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Synthesis, Characterization and antimicrobial activity of Transition Metal Complexes of Pyrrole-2-carboxaldehyde with Leucine

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Abstract:

The Schiff base ligand was prepared by condensation of pyrrole-2-carboxaldehyde with leucine. Cu(II), Co(II), Mn(II), Zn(II) and Ni(II) complexes of above ligand was synthesised as well. The synthesised ligand and complexes have characterized by IR, UV, ^{1}HNMR ESI-mass, Powder XRD, SEM and EDAX. The antimicrobial activity of the synthesized ligand and its complexes have been tested for their antibacterial activity against bacterial species Basillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae , Pseudomonaos aeruginosa, Staphylococcus aureus and fungal species Candida albicans and Aspergillus niger. The result found that the metal complexes were more active than the ligand.

Key words:

Pyrrole-2-carboxaldehyde, Leucine, metal complexes, XRD, SEM.

1. Introduction

The transition metal ions play significant roles in various enzymes functions. Certain drugs play a vital role as bio-ligands in the biological systems. Similarly, nitrogen containing bases such as derivatives of pyrrole, pyridine, pyrimidine ,pyrazine and purine have a vital role as bio-ligands. Imine or azomethine groups are present in various natural, naturally derived and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [1-3]. Due to the presence of the imine group, the electron cloud of the aromatic ring and electronegative nitrogen, oxygen and sulfur atoms in the Schiff bases molecules, these compounds effectively prevent corrosion of mild steel, copper, aluminum and zinc in acidic medium [4].The metal complexes of Schiff bases derived from heterocyclic compounds have been the centre of attraction for many researches in recent years. This may be attributed to their stability,biological activity and potential applications in many fields of science and technology. Schiff base amino acid complexes act as good chelating agents and behave as efficient biologically active and cytotoxic agents. Studying the interaction between transition metal complexes and DNA has attracted many interests due to their importance in cancer therapy, design of new types of pharmaceutical molecules and molecular biology.The complexes of transition elements with Schiff bases have wide applications in food industry, dye industry, catalysis, fungicidal, agrochemical, anti-inflammable activity, antiradical activities and biological activities. Among these, heterocyclic Schiff base ligands and their complexes possess great importance due to their pharmacological properties. In this paper the metal complexes of Cu(II), Co(II), Mn(II), Zn(II) and Ni(II) with the Schiff base derived from pyrrole-2-carboxaldehyde with leucine have been synthesized. The ligand and the metal complexes have been characterized by IR UV, ESI-mass, powder XRD, SEM and EDAX. The ligand and their metal complexes have been screened for their antimicrobial activities using the well diffusion method against the selected bacteria and fungai.

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2. Experimental

2.1 Material and Methods

All reagents were analytical grade producers (Aldrich) and used without further purification. Chemical analysis of Carbon, Hydrogen and Nitrogen was performed using a Elemental vario EL III CHN analyzer. IR spectra of the ligand and complexes were recorded in the range of 4000-400cm^{-1} on JASCO FTIR-4100 Infrared spectrophotometer by KBR disc technique. UV-visible spectra was recorded using spectrophotometer with DMSO as the solvent in the range of 200-700 nm, ^{1}H NMR spectra were recorded in DMSO–d6 on a Bruker Avance 111,400 MHZ Spectrometer. X-ray diffraction study of some metal complexes were obtained onRigaka D-Max C-Xray diffractometer, Scanning Electron microscope images were recorded in JEOL model JSM -6390V electron microscopy.

2.2 Synthesis of Schiff base ligand potassium(Z)-2-(((1H-pyrrol-2-yl)methylene) amino)-4-methylpentanoate**.**

Pyrrole-2-carboxaldehyde (0.01 mol) is dissolved in 20 ml MeOH and added 20 ml methonic solution of L-leucine (0.01 mol) containing KOH (0.01 mol). The solution obtained was heated at 60° for 9 hours. Brownish yellow solution was formed. The volume of the solution is reduced to half. Filtered the precipitate, washed with ether followed by ethanol and dried in desiccators.

2.3 Synthesis of Schiff base metal complexes:

To the hot methanolic solution of Schiff base ligand (0.01 mol), and the methanolic solution of metal ions ($CoCl_2.6H_2O$, $CuCl_2.2H_2O$, $NiCl_2.2H_2O$, $MnCl_2.2H_2O$, $ZnCl_2$) was added drop by drop at 60° in 1:2 (metal:ligand) molar ratio. The mixture was then refluxed for 1 hour the intensity of the colour becomes translucent. The resulting mixture was filtered out, washed repeatedly with ether and dried in desiccator.

2.4 Antimicrobial activity:

2.4.1. Test organisms:

Bacterial species Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonos aeruginosa, Staphylococcus aureus and fungal species Candida albicans and Aspergillus niger were used as test organisms.The Schiff base ligand and its metal complexes were screened against bacterial species such as Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonos aeruginosa, Staphylococcus aureus and fungal species like Candida albicans and Aspergillus niger in agar well diffusion method. The Solvent used for dissolving the synthesised compounds was DMSO.

2.4.2.Experimental methods:

Muller hinton agar medium (20ml) was poured into each petri plate, and plates were swabbed with 100 µl inoculated of the test microorganisms and kept for 15 minutes for adsorption . Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates, and these were located with a 100µl solution of each compound in DMSO. All the plates were incubated 37° for 24 hrs .After incubation , the inhibition growth was analysed and the results were recorded.

3. Results and Discussion:

The analytical data and some physical properties of the ligand and its metal complexes are noted in table 1. The data shows that the complexes are formed in the ratio 1:2 (M : L). The resulting metal complexes were found to be stable at room temperature and insoluble in common solvents such as EtOH, MeOH but soluble in DMF and DMSO.

Table: 1 Analytical data and some physical properties of the ligand and metal complexes.

Compound	Empirical Formula	Analytical data %				Molar Conductance (Ω^{-1} cm^2 mol^{-1}) DMSO	Colour	Mel. point
		Carbon	Hydrogen	Nitrogen	Metal			
PMAMP	$C_{11}H_{15}KN_2O_2$	53.63(C) 53.10 (A)	6.74(C) 6.98 (A)	11.37 (C) 11.12 (A)	-	-	Black	220
[Cu(PMAMP)$_2$]	$C_{22}H_{30}CuN_4O_4$	55.27 (C) 55.88 (A)	6.33 (C) 6.76 (A)	11.72 (C) 11.55 (A)	13.29 (C) 13.35 (A)	10.41	Blue	240
[Co(PMAMP)$_2$]	$C_{22}H_{30}CoN_4O_4$	55.87(C) 55.38 (A)	6.39 (C) 6.67 (A)	11.83 (C) 11.88 (A)	12.45 (C) 12.60 (A)	15.60	Pink	230
[Mn(PMAMP)$_2$]	$C_{22}H_{30}MnN_4O_4$	56.29 (C) 56.91 (A)	6.44 (C) 6.62 (A)	11.93 (C) 11.48 (A)	11.70 (C) 11.96 (A)	12.05	Dirty White	245
[Zn(PMAMP)$_2$]	$C_{22}H_{30}ZnN_4O_4$	55.06 (C) 55.86 (A)	6.30 (C) 6.48 (A)	11.68 (C) 11.96 (A)	13.62 (C) 13.66 (A)	8.5	Dirty White	250
[Ni(PMAMP)$_2$]	$C_{22}H_{30}NiN_4O_4$	55.84 (C) 55.40 (A)	6.39 (C) 6.54(A)	11.84 (C) 11.08 (A)	12.40 (C) 12.56 (A)	8.0	Ash color	233

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3.1 IR Spectra

The important IR spectral data of the Schiff base ligand and its complexes are given in Table- 2. The IR spectrum of the free Schiff base ligand exhibits a sharp band at 1630 cm^{-1} due to the azomethine group. On complexation, this band was shifted to a lower frequency in the 1627 - 1620 cm^{-1} range indicating the coordination of the azomethine nitrogen atom to the metal ion(5). The band at 1589 and 1404 cm^{-1} can be respectively described to asymmetric Carboxyl $\nu_{as}(COO^-)$ and symmetric Carboxyl $\nu_s(COO^-)$ groups. During complexation they were shifted to lower frequency by ~ 20 – 50 cm^{-1} range indicating the linkage between the metal ion and Carboxylato oxygen atom(10). The large difference between the $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ value of ~ 200 cm^{-1} indicates the monodentate binding nature of the carboxylato group (6) in the complexes. A characteristic band at 3250 cm^{-1}, due to (N-H) of pyrrole, was observed in the spectra of the ligand .The Cu(II), Co(II), Mn(II), Zn(II) and Ni(II) complex show band at ~ 3240 cm^{-1} which is assigned for stretching frequency of N-H of pyrrole. The stretching vibration due to N-H of pyrrole of ligand remains unchanged, indicating that the N-H group is not involved in coordination The IR spectra of the metal complexes also show some new bands in 532-594 and 424-470 cm^{-1} regions which may be due to the formation of M-O and M-N bands(7). Accordingly , one can deduce that the ligand binds the metal ion as bidentate fashion. The bonding sites are the azomethine nitrogen and the carboxylato oxygen atoms. The IR spectra of Schiff base and Cu(II) complex shown in Fig. 1,2.

Table - 2 IR spectral data of the ligand and its complex(cm^{-1})

Compound	C = N	ν_s COO^-	$\nu_{as}COO^-$	M-O	N-H	M-N
PMAMP	1630	1404	1589	-	3250	-
$[Cu(PMAMP)_2]$	1620	1465	1550	570	3240	439
$[Co(PMAMP)_2]$	1620	1458	1535	594	3245	470
$[Mn(PMAMP)_2]$	1620	1411	1580	532	3250	447
$[Zn(PMAMP)_2]$	1620	1411	1550	563	3240	432

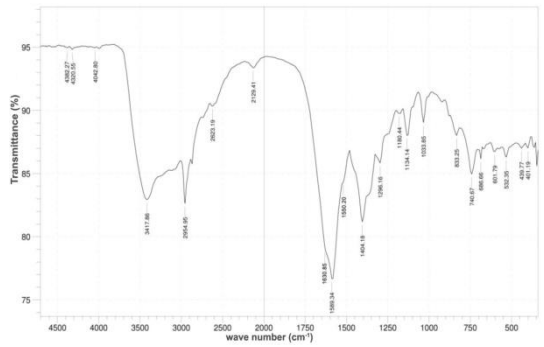

Figure 1 : IR Spectrum of the Ligand

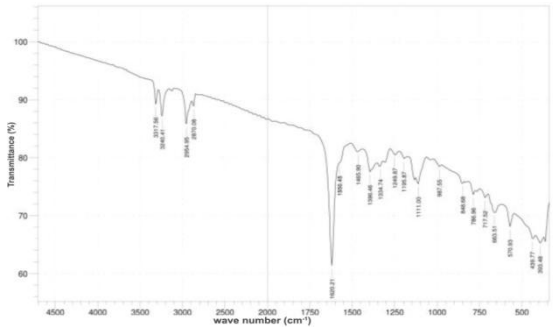

Figure 2 : IR Spectrum of the $[Cu(PMAMP)_2]$

3.2 Electronic Spectra

The electronic spectra of the complexes were recorded from freshly prepared solution of DMSO at room temperature. The electronic spectrum of Schiff base ligand there are two absorption bands at 219, 289 nm assigned to $\pi - \pi*$ and $n - \pi*$ transitions. These transitions are also found in the spectra of the complexes, but they are shifted towards longer wavelength from ligand to complex, indicating coordination of ligand to metals through the azomethine moiety. The spectrum of the copper (II) complex displayed a band at 275 and 580 nm. It assigned to $^2B1_g \rightarrow {}^2A1_g$ transition which is characteristic of distorted square planar structure (8).The magnetic susceptibility value of the Cu(II) complex is 1.84. The cobalt complex shows a d-d band at 620 nm assigned as

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$^1A1_g \rightarrow {}^1B1_g$ transition, which confirms square-planar geometry. This is further confirmed by its magnetic susceptibility value (3.35 BM) (9). The spectrum of Mn(II) complex contains bands at 343 nm and 455 nm, which corresponds to $^6A_{1g} \rightarrow {}^4E_{1g}$ (D) and $^6A_{1g} \rightarrow {}^4T_{1g}$(G) transitions. Similar observations has been made in the case of tetrahedral manganese (II) complexes (10,11).The magnetic moment value of Mn(II) complex is 5.84 BM. Generally Zn(II) complexes does not exhibit any d-d- transition due to its completely filled d_{10} electronic configuration so it exhibit charge transfer spectra. The Zn(II) complex showed a band at 440 nm attributed to LMCT, which is compatible with this complex having a tetrahedral geometry . The electronic spectrum of Ni(II) complex exhibited one absorption bands at 530 nm which may be assigned to the transition, $^3T1(F) \rightarrow {}^3T1(P)$ which indicates the tetrahedral geometry around Ni(II) ion. The magnetic moment value of Ni(II) complex is 3.62BM. The UV spectrum of Schiff base ligand and copper (II) metal complex are indicated in Figure 3 & 4

Table 3 Electronic Spectral Data of ligand and its metal complexes

Compound	Absorption nm	Band Assignment	Geometry and Magnetic Moment
PMAMP	219, 289	π - π*, n - π*	-
$[Cu(PMAMP)_2]$	275, 580	π - π*, $^2B1_g \rightarrow {}^2A1_g$	Distorted, Square planar 1.84BM
$[Co(PMAMP)_2]$	620	$^1A1_g \rightarrow {}^1B1_g$	Square planar, 3.35 BM
$[Mn(PMAMP)_2]$	343,455	$^6A_{1g} \rightarrow {}^4E_{1g}$ (D), $^6A_{1g} \rightarrow {}^4T_{1g}$(G)	Tetrahedral ,5.84 BM
$[Zn(PMAMP)_2]$	435	LMCT	Tetrahedral Diamagnetic
$[Ni(PMAMP)_2]$	530	$^3T1(F) \rightarrow {}^3T1(P)$	Tetrahedral 3.62 BM

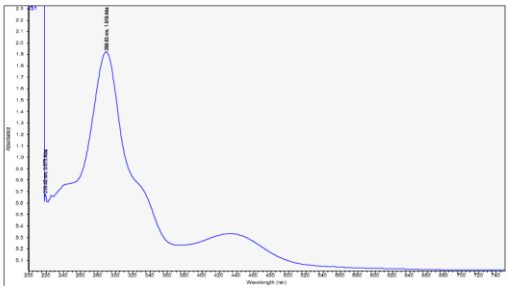

Figure 3 : UV- Visible Spectrum of the PMAMP

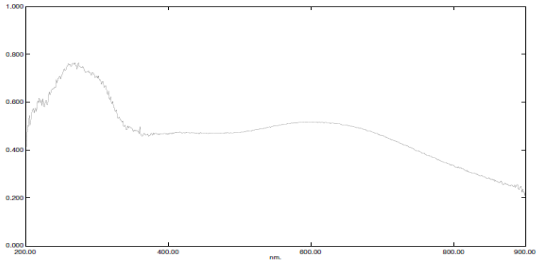

Figure 4 : UV- Visible Spectrum of the $[Cu(PMAMP)_2]$

3.3 ^{1}H NMR Spectra

The ^{1}H NMR spectrum of the complex was recorded in DMSO-d_6. The spectrum of the ligand showed the aromatic protons in the range δ 6.82 ppm and these remains almost at the same position in the spectra of the metal complexes. peaks in the region δ 6.35 - 6.36 ppm were assigned to a pyrrole hydrogen.

The coordination of the azomethine nitrogen was assigned by the downfield shifting of the azomethine proton signal from 7.95ppm in free ligands to 8.30 ppm in their zinc complex. The downfield shifting of azomethine proton in Zn(II) complexes was attributed to the discharging of electronic cloud towards the Zn(II) ion. These observations suggest that the ligand coordinate to the Zn(II) through the azomethine nitrogen and carboxylato oxygen atom. A peak in the region 0.86 ppm characteristic of methyl group are present in the spectrum of the complex. Signals 1.56ppm were attributed to the methylene group are presented in the complex. The methine group appeared as the signals at 1.60ppm. A singlet peak at 11.40 ppm due to NH of pyrrole ring(12). The ^{1}H NMR spectrum of the ligand and Cu complex is shown in Fig. 5,6.

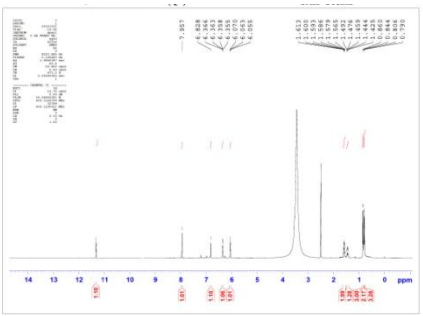

Figure 5: ^{1}H NMR spectrum of the PMAMP

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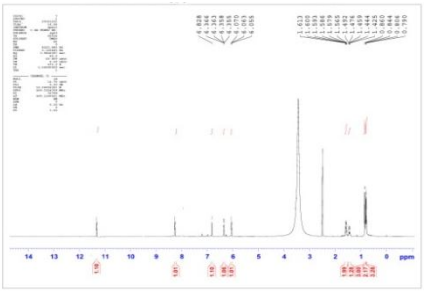

Figure 6 : ^{1}H NMR spectrum of the [Zn(PMAMP)$_2$]

3.4 Mass spectrum

The mass spectrum of Schiff base ligand showed of a molecular ion peak at m/z 246 and it corresponds to [M^+] ion was given to Fig 7.The observed peaks are in good agreement with their empirical formula as obtained from the analytical data.

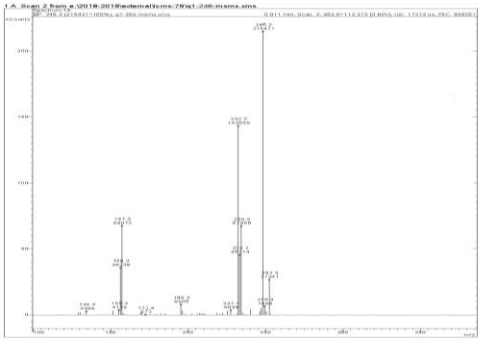

Figure 7: Mass spectrum of PMAMP

The mass spectrum of the Copper(II) complex confirms the stoichiometry of the complex. In the mass spectrum of copper(II) complex showed molecular ion peak at m/z = 478 [M^+] was given Fig 8. Elemental analysis values are in close agreement with the values calculated from molecular formula assigned to these complexes. Which are further confirmed by the ESI- mass spectral study of the complexes.

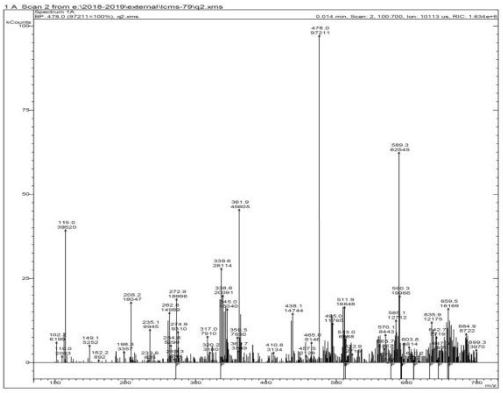

Figure 8: Mass spectrum of [Cu(PMAMP)$_2$]

3.5 Thermo gravimetric studies

The thermal behaviour of the copper(II) complex have been made in the temperature range of 40°C to 800°C at a heating rate of 20°C/min. The thermogram indicates that the complex is stable upto 200°C and then begins to decompose in two stages. In the present study Cu(II) complex decomposes in two stages, in the first stage (271°C) it shows weight loss (22.3%) corresponding to high temperature range this indicates that amino acid. Which is an endothermic process as the DTA curve indicates. In the second stage (750°C) it shows the weight loss upto (20%) and that corresponds to the organic ligand moiety leaving the metal oxide residue. It is an endothermic peak as shown in Figure 9.

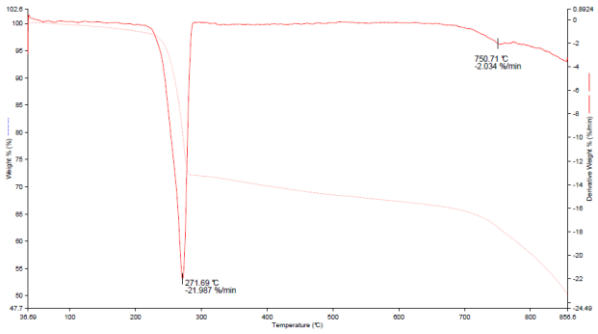

Fig 9:TG/DTA spectrum of the [Cu(PMAMP)$_2$]

Based on the observations in elemental analysis IR spectra, UV-Visible spectra,1HNMR spectra ESI-mass and TG/DTA spectral studies, the proposed structure of Schiff base ligand and complexes.The Schiff base ligand and complexes are shown in Fig 10,11and 12.

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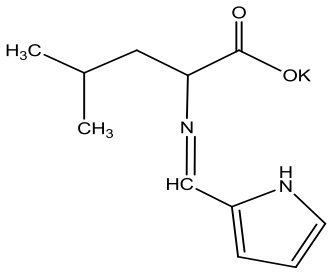

Fig 10:Structure of the ligand

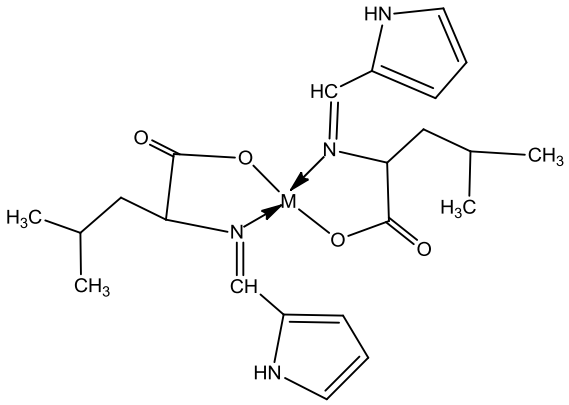

Fig 11: Structure of the Cu(II) and Co(II) complexes

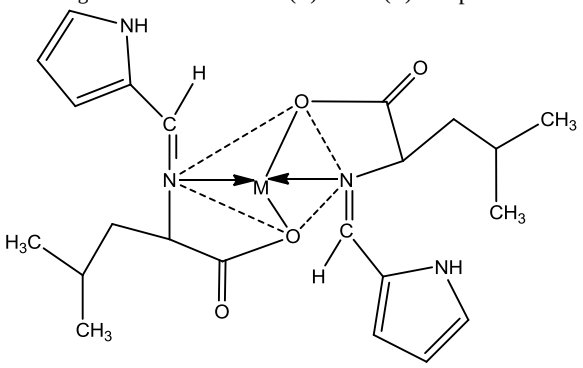

Fig 12: Structure of the Mn(II), Zn(II) and Ni(II) complexes

3.6 XRD

The powder XRD patterns of Cu(II) complex are recorded over the 2θ = 0-80A^o range .From the data, the complexes show sharp peaks indicating their crystalline nature. The average crystalline size of the complex d_{XRD} were calculated using scherrer's formula (13).The Cu (II) complex have an average size of 23.08 nm, suggesting that the complex are in a nanocrystalline phase. The Ligand have an average size is 19.55 nm that the ligand is a nano crystalline phase. The powder XRD pattern of Ligand and Cu (II) complex is shown in Figure.13,14. Comparing the XRD pattern of the ligand and Cu(II) complex, addition peaks at 54.20, 58.50, 66.00, which clearly indicate that copper is coordinated to the ligand during complexation (14).

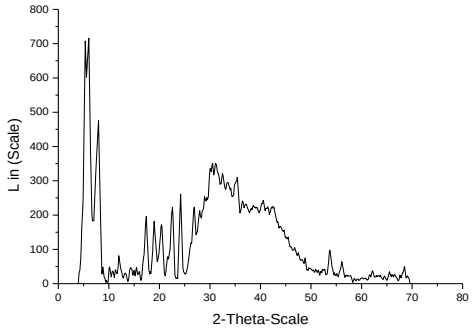

Figure 13: XRD pattern of the $[Cu(PPMACB)_2H_2O.Cl]$

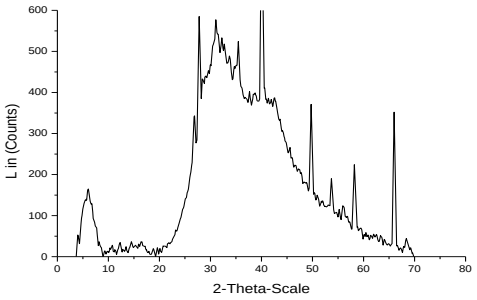

Figure 14 : XRD pattern of the PPMACB

3.7 SEM (Scanning Electron Microscope)

The surface morphology of ligand is shown in Figure 15. The SEM images of Schiff base ligand has small grains morphology. This arrangement showed a thickness in the submicron regime. The average size of particle is 50.77nm. However particles with size less than 100 nm were also absorbed which groups to form agglomerate of larger size.

The surface morphology of Cu(II) complexes is shown in Figure 16. The SEM images of the complex has stone like morphology. The average size of the particle is 73.33 nm. The particle size is less than 100 nm which groups to form agglomerate of larger size.

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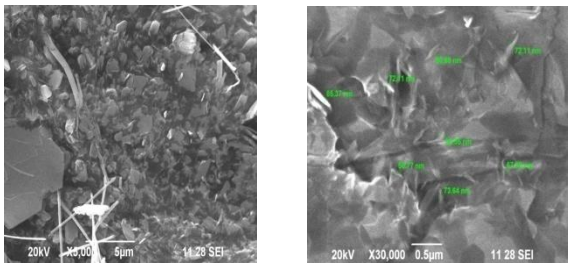

Fig 15 : SEM Photograph of the PMAMP

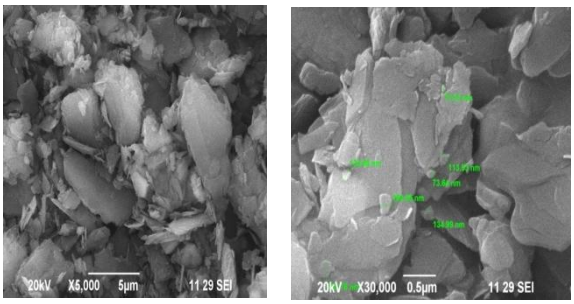

Fig 16: SEM Photograph of the $[Cu(PMAMP)_2]$

3.8 EDAX Analysis

EDAX can be used to determine which chemical elements present in the sample ,and can be used to estimate their relative abundance. Elemental maping of a sample and image analysis are also possible as showed in Figure 17,18. The EDAX result of ligand showed that the atomic percentage of carbon, nitrogen, oxygen and potassium are 54.63, 13.35, 27.32, 5.15 % respectively while that of Cu(II) complex were found to be 55.81, 11.78, 13.04, 13.70 % for carbon, nitrogen, oxygen, and copper respectively.

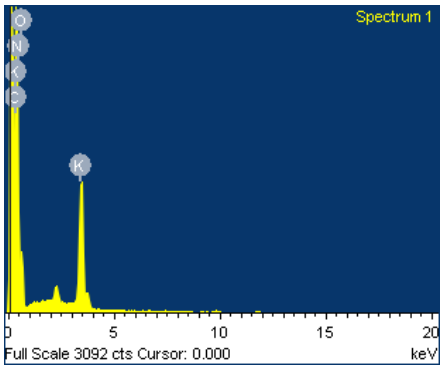

Fig 17: EDAX photograph of the PMAMP

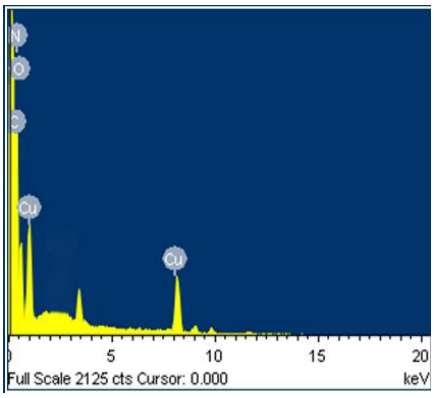

Fig 18: EDAXPhotograph of the $[Cu(PMAMP)_2]$

Composition of C & N percentage in EDAX agrees well with CHN analysis which confirm that Complexation has occurred effectively in 1:2 ratio of its support elemental composition.

3.9 Antimicrobial activity

3.9.1 Antibacterial activity

The Schiff bases PMAMP and their metal complexes were screened for bacterial activity against the bacterial species such as Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumonia, and E. coli. The activities of the complexes were compared with the standard drug Amikacin.The antibacterial activity are presented in the figure 19.

The result indicate that the ligand show moderate activity against all the five bacterial species. The Cu(II) complex showed higher activity against K. Pneumonia and E.coli than the corresponding Schiff base ligand as well as the control Amikacin and moderate activity against B. Cereus and P. aeruginosa .Mn(II) showed higher activity against E.coli than the corresponding Schiff base ligand and other complex containing less activity against all other species. Co(II),Zn(II) and Ni(II) complex showed lesser activity against B.substilis, B. cercus and S. aureus. Zn (II) exhibit moderate activity against P.aeruginosa, P. aeruginosa, S. aurus.

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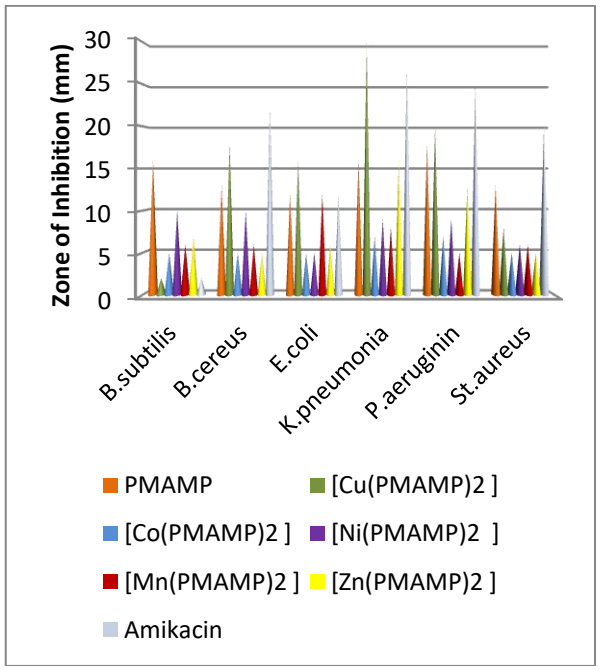

Fig 19 : Antibacterial Activities of PMAMP and its metal Complexes

3.9.2 Antifungal activity

The Schiff base PMAMP and their metal complexes were screened for fungal activity against the fungal species such as Candida albicans, Aspergillus niger. The activities of the complexes were compared with the standard drug Nystatin. Complexes exhibited more activity than the ligands because complexation increases the antifungal activity.

The result indicate that the ligand PMAMP show moderate activity against C. albicans and A. Niger. Cu(II) complexes show highest activity against C. albicans followed by Co(II), Mn(II), Zn(II) and Ni(II) compexes and standard drug nystatin. Cu(II) and Zn(II) complexes have high activity against A. niger, compared with other complexes.

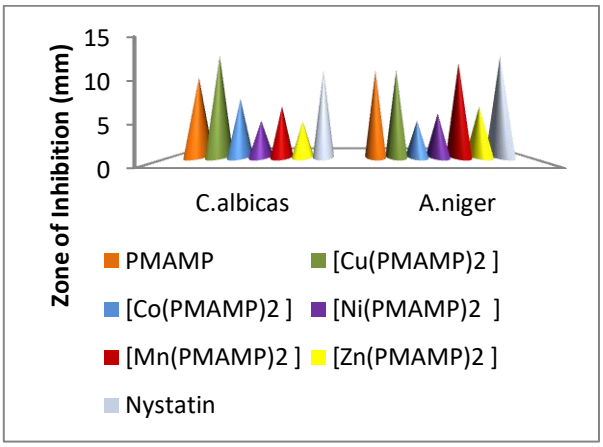

Fig 20 : Antifungal Activities of PMAMP and its metal Complexes

It is found that metal complexes have higher antibacterial activity than the schiffbase ligand (15). Such increased activity of the metal complexes can be explained on the basis of overtone's concept and chelation theory(16,17). On chelation the polarity of the metal ion will be reduced due to the overlapping of the ligand orbital and partial sharing of positive charge of the metal with donor groups(18). Itincreases the delocalization of -electrons over the whole chelate ring and enchances the lipoplicity of the complexes. This increased lipoplicity enchances the penetration of the complexes into lipid membranes and block the metal binding sites in the enzymes of microorganisms. The antimicrobial activity depends on the molecular structure of the compound, the solvent used (19) and the species screened under consideration(20).

Conclusion:

Schiff base and its complexes were prepared and characterized using the elemental analysis, molar conductance, magnetic measurement, TG/DTA, SEM, EDAX, XRD, IR and electronic spectral analysis. IR spectral data shows that the ligand act as bidentate, coordinating through azomethine nitrogen and carboxylato oxygen atoms. Electronic spectral studies reveal that octahedral geometry for Cu(II) and Co(II) complexes. Mn(II), Zn(II) and Ni(II) complexes exhibit tetrahedral geometry. XRD and SEM analysis explains the crystalline and morphological structure of the complexes. The antimicrobial activitiy of the Schiff base and their complexes have been also studied. The results reveal that the Cu(II) complex are showing high activity than the free ligand. .

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