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Synthesis, Characterization and Antimicrobial Activities of N-Salicylideneaniline and Its Cu^{2+}, Co^{2+}, Cr^{3+} and Zn^{2+} Complexes

M. S*[1]. Iorungwa, E. N[1]. Iornumbe, M. I[2]. Terhemen, H[1]. Abuh, P. D[1]. Iorungwa, and N.R[3]. Akpa – Igwe

[1]Inorganic /Physical Chemistry Research Group, Joseph Sarwuan Tarka University, Makurdi, Benue, Nigeria.
[2]Department of Chemistry, Federal College of Education, Obudu, Nigeria

[3]Department of Chemistry, Nigeria Defence Academy, Kaduna, Nigeria

***Correspondence E-mail:** saviour.moses@gmail.com

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ABSTRACT

The Schiff base ligand N-Salicylideneaniline(SALAN) was prepared by condensation reaction between Salicylaldehyde and Aniline in alcohol medium. N-Salicylideneanilineand its Cu(II), Co(II), Cr(III) and Zn(II) metal complexes were synthesized through condensation reaction achieved by microwave assisted technique. The physico-chemical properties were determined and both the Schiff base and its metal complexes were characterized by FTIR, UV, ^{1}HNMR and tested for their microbial activity. FTIR results showed SALAN(1274.7, 1613.9)cm^{-1}, SALAN/Cr(1293.4, 1610.2)cm^{-1}, SALAN/Co(1293.7, 1613.9)cm^{-1}, SALAN/Cu(1244.9, 1591)cm^{-1} and SALAN/Zn(1297.1, 1613.9)cm^{-1}which revealed complexation arising from the oxygen and nitrogen atoms of the azomethine group. The melting point measurement revealed that the Schiff base has the lowest melting point of 34 ◦C. Electrical conductivity measurement (µs/cm)are 92.7 (SAL/Cr), 105.8 (SAL/Co), 119.7 (SAL/Cu) and 245.5 (SAL/Zn). This indicates that the complexes are non-electrolytic in nature. The antimicrobial susceptibility test revealed that the metal complexes have higher inhibiting potential against the test microbes as compared to the uncomplexed Schiff base.
Keywords: Synthesis, Characterization, Antimicrobial studies, Salicylideneaniline, Metal complexes

Introduction

The synthesis of Schiff base ligands and their metal complexes is of special interest to chemistry, medicine, biological science, pharmaceutical industry and material science. The vast applications of Schiff bases and their complexes are impacted by the presence of nitrogen and oxygen atoms in the molecules of the Schiff base [1]. Schiff bases are important organic compounds that contains azomethine group(-HC=N) obtained as the condensation product of primary amine and ketone or aldehyde [2]. The azomethine nitrogen atom has a lone pair of electrons which enablesthe ligand to coordinate with a metal ion [3]. Structurally, a Schiff base is a nitrogen analogue of an aldehyde or ketone in the carbonyl group which has been replaced by an imine or azomethine group [4]. The nitrogen atom in the azomethine group is basic and exhibits pi-acceptor properties. For this reason, Schiff bases have been considered important ligands due to their coordination chemistry and they can be easily prepared and linked with different kind of metal ions [5].

They are used in food and dyes industries, material science, agriculture, analytical chemistry, catalysis, polymer sciences, medical and biological science as antimicrobial agents, anticancer, antiseptic, antidiarrhea, antiulcer agents, corrosion inhibitor and as myocardial perfusion imaging agents [6].

In recent years attention has been shifted to preparation of Schiff base because of their important activities against various micro-organisms. However, the antimicrobial activity varies from one structure of the amide and aldehyde to another. Structures involving heterocyclic ones tend to exhibit greater antimicrobial activity. Thus, some Schiff bases do not act against certain micro-organism and in such cases the antimicrobial activities are enhanced through complexation [7]. This enhancement is due to the ability of Schiff bases to form stable complexes with metal ions. The growing interest of biological activities of Schiff base has led to the synthesis of N-salicylidene-Aniline and its derivatives which exhibit thermosalient effect and properties against micro-organism and corrosion [8].

This research article highlights the synthesis, characterization and antimicrobial activity of N-salicylideneaniline and its Cr^{3+}, Co^{2+}, Cu^{2+} and Zn^{2+} metal complexes.

MATERIALS AND METHODS

Reagents and Solvents

The reagents and solvents used were salicylaldehyde, aniline, colbalt(II)chloride,copper(II)chloride,chromium(II)chloride, zinc(II)chloride,ethanol, acetone, methanol, diethyl ether andn-butanol. All the chemicals were of analytical grade obtained from BDH and used without further purification.

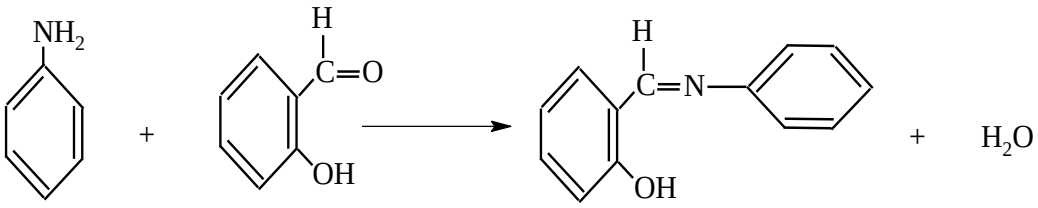
Apparatus and Instruments

The apparatus and instruments used include werePw 184 weighing balance, microwave oven, Electrothermal digital melting point apparatus, Agilent Carry 300 UV - visible Spectrophotometer, carry 630 FTIR spectrophotometer and Bruker Avance III 400MHz spectrophotometer.

Methods

Synthesis of SALAN

SALAN was synthesized using the method reported by Ejelonu*et al,*[9] and Iorungwa *et al.*[10](Scheme 1). Exactly 1.76 mL (0.02mol) of salicylaldehyde and 1.92 mL (0.02mol) of aniline were separately dissolved in 20 mL ethanol and stirred vigorously. At room temperature, the two solutions were mixed and effectively stirred to ensure complete solubility. Then the mixture was placed in microwave oven set at 120W for 15 min. Oily yellow solution was obtained and allowed to cool overnight in order to obtain crystals of N-Salicylideneaniline. The crystals were washed with ethanol and dried in an oven at 40◦C.



Scheme 1: Synthesis of SALAN

Synthesis of Metal Complexes

Cr(III), Co(II), Cu(II) and Zn(II) complexes of N-Salicylideneaniline were synthesized using the method adopted by Iorungwa *et al* [10]. Exactly 2.0g of SALAN and 2.0g of the metal salt were each dissolved in 10 mL ethanol. The metal salts were added to the ligand solution in drops and stirred effectively for 1h at room temperature. The mixture was then placed in a microwave set at 120W for 30min for the complexation reaction to complete. The solution was cooled in an ice bath and the crystals obtained were dried at room temperature.

Characterization of SALAN and its Cr(III), Co(II), Cu(II) and Zn(II) Complexes

Solubility Test

The synthesized Schiff base and its Cr(III), Co(II), Cu(II) and Zn(II) were added to 10 mL each of the following solvents; distilled water, dimethyl ether, methanol, ethanol, acetone, n-hexane and shaken vigorously to determine its extend of solubility in the solvent. The entire solute dissolved to give a homogenous mixture after shaking the sample (CS). However, some samples were sparingly soluble (SS) and some were insoluble (INS).

Melting Point Determination

The melting point of SALAN and its metal complexes were determined using electro thermal digital melting point apparatus. SALAN and its metal complexes were separately ground into powder with mortar and pestle. Powdered sample each of the Schiff base ligand and metal complex were placed in different capillary tubes inserted into the heating block and heated. The temperature at which each of the samples melted was read from the digital screen and recorded as the melting point.

Molar Conductivity

The molar conductivities of SALAN and its metal complexes were obtained using HACH Sension5 for Conductivity. The conductivity measurement was performed to check the electrolytic nature of the Schiff base and its metal complexes.

Infrared Spectra Data

Fourier-transform infrared (FTIR) spectra of the synthesized Schiff base and its metal complexes were obtained using carry 630 FTIR spectrophotometer. SALAN along with it metal complexes was placed on the slide in the sample chamber of the spectrophotometer with the distance of 7 mm between the slide and the lens. The spectrophotometer scanned the sample and suitable peaks were obtained (resolution: 8, 32 sample scan, range 4000 – 650 cm^{-1}).

Electronic Spectra Data

The ultraviolet-visible spectra of the prepared SALAN and its metal complexes were obtained using acetone as solvent from Agilent Carry 300 UV - visible spectrophotometer in the visible region between 200 – 800 nm using a glass cell of 1cm path length.

^{1}HNMR Spectra

The ^{1}HNMR spectral of SALAN was recorded using Bruker Avance III 400MHz spectrophotometer at room temperature, using TMS as reference. Chemical shift values (δ) were reported in parts per million (ppm) relative to

TMS and coupling constants are given in Hz. The solvent used for these measurements was $CdCl_3$. Multiplicities are given as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), and multiplet (m).

Antimicrobial Activity Study

The antimicrobial activity studies of SALAN and its metal complexes were performed in the Microbiology Laboratory of Joseph Sarwuan Tarka University Makurdi. SALAN ligand and the metal complexes were screened against five (5) bacteria (*Staphylococcus aureus, Salmonella typhi, Escheriachia coli, Bacillus specie and Proteus specie*) and three (3) fungi (*Aspergillus specie, Microsporumspecie and Trichophytumrubrum*) by agar well diffusion technique as reported by Iorungwa *et al.,* [10].

Antimicrobial Susceptibility Testing

The microorganism cultures were incubated by inoculation into agar nutrient for 24 h at 38 °C for bacteria and 48 h at 34 °C for fungi. Ciprofloxacin (control), fluconazole (control), Schiff base ligand and each of the metal complexes was dissolved in DMSO and solutions of the concentration were prepared separately. A sterile cork borer of 6 mm in diameter was used to bore a well on the agar medium inoculated with microorganism. The well was filled with about 20 µL of test solution using a micropipette and labeled appropriately. The plate was allowed to stand for 30 mins to enable the test solution diffuse well into the agar. The plate was then incubated at 37◦C, 24 h for bacteria and 30◦C 72 h for fungi. During the time of incubation, the test solution diffused and the inhibition zones formed on media were measured in mm.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC)

The MIC was determined using agar-dilution technique as reported by Iorungwa *et al* [10].Varying concentrations of the SALAN ligand and the metal complexes were prepared in an agar by mixing different proportions of the Muller Hinton agar and each of the stock solutions of the ligand and the complexes. The following combinations were done 6 mL: 6 mL, 3 mL: 9 mL, 1.5 mL: 10.5 mL and 0.75 mL: 11.25 mL of antimicrobial agent and Muller Hinton agar respectively thereby giving the following percentage concentrations 50%, 25%, 12.5% and 6.25%, respectively. The organisms were spot-inoculated on all the concentrations and incubated. After 24h and 72h for bacteria and fungi incubation, the plates were checked for growth. The minimum concentration at which the organism completely inhibited was recorded as the minimum bactericidal or fungicidal concentration (MBC/MFC) while the minimum concentrations at which the organism was not inhibited but did not expand beyond the spot of inoculation was recorded as the MIC.

Results and Discussion

Physical Properties of the SALAN ligand and its Metal Complexes

The reaction between salicylaldehyde and aniline in ethanol medium produced a crystalline coloured Schiff base ligand. The synthesized SALAN ligand and chromium(III), cobalt(II), copper(II) and zinc(II) chloride produced metal complexes that where crystalline and coloured in nature with high percentage yield of about 68.3 – 85.4% and pH range of 2.3 – 5.3 (**Table 1**). The metal complexes melt in the range of 34 ◦C – 287.8 ◦C and above 400 ◦C(**Table 2**). The ligand is completely soluble in both water and organic solvents (diethyl ether, methanol, ethanol, acetone and n-butanol). The metal complexes are slightly soluble in water except the Cr(III) complex. Cr complex was slightly soluble in diethyl ether and acetone but completely soluble in methanol, ethanol and n-butanol. Co complex was completely soluble in acetone, insoluble in diethyl ether and slightly soluble in ethanol, methanol and n-butanol. Cu complex was slightly soluble in methanol, ethanol, n-butanol and acetone but completely insoluble in diethyl ether. Zn complex was completely soluble in ethanol, acetone and n-butanol, slightly soluble in diethyl ether and methanol (**Table 3**). The ligand and the metal complexes gave electrical conductivity values in the range of 82.5 – 245.5 μs/cm.

Table 1: Colour, percentage yield and pH of SALAN and its metal complexes

Ligand/Metal complexes	Colour	Yield (%)	pH
SALAN	Yellow	86.1	5.3
SALAN/Cr	Deep Green	68.3	2.3
SALAN/Co	Green	83.1	4.8
SALAN/Cu	Dark brown	71.2	2.8
SALAN/Zn	Yellow	85.4	4.1

Table 2: Melting point of ligand and metal complexes

Ligand/Metalcomplexes	Meltingpoint (°C)
SALAN	34
SALAN/Cr	Above 400
SALAN/Co	Above 400
SALAN/Cu	98.9
SALAN/Zn	287.8

INS = Insoluble SS = slightly soluble CS = Completely Soluble

FTIR Spectra Result of SALAN and its Metal Complexes

The FTIR spectra of the synthesized SALAN ligand and its metal complexes were recorded in the region of 650 – 4000 cm^{-1}(Table 3). The table indicate regions of absorptions due to C – O, C = N, O – H, N – H Stretching band. In the metal complexes, the band characterizing azomethine group shifted to 1610.2 cm^{-1} in Cr(III) and Co(II), 1591.6 cm^{-1} in Cu (II) while there is no shift of 1613.9 band in Zn(II) complex. The shift from 1613.9 cm^{-1} suggests the coordination of azomethine nitrogen to the metal ion (Iorungwa, 2020). The lowering of band is due to the reduction of electron density in the azomethine link. The band at 1274.7 cm^{-1} is assigned to phenolic C – O stretching in the ligand. In the metal complexes the band shifted to higher and lower symmetric and asymmetric bands and M – O and M – N vibrations. Comparison of metal complexes spectra with the ligand is relevant in depicting the site of coordination. The FTIR spectra of the ligand has a strong band around 1613.9 cm^{-1} which is characteristic of azomethine (C=N)

frequencies from 1274.7 cm^{-1} to 1293.4 cm^{-1} (Cr(III) complex), 1293.4 cm^{-1}(Co(II) complex), 1244.9 cm^{-1}(Cu(II) complex) and 1297.1 cm^{-1} (Zn(II) complex). The band at 3634.9 $cm^{-1}$for the ligand corresponds to O – H stretching. The spectrum of individual complex showed shift to a lower wave number indicating deprotonation of the hydrogen atom in OH of the ligand. Coordination is therefore explained to have occurred via the azomethine nitrogen and phenolic OH of the ligand molecule [11].

Table 3: Important FTIR bands of the ligand and its complexes

Ligand/Metal complexes	C – O	C = N	O – H	M – O	M – N
SALAN	1274.7	1613.9	3634.9		
SALAN/Cr	1293.4	1610.2	3034	741	
SALAN/Co	1297.1	1610.2	3038	752	682.1
SALAN/Cu	1244.9	1591	3049	834	752.9
SALAN/Zn	1297.1	1613.9	3391.9	749	

Ligand/Metal complexes	Distilled water	Diethyl ether	Methanol	Ethanol	Acetone	n-Butanol
SALAN	CS	CS	CS	CS	CS	CS
SALAN/Cr	CS	SS	CS	CS	SS	CS
SALAN/Co	SS	INS	SS	SS	CS	SS
SALAN/Cu	SS	INS	SS	SS	SS	SS
SALAN/Zn	SS	SS	SS	CS	CS	CS

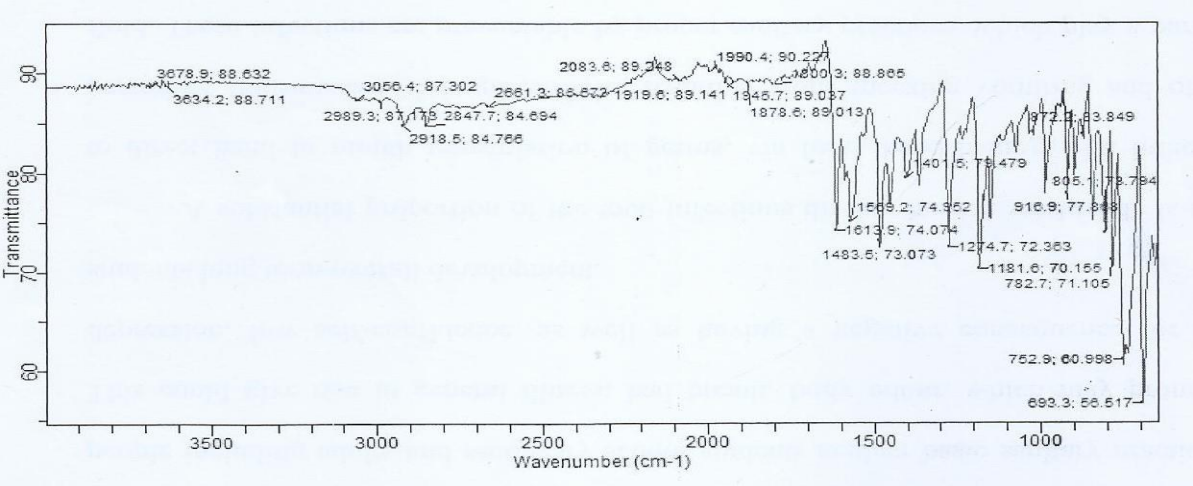


Figure 1: FTIR Spectra of SALAN

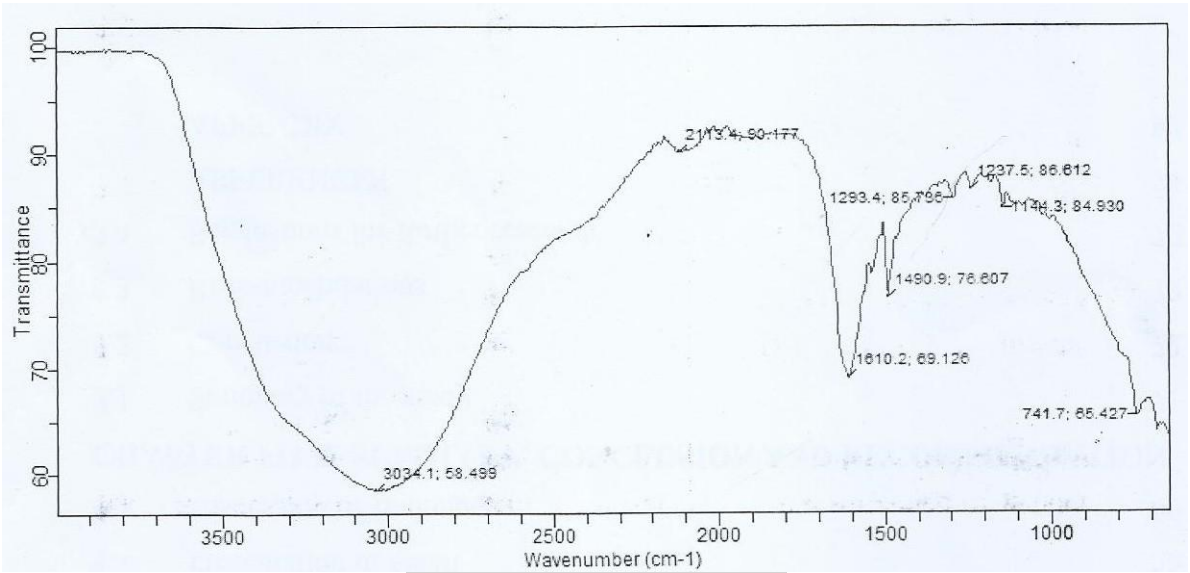


Figure 2: IR Spectra of SALAN/Cr

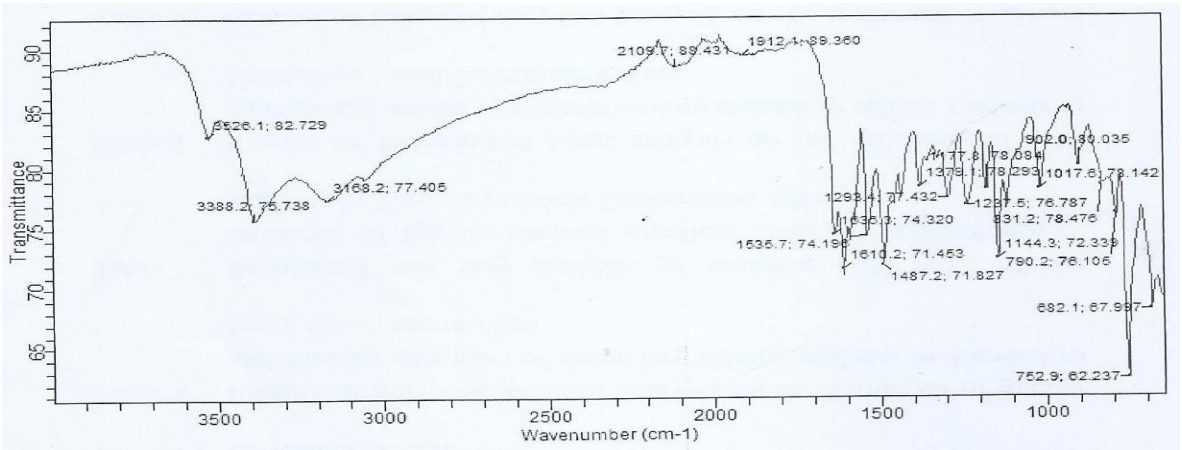


Figure 3: IR Spectra of SALAN/Co

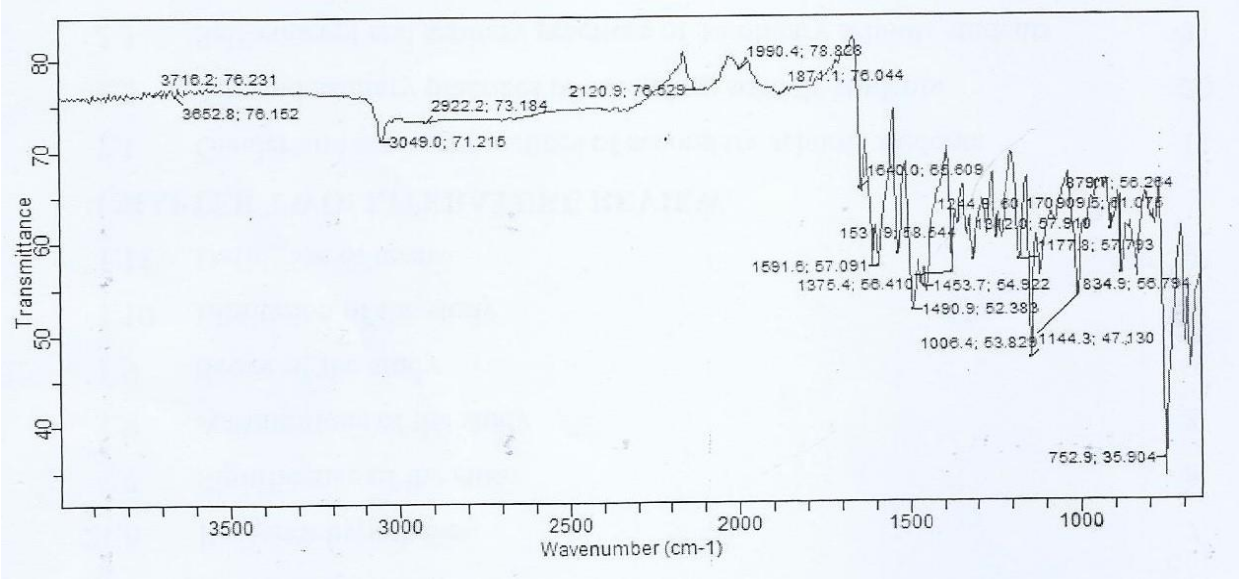


Figure 4:FTIR Spectra of SALAN/Cu

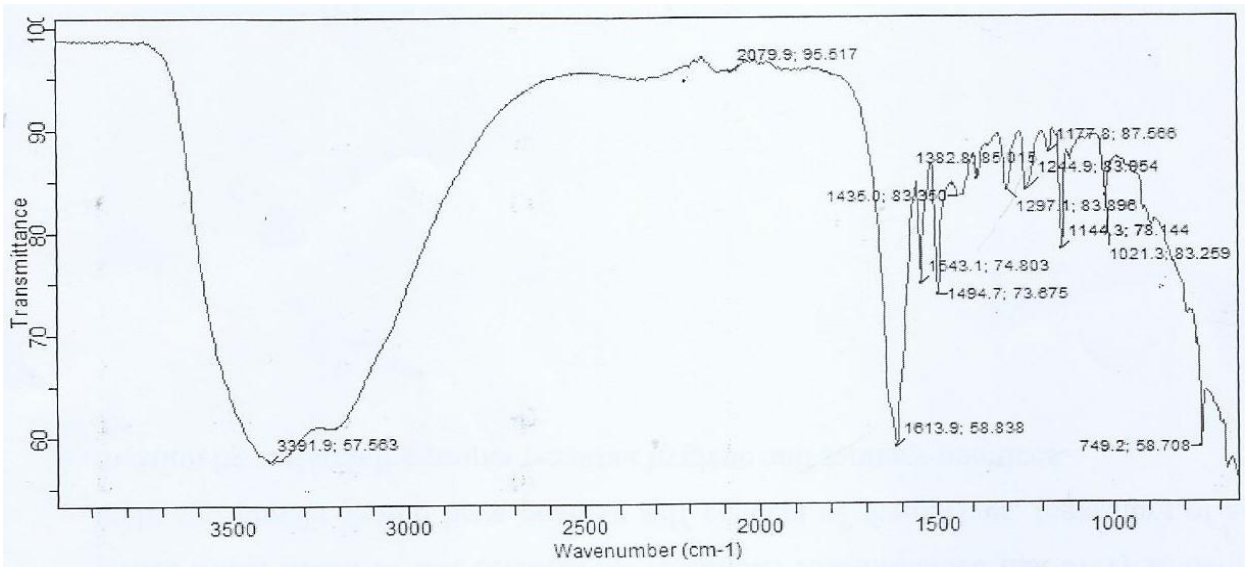


Figure 4: FTIR Spectra of SALAN/Zn

Electronic Spectra result of SALAN and its Metal complexes

The structure of the complexes was elucidated based on the absorption bands they exhibited. The electronic spectra of the SALAN ligand and the metal complexes are presented (Table 4). The most important transitions for analysis are the intense π→π* transitions (higher energy) and the (lower energy) n→π* transitions.

The UV-visible absorption spectra of all the metal complexes to some extent showed a shift of absorption band to longer wavelength (red shift) compared to that of the ligand which indicates complexation [12 – 13]. The absorption spectra of Cu(II) and Zn(II) complexes showed similarity in their structures and generally showed the characteristic band of the ligand with some changes both in frequencies and intensities.

The electronic spectrum of the ligand showed a single band at 268 nm (37,313 cm^{-1})due to π→π* transition of the chromophore (-C=N-) partly due to conjugated π bond of phenyl ring [14 15].The electronic spectrum of the Cr(III) complex showed a band at 425 nm (23,529 cm^{-1}). The low energy is associated with n-π* transitions

between the lone pair of electrons of p-orbital of N-atom in C=N group and a Cr^{3+}. The electronic spectrum of Co(II) complex showed a single band at 674 nm (14,837 cm^{-1}). The lower energy transition is assigned to n-π*. The electronic spectrum of Cu(II) complex showed a band at 273 nm (36,630 cm^{-1}) assigned to π→π*. In the electronic spectrum of Zn(II), a single band was seen at 271 nm (36, 900cm^{-1}) assigned to toπ→π*

Table 4: Electronic and magnetic moment data for the ligand and its metal complexes

Ligand/Metal complexes	λ_{max} (nm)	λ_{max} (cm^{-1})	Assignment	Geometry
SALAN	268.00	37,313	π - π^*	
SALAN/Cr	425.00	23,359	n → π^*	
SALAN/Co	674.00	14,837	n → π^*	
SALAN/Cu	273.00	36,630	π - π^*	
SALAN/Zn	271.00	36,900	π - π^*	

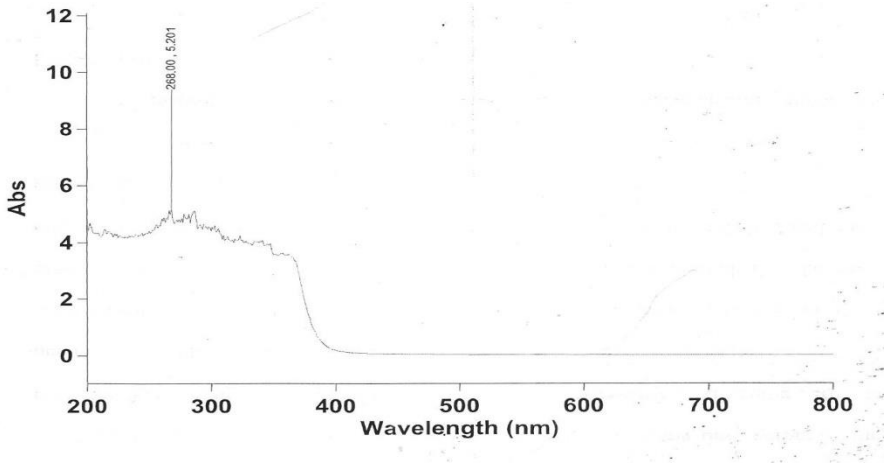


Figure 6: Electronic Spectral of SALAN

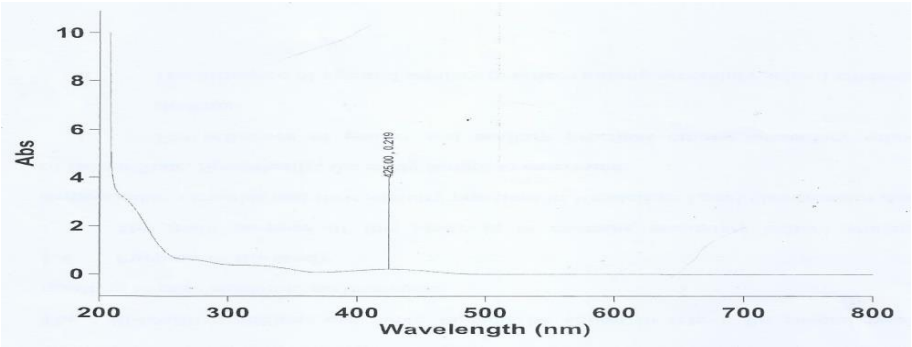


Figure 7: Electronic Spectral of SALAN/Cr

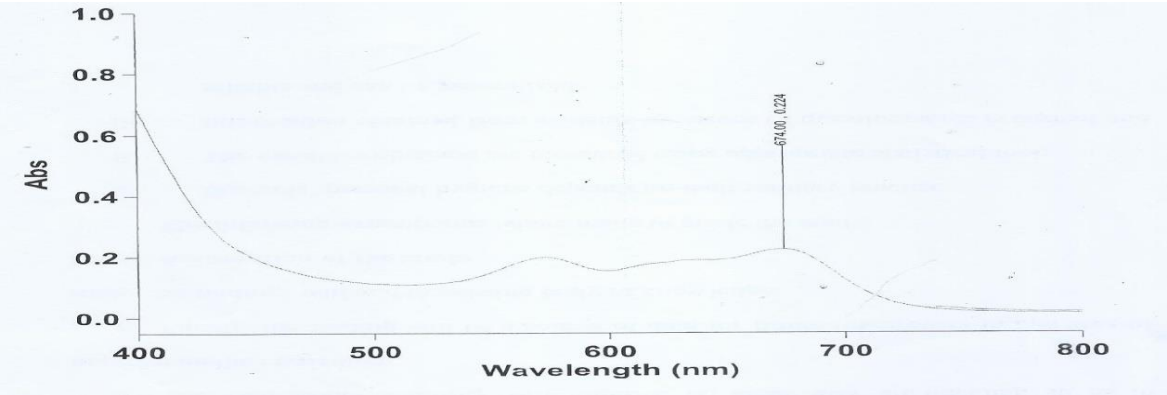


Figure 8: Electronic Spectral of SALAN/Co

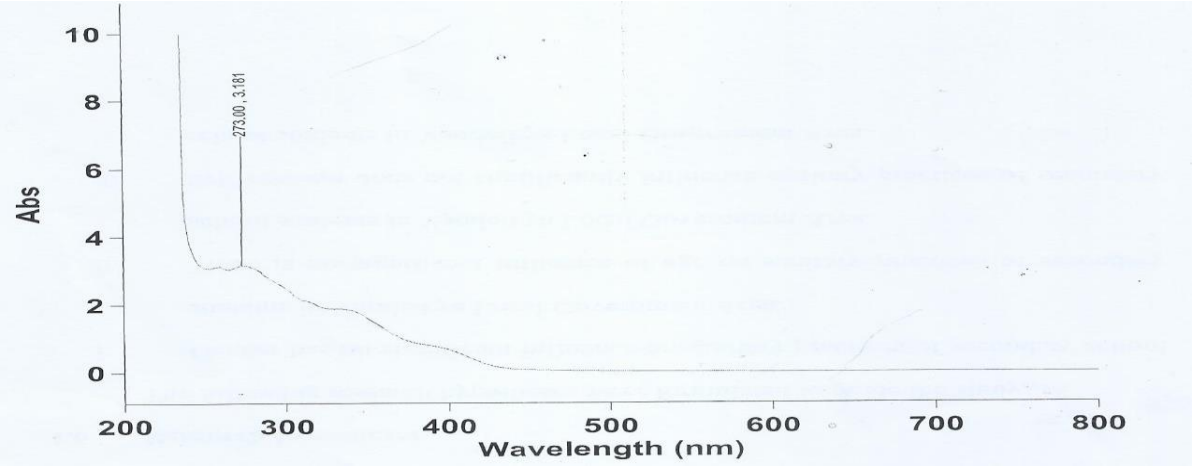


Figure 9: Electronic Spectral of SALAN/Cu

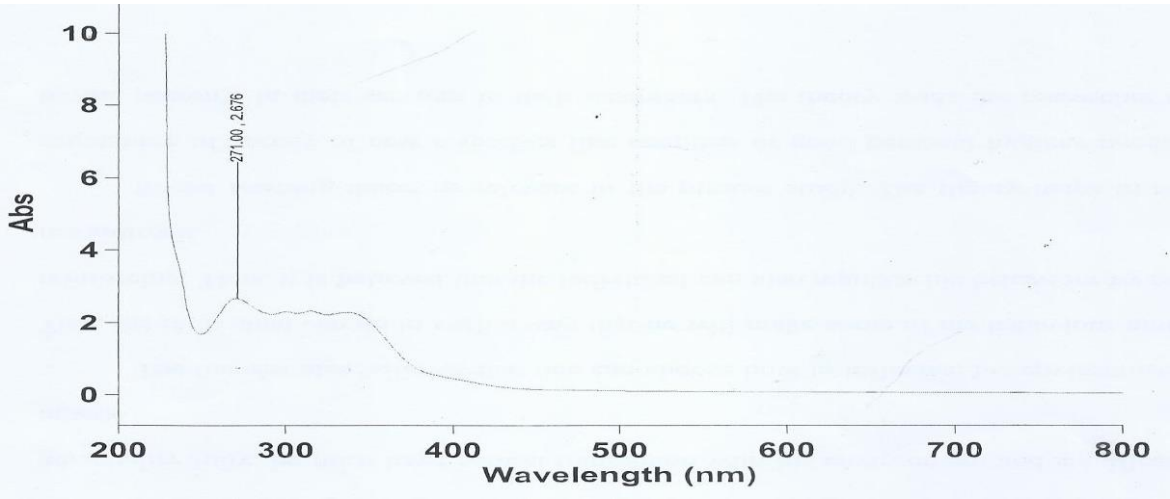


Figure 10: Electronic Spectral of SALAN/Zn

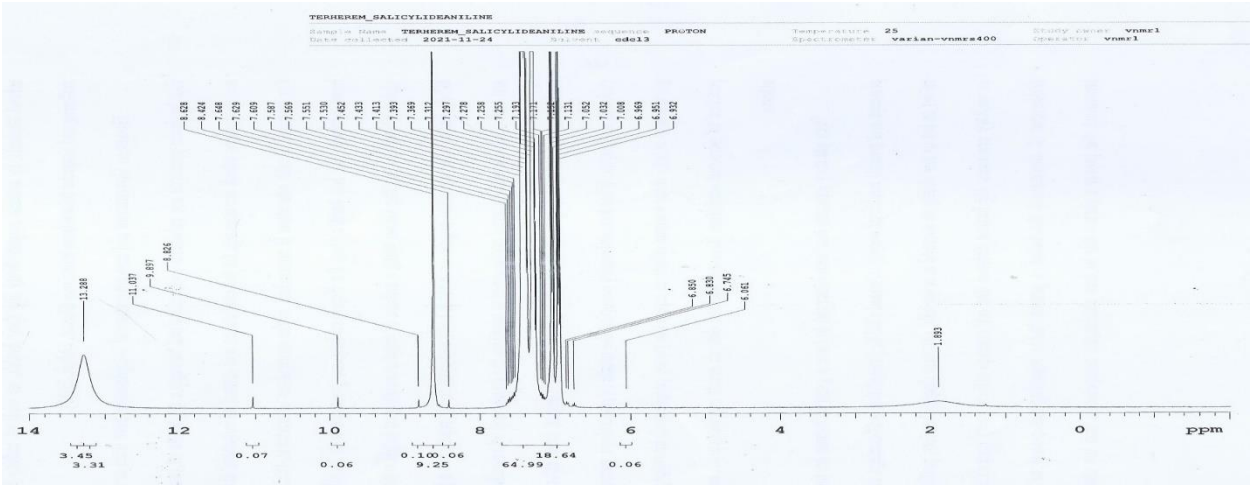


Figure 11: ^{1}HNMR of SALAN

Zone of Inhibition

The zone of inhibition of SALAN and its Cr^{3+}, Co^{2+}, Cu^{2+} and Zn^{2+} complexes against the microbial species used, at 150 μg/mL and 250 μg/mL measured in millimetre is reported (Table 4). This was evaluated by Agar-well diffusion technique described by Iorungwa *et al*, [10]. The zones of inhibition of the ligand and complexes against the microbial species are compared using a statistical 3D bar graph (Figure 12) to elucidate their potential activities. On comparing the biological activity of the Schiff base and its metal complexes with the standard bactericide and fungicide, it showed that someof the metal complexes had good activity as compared to the standard drugs and all the complexes were more active than the ligand. The ligand showed no activity against *Escherichia Coli,* Cr(III) complex showed no activity against *Aspergillus specie*, Zn(II) complex showed no activity against *Escherichia coli, Bacillus specie, Proteus specie* and *Aspergillus specie.*

At 150 μg/mL and 250 μg/mL the Schiff ligand showed greater activity against *Trichophytum rubrum* (fungi) and *Staphylococus aureus* respectively. Cr(III) complex showed greater activity against *Salmonella typhi, Proteus specie and E. coli* respectively. Among the complexes, Cu(II) complex showed greater against bacteria and fungi. Apart from P*roteus specie*, the complexes had activity against all other microorganisms as compared to the standard drugs.

Minimum Inhibition Concentration

From the results presented in Table 9, the ligand and most of the metal complexes exhibited good activity against microorganisms at (MIC≤ 150) compared to the ligand. These could become promising antimicrobial agents with potential applications in pharmaceutical industry for controlling pathogenic bacteria and fungi. Thus, qualify these compounds as suitable initiating agent for drugs formulation.

Cr(III) complex showed greater activity at low concentration of 3.13 μg/mL against S*almonella typhi,* 6.25 against*Staphyloccocus aureus*, *Bacilluss specie, Microspurum specie and Triphytum rubrum.*Co(II) complex showed minimum inhibition concentration against *Eschorichia coli*. Cu(II) complex showed MIC against S*almonella typhi.* Zn(II) complex no activity against *Eschorichia specie, Bacillus specie, Proteus specie* and*Aspergillus specie.*

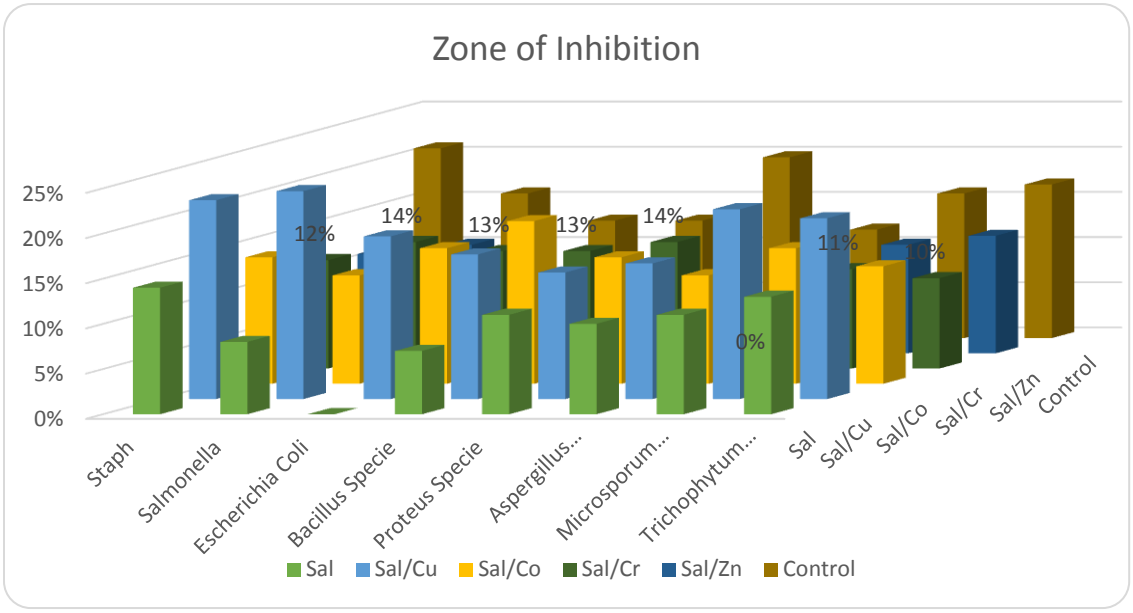


Figure 12: Zone of inhibition

Conclusion

The Schiff base ligand (SALAN) and its Cr^{3+}, Co^{2+}, Cu^{2+} and Zn^{2+} were successfully synthesized in ethanol medium using microwave assisted technique. The synthesized Schiff base and the metal complexes had gave percentage yield. The physiochemical and spectroscopic parameters were characterized in terms of pH, melting point, electrical conductivity, solubility test, UV-Vis,FTIR and ^{1}HNMR spectrophotometric analysis. The result of characterization revealed complexation between the Schiff base ligand and its metal complexes. The ligand and its metal complexes were assayed for their anti-microbial effect against five bacteria and 3 fungi and they showed great inhibition against the growth of the test microbes.

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