a(Å)	4.91	5.19
b(Å)	4.91	4.51
c(Å)	5.43	29.97
α(°)	90	90
β(°)	90	90
γ(°)	120	120
Volume($Å^3$)	130.907	701.505

Proposed Structures of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L) and its Copper(II) Complex

From the elemental analysis, observed magnetic moment and the spectral data, structures of the synthesized compounds are suggested as shown in figure1 The structures assigned to these compounds are confirmed by the effective magnetic moment, IR, GC-MS, XRD and UV-Vis. Spectroscopic data and by analysis of the analogous structures available in the literature (Turdor *et al.*, 2007)

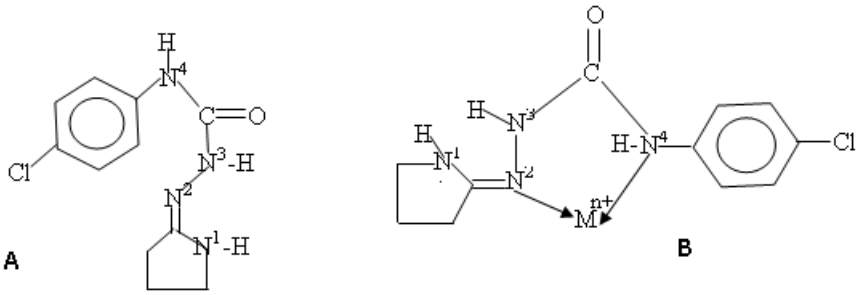


Fig. 7: Proposed Structures of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L)(A) and its Cu(II) Complex(B).

The result of antifungal screening revealed that the free ligand and its copper(II) complex did not show appreciable inhibition activity against *C. albicans* and *A.flavus*. In the case of *A. flavus*, the observed resistance against the ligand and its Cu(II) complex could be attributed to the development of biofilms which provide temporary antifungal drug resistance and protects the pathogen in the hostile environment. This is a very common behaviour among Aspergillus spp. (Paul *et al*., 2017). The underlying mechanism of resistance in *C. albicans* as observed against the ligand and its copper(II) complex could be attributed also to the ability of the organism to bring about alterations in drug targets, due to mutation in target which reduces binding of drug to the target organism (Sanglard, 2016). Again, the free ligand did not record appreciable activity against all bacteria tested, while its copper (II) complex showed inhibitory activities against *B. Subtilis*, *E.coli*, *K. Pneumonae*. From antibacterial activities data, the complex was more potent antibacterial agent than the free ligand against one or more microorganisms. This is attributable to the hydrophobic nature of the Schiff base ligands, which restricts their permeation to the cells and tissues. In addition, chelation enhances biochemical potential of bioactive organic species (Nair *et al*., 2012). When compared to standard drugs, copper (II) of the ligand was a better antimicrobial agent than fluoconazole but showed lesser inhibitory activity than ampicilin at concentrations studied.

Table 5: Antimicrobial Activities of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L) and its Cu(II) Complex.

S/N	Compounds	Dosages µg/ml	*S. aureus*	*B. Subtilis*	*E. Coli*	*K. Pneumoniae*	*C. albicans*	*A. Flavus*
1.	$C_{11}H_{13}ON_4Cl$(L)	1000 500	2 1	3 -	3 1	- -	4 -	- -
2.	$CuL(H_2O)_3\ NO_3$	1000 500	- -	9 6	9 7	9 6	2 1	- -
3.	DMSO	100%	-	1	-	-	-	-
4.	AMPICILIN	100	8	14	10	-	-	-
5.	FLUOCONA.	25	-	-	-	-	-	-

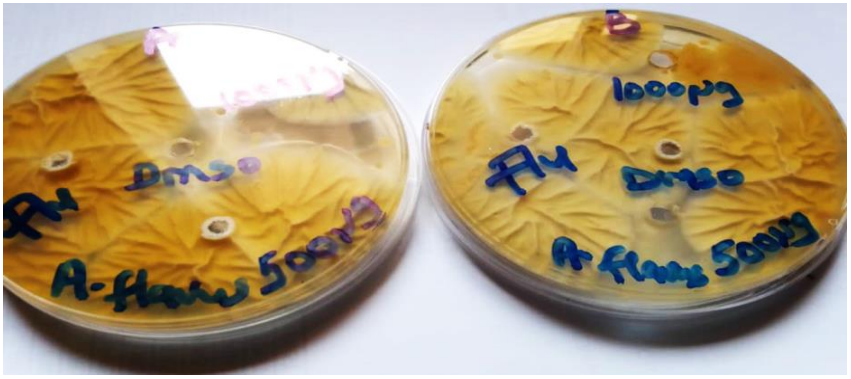


Fig. 8: Antifungal Activities of the ligand(A) and its Cu(II)(B) against A. flavus by disc diffusion method.

In comparison with the ligand which is rated inactive against all organisms studied due to its higher MIC and MBC values (table 6), potent bateriacidal behaviour of the metal complex could be attributed to the fact that chilation increases solubility, conductivity and π-electron delocalization in metal complexes (Wissner *et al*., 2000). This is in consonance with the finds of Yousif et al., (2013). The higher performance of copper(II) complex could also be a consequence of redox processes for copper compounds, generating Cu(I) and Cu(0) species during the intracellular enzymatic reduction which increases the possibility of producing reactive oxygen species (ROS), which are highly associated with cellular death of pathogens and microorganisms (Chetan *et al*., 2013).

Table 6: MIC and MBC Values of Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L) and its Cu(II) Complex.

		MIC						
S/N	Compounds	(MBC)	*S. aureus*	*B. Subtilis*	*E. Coli*	*K. Pneumoniae*	*C. albicans*	*A. Flavus*
1.	$C_{11}H_{13}ON_4Cl$(L)	MIC MBC	500 >500	500 >500	500 >500	- -	250 500	- -
2.	$CuL(H_2O)_3\ NO_3$	MIC MBC	- -	62.5 125	125 250	125 250	250 500	- -

3.	AMPICILIN	MIC MBC	4 -	8 -	8 -	6.25 -	- -	-
4.	FLUCONA	MIC MBC	- -	- -	- -	- -	125 -	- -

4. CONCLUSION

The ligand Pyrrolidin-2-ylidene-2,(4-chlorophenyl)semicarbazone (L) and its Copper(II) complex were synthesized and fully characterized. The ligand coordinated to the copper (II) ion through metal-oxygen and metal-nitrogen bond formation to afford the corresponding complex. The metal ion was six coordinated in the complex and possessed octahedral geometry. Among organisms tested, copper (II) complex of the ligand showed to be a more potent antibacterial agent against *E. coli*, *K. pneumonia*, *B. subtilis*. The complex exhibited better antimicrobial activity than the free ligand. Though the complex showed better inhibitory activity than fluoconazole, ampicilin was a more potent antimicrobial agent than the complex at concentrations studied.

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Conflict of interest

The authors hereby declare that no conflicting interest exist.

5. REFERENCES

1. Abraham, R. and Mobli M. Modeling 'HNMR spectra of organic compounds: Theory, applications and NMR prediction software. *Wiley, NewYork*. ISBN 0-470-7230, 2008; 11-17.
2. Agarwal, R.K., Singh, L. and Sharma, D.K. Synthesis, spectral and biological properties of copper (II) complexes of thiosemicarbazone of Schiff-bases derived from 4-aminoantipyrine and aromatic aldehydes. Polish Journal of Chemistry, 2005; 68, 87: 1-887.
3. Ali, U., Taj, M.B., Raheel, A., Alelwani, W., Tirmizi, S.A. A Min. Review on mixed chelation Parameters. Biomed. J Sci and Tech. Res, 2019; 20(4): 15287-15292.
4. Bahl, A. and Bahl, B.S. A Textbook of Organic Chemistry. S.Chand and Company, Ram Nagar, New Delhi-110055, 2010; 11: 973.
5. Balouiri, M., Sadiki, M. and Ibnsouda, S. K. Methods for In Vitro Evaluating Antimicrobial Activity: A review, J. Pharm. Anal, 2016; 6: 71–79.
6. Belicchi, F., Gaspari F., Pelizzi P. and Tarasconi, R. Study of transition elements on Ligands. Dalton Transition, *Journal of Chemistry Society, 1992;* 53(1): 2153-2 184.
7. Bindu, P., Kurup, M.R.P., Satyakeerty, T.R. Polyhedron, 1998; 18: 321.
8. Chetana, P.R., Somashekar, M.N., Srinatha, B.S., Policegoudra, R.S., Aradhya, S.M., and Ramakrishna Rao. Synthesis, Crystal Structure, Antioxidant, Antimicrobial, and Mutagenic Activities and DNA Interaction Studies of Ni(II) Schiff Base 4-Methoxy-3-benzyloxybenzaldehyde Thiosemicarbazide Complexes. Hindawi Publishing Corporation ISRN Inorganic Chemistry, 2013; Article ID 250791, 11 pages http://dx.doi.org/ 10.1155/2013/250791
9. Cocco, M.T., Congiu, C., Onnis, V., Pellerano M.L. and De Logu, A. synthesis and antimycobacterial activity of new S-alkylisothiosemicarbazone derivatives. *Journal of flioorganic and Medicinal Chemistry, 2002;* 10: 501-506.
10. El-Sherif, A.A. and Eldebss, T.M. Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu(II), Ni(II), Mn(II), Co(II) and Zn(II) complexes with Schiff base derived from 5-bromosali-cylaldehyde and 2-aminomethylthiophene. Pectrochim Acta A MolBiomolSpectrosc, 2011; 79(5): 1803-14.
11. Joseph, M., Kuriakose, M., Kurup, M.R.P., Suresh, E., Kishore, A., Bhat, S.G. Structural, antimicrobial and spectral studies of copper(II) complexes of 2-benzoylpyridine N(4)-phenyl thiosemicarbazone. Polyhedron, 2006; 25: 61-70.
12. Leovac, V.M., Jevtovic, V.S., Jovanovie, L.S. and Bogdanovic, S. Metal complexes with Schift-based ligands- pyrodoxal and semicarbazide-based derivatives. *Journal of Serbian Chemistry Society*, 2005; 70 (3): 394-399.
13. Lima, R.I., Firo, L.W, Carneiro, T.M. and Beraldo, A. Nickle (II), copper(I) and copper (II)'?np1exes of bidentate heterocyclic thiosemicarbazones. Journal of *Brazilian Chemistry Society, 1999;* 10(3): 185-188.
14. Marina, C., Mirta, R., Ivica, D., Gerald, G. and Boris, K. Oxovanadium (V) coplexes of salicylaldehyde and 3-methoxysalicylaldehyde 4-phenyithiosemicarbazone: Formation of two new thiazoline compounds. Journal of Croadca Chemical society, 2007; 80(3): 583-590.
15. Martin, S., Cuesta, P., Rizo, P. and Cortesc. M. A computer program based on parallel line assay for analysis of skin test. *Journal of Allergy*, 2007; 52: 77-100.
16. Molavi, A. Metal complexes in traditional medicine of South Africa. National Geographic Newspaper Review, 2003; (32): 23-34.

17. Nair, M.S., Arish, D., Joseyphus, R.S. Synthesis, characterization, antifungal, antibacterial and DNA cleavage studies of some heterocyclic Schiff base metal complexes. J Saud Chem Soc, 2012; 16: 83-8.
18. Offiong, O.E., Martelli, S. Antibacterial activity of metal complexes of benzyil and benzoin thiosemicarbazone. Journal of Farmacology, 1994; 49: 513-518.
19. Offong, O.E. and Martelli, S. Synthesis and biological activity of novel metal complexes of 2-acetylpyridine thiosemicarbazones. Journal of Farmaclogy, 2006; 50(9): 625-632.
20. Omar, A.M., Eshba, N.H. and Salama, H.M. Synthesis of some substituted isatin-B-thiosemicarbazones and isatin-B-hydrazonothiazoline derivatives as potential antiviral and antimicrobial agent. Journal of Pharmaceutical, 2001; 31: 701-709.
21. Paul, S., Ludena, Y., Villena, G.K., Yu, F., Sherman, D.H., Gutierrez-Correa, M. High-quality draft genome sequence of a biofilm forming lignocellulolytic Aspergillus niger strain ATCC 10864. Stand Genomic Sci, 2017; 12(1): 37.
22. Płowaś, I., Świergiel, J., Jadżyn, J. Electrical conductivity in dimethyl sulfoxide + potassium iodide solutions at different concentrations and temperatures, Journal of Chemical & Engineering Data, 2014; 59: 2360-2366, doi: 10.1021/je4010678
23. Raman, N., Raja, S.J., Sakthivel, A. J Coord Chem, 2009; 62: 691.
24. Reddy, V., Patil, N., Reddy, T. and Angadi, S.D. Synthesis, characterization and biological activities Cu(II), Co(II), Ni(II), Mn(II) and Fe(III) complexes with Schiff base derived from 3-(4-chlorophenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole. E-Journal of Chemistry, 2018; 5, 3: 529-538.
25. Sanglard, D. Emerging threats in antifungal-resistant fungal pathogens. Front Med, 2016; 3: 11.
26. Sulekh, C., Umendra, K. and Verma, H.S. synthesis, spectral nature and antifungal activity of di(p-chlorophenyl)-thiosemicarbazone and its Cr(III) and Co(II) metal complexes. *Oriental Journal of Chemistry*, 2003; 19(2): 355-360.
27. Tudor, R., Aurelian, G., Anca, N. and Rodica, G. Complexes of $3d^n$ metal ions with thiosemicarbazone: synthesis and antimicrobial activity, Oriental Journal of Chemistry, 2007; 52: 782-832.
28. Vandana, C.S. (2014). Synthesis, spectroscopic, anticancer and antibacterial studies of Ni(II) and Cu(II) complexes with 2-carboxybenzaldehyde thiosemicarbazone, Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 2014; 129: 333-338. doi: 10.1016/j.saa.2014.02.141
29. West, D., Padhye, S., Sonawane, P. In Structure and Bonding, Springer-Verlag: New York, 1991; 76: 1-49.
30. Wissner, A., Berger, D.M., Boschelli, D.H. 4-anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors of epidermal growth factor receptor kinase and bisisosteric relationship to the 4-anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors. Journal of Medicinal Chemistry, 2000; 43, 17: 3241-3256.
31. Yekeler, H. Solvent effects on dimeric self association of 2-pyrrolidone: an ab initio study. J Comput. Aided Mol. Des, 2001; 15(4): 287-95.
32. Yousif, E., Majeed, A., Al-Sammarrae, K., Salih, N., Salimon, J. and Abdullah. Metal Complexes of Schiff Base: Preparation, Characterization and antibacterial activity. Arabian Journal of Chemistry, 2013; 06: 006.
33. Zahid, H.C., Humayun, P., Khalid, M.K. and Claudiu, T.S. Organometallic-based antibacterial and antifugal compounds: transition metals of 1,1‘-diacetylferrocene-derived thiosemicarbahydrazone, carbohydrazone, thiosemicarbazone and semicarbazone. Journal of *Enzyme Inhibition and Medicinal Chemistry*, 2005; 19(3): 81-98.