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Synthesis, Characterization and Antimicrobial Activities of Mixed Ligand Divalent Metal Complexes of N-Phenyl Dithiocarbamate with Alanine - Schiff Base

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Abstract: Synthesis, characterization and antimicrobial activities of mixed ligand metal complexes of N-Phenyl dithiocarbamate with schiff base of Mn(II), Fe(II), and Pb(II),Co(II),Ni (II),Cu (II),Zn(II) and Cd (II) metal ions were carried out in this study. The ligands and their respective metal complexes were characterized by FTIR, UV spectroscopy, and elemental analysis. The data from the elemental analysis suggest a good agreement between the calculated and the experimental values for the elements viz; C, H, N and S. Also, the UV-Visible and FTIR spectroscopic data revealed that the metal complexes coordinate through the nitrogen and oxygen atoms of the alanine Schiff base and via the two sulphur atoms of the N-Phenyl dithiocarbamate ligands which serve as bidentate sites for the ligand. The antimicrobial 1 studies revealed that the synthesized ligands and their metal complexes recorded strong potency against the pathogens being investigated, suggesting that they could serve as good antimicrobial agents against the selected pathogens.

Keywords: Mixed ligand; Metal complexes; N-Phenyl dithiocarbamate; Alanine schiff base; Antimicrobial activity.

1. Introduction

Coordination complexes with mixed ligands are of considerable importance in the field of metalloenzymes, and are known to possess various biological activities [1]. Dithiocarbamates are highly versatile monoanionic chelating ligands which form stable complexes with all the transition elements, the attribute of being able to form complexes with all metal is being attached to the presence of the sulphur atoms and the delocalization of a positive charge from the metal to the outside boundary or the surface of the complexes [2, 3]. They are easily prepared from primary or secondary amines and depending upon the nature of the cation and a show good solubility in water and polar organic solvents.[4].

Dithiocarbamates are lipophilic and generally bind to metals in a symmetrical chelate fashion but examples of other coordination modes are known with the monodentate and anisobidentate models being most prevalent. [5].

Aniline is an organic compound with a phenyl group attached to an amino group. Aniline is the prototypical aromatic amine, a precursor of many industrial chemicals, mainly used in the manufacture of methylene diphenyl diisocyanate, a precursor to polyurethanes [6].

Schiff base play important roles in coordination chemistry as they easily form stable complexes with most transition metal ions. Many Schiff bases have biological activities which include antifungal and antibacterial activities, metal complexes have higher antimicrobial activity than free ligand as complexation increases the antimicrobial activity [6, 7].

Metal complexes of Schiff bases have been investigated because they play an important role not only in the development in coordination chemistry, but also in catalysis, enzymatic reaction, magnetism, molecular architectures [8] and exhibit biological application and pharmaceutical activities. Chohan, *et al.* [9]. The pharmacological activities of these metal compounds depend on the metal ion, its ligand and the structure of the compounds. These factors are responsible for reaching them at the proper target site in the body [10].

Schiff bases have gained an important role due to their physiological and pharmacological activities. It is also used in catalysts, antioxidative activity, for medicine as antibiotics, anti-inflammatory agents and industry for anticorrosion properties [8].

The Schiff base ligand has less activity in comparison with their complexes against micro organisms. This is due to the fact that the metal chelates have more antibacterial activity than uncomplexed ligand because of chelation theory [11]. It has been maintained that schiff base ligands and their the metal complexes are considerably active against the growth of the micro organism [11]. This paper is aimed at the synthesis, characterization and antimicrobial activities of mixed ligand Divalent metal complexes of N-Phenyl dithiocarbamate with schiff base .

2. Materials and Methods

2.1. Reagents and Solvents

All reagents and solvents were of analytical grade and used without further purification, and they were Sigma-Aldrich and Schlau products. These include; toluene, diethyl ether, ethanol, chloroform, propan-2-ol, xylene, DMSO, acetone, aniline, ammonium hydroxide, carbon disulphide, diphosphorus pentoxide, aqueous ammonia, sodium hydroxide, salicylaldehyde, deionized water, lead chloride, manganese sulphate and iron sulphate.

2.2. Synthesis of N-Phenyl Dithiocarbamate Ligand

N-phenyl dithiocarbamate ligand was synthesized by measuring 9.20 cm³ of aniline into two-necked round bottom flask in an ice bath placed on a magnetic stirrer. This was followed by the of 25.00 cm³ ammonia solution (NH₄ OH) into the flask. After 10 minutes, 6.50 cm³ carbon disulfide solution was added drop-wisely to the mixture in the reaction flask. The reaction was allowed to continue with stirring for 7 hrs. At the completion of 7 hrs, the product formed was filtered and washed with deionized water, and dried using diphosophorus pentoxide and sodium hydroxide in vaccum dessicator.

2.3. Synthesis of Schiff Base Ligand

Schiff base ligand was synthesized by adding 2.2 ml of salicylaldehyde and 2.0 ml of aniline together into a onenecked round bottom flask.10 ml of ethanol and boiling chips was added to the mixture in the flask and then allowed to reflux for 1 hour on addition of 10 drops of 5% HCl solution. After 1hour, another 10 ml of ethanol was added and then the refluxed mixture in the flask was transferred into a clean beaker, leaving the boiling chips behind. The mixture in the beaker was allowed to cool to room temperature and then placed in an ice bath for 10 minutes. As the temperature of the mixture reduced, small chunks of ice was added and the inner wall of the beaker was scratched with a glass rod. The beaker containing the mixture was kept chilled, and more chips of ice were added. The mixture was kept for 5 minutes in the ice bath as soon as the crystals began to form. The product containing the crystal was filtered using pipette filtrations and washed twice with 10 ml of water. The residue was then recrystallized by dissolving it in warm ethanol, followed by drop wise addition of water with intermittent scratching of the inside walls of the container with a stirring rod. Product formed was filtered and dried over silica gel in a vaccum dessicator [12].

2.3.3.1. Synthesis of Divalent Metal Complexes

The metal complex was prepared by addition of 1.00mmol (0.197g) aniline schiff base ligand with continuous stirring in 30.00 cm³ deionized water in an analytically clean beaker for few minutes. 1.0mmol (0.04 g) NaOH dissolved in 20 cm³ deionized water was added to the beaker, and the mixture was stirred for one hour. 1.00 mmol metal salts of [(0.3620g of lead (II) chloride (PbCl₂), 0.151g of manganese (II) sulphate monohydrate (MnSO₄, H₂O), and 0.2832g of iron (II) tetraoxosulphate (VI) heptahydrate (FeSO₄. 7H₂O) , Cobalt (II)chloride hexahydrate(CoCl₂.6H₂O),Nickel(II) nitratehexahydrate(Ni(NO₃).6H₂O), Copper(II)chloride (CuCl₂.2H₂O), Zinc(II)chlorideZnCl₂ and Cadmiun(II) chloride monohydrate (CdCl₂.H₂O) weighed separately and dissolved in 30 cm³ of deionized water and were respectively added to the individual mixture while stirring continued for about 1 minute. The mixture was further allowed to stir for two hours. Finally, 1.00 mmol (0.1860g) aniline dithiocarbamate ligand dissolved in 30 cm³ deionized water was added to the mixture. The resulting mixture was then allowed to stir for three hours. The precipitate formed was filtered and washed with water, and the residue was kept in a clean crucible, and dried over silica gel in a dessicator until constant weight obtained.

 $\label{eq:state-1.1.} \begin{array}{l} Figure -1.1. \ Divalent \ metal \ complexes \ of \ N-Phenyl \ dithiocarbamate \ with \ schiff \ base \ . \\ Where \ M= \ METAL, \ Fe(II), \ Pb \ (II) \ , \ Mn \ (II), \ Co(II), \ Ni(II), \ Cu(II), \ Zn(II) \ and \ Cd \ (II). \end{array}$



Aniline metal complex

3. Antimicrobial Activities

The antimicrobial study was done to determine the antimicrobial properties of the synthesized ligands and their respective mixed ligand metal complexes. It was carried out on ten different fungi and bacteria species respectively.

Ten different bacteria and fungi species were collected from Obafemi Awolowo University Ile-Ife Teaching Hospital (OAUTH) Nigeria, they were sub-cultured on nutrient agar for bacteria and potato dextrose agar for fungi. Sterile swabs were then used to seed the set agar plates with each test organism, then a 8 mm diameter cork-borer was used to bore well, each well was then filled with the solution of each test compound or metal complex.

Each of the compounds was made into solution by constituting it with 30% dimethyl sulphuroxide (DMSO). For bacterial study, concentrations of 30mg/ of 30% DMSO, 50 mg/ ml of 30% DMSO of ampicillin and mycotine were used (as positive control) for both bacteria and fungi, and 30% DMSO as negative control. The plates were then inoculated for 24hrs, during this period the compound and antibiotics diffused into the growth medium. For fungal test each of the test organisms were transferred to a liquid medium nutrient broth, they were inoculated for 18hrs and then used for the tests.

4. Results and Discussion

The melting point results obtained were observed to range between (261-290) °C for the synthesized metal complexes. Solubility tests results for the synthesized ligands and their metal complexes of [Mn (II), Fe (II), Pb (II),Co(II),Ni(II), Cu(II), Zn(II) and Cd(II)] were obtained using various solvents. These included dimethyl sulphuroxide (DMSO), ethanol, toluene, trichloromethane, etc. available data from the solubility tests revealed that the *N*- phenyl dithiocarbamate ligand was soluble in both water and organic solvents, while the *N*-salicyldehyde schiff base and the metal complexes were mostly soluble in organic solvents. Their degree of solubilities in organic solvents also varied from metal complex to metal complex. These findings suggest that the schiff base and different metal complexes are non polar compounds [8].

Metal complex	Colour	%yield	M. pt ⁰ C	С	Н	N	S
[Mn(II)Anil-PhDTC	Dark brown	57	264	56.15	3.75	6.58	14.96
				(56.13)	(3.74)	(6.55)	(14.96)
[Pb(II)Anil-PhDTC	Black	63	261	56.91	3.85	6.65	15.14
				(56.75)	(3.78)	(6.62)	(15.97)
[Fe(II)Anil-PhDTC	Brown	55	263	56.81	3.92	6.57	15.22
				(58.70)	(3.76)	(6.64)	(15.21)
[Cu(II)Anil-PhDTC	Dark brown	67	292	56.15	3.75	6.52	14.95
				(56.13)	(3.74)	(6.55)	(14.97)
[Co(II)Anil-PhDTC	Black	54	290	56.91	3.82	6.64	15.15
				(56.97)	(3.78)	(6.62)	(15.13)
Ni(II)Anil-PhDTC	Dark brown	52.5	286	56.17	3.62	6.58	15.01
				(56.15)	(3.65)	(6.52)	(15.05)
[Zn(II)Anil-PhDTC	Pale yellow	48	268	55.89	3.73	6.62	15.13
				(55.94)	(3.81)	(6.64)	(15.16)
Cd(II)Anil-PhDTC	Milk yellow	52	273	50.37	3.36	5.88	13.43
				(50.41)	(3.42)	(5.92)	(13.45)

Table-II. Solubility Tests Of	The Ligands And	Metal Complexes.
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Solvent	Alanine Schiff base	N-pheyl DTC	Mn(II) Complex	Pb (II)	Fe (II)	Cu(II)	Co(II)	Ni (II)	Zn (II)	Cd (II)
Water	Soluble	Sparingly Soluble	Insoluble	Insoluble	Insoluble	Insoluble	insoluble	Insoluble	Insoluble	Insoluble
Toluene	Sparingly soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Insoluble	Soluble	soluble	Partially Soluble	Sparingly Soluble	Insoluble
Chloroform	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Soluble	soluble	Sparingly Soluble	Sparingly Soluble	Insoluble
Ethanol	Soluble	Soluble	Partially Soluble	Sparingly Soluble	Soluble	Soluble	Soluble	Partially Soluble	Partially Soluble	Soluble
Xylene	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble	Sparingly Soluble	Sparingly Soluble	soluble	Sparingly Soluble	Sparingly Soluble	Insoluble
Acetone	Soluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Sparingly Soluble	Soluble	Soluble	Insoluble	Soluble	Sparingly Soluble
Diethylethe r	Soluble	Soluble	Partially Soluble	Sparingly Soluble	Sparingly Soluble	Soluble	Soluble	Sparingly Soluble	Soluble	Soluble
Propan-2-ol	spaingly	Soluble	Partially Soluble	Sparingly Soluble	Sparingly Soluble	Soluble	Soluble	Sparingly Soluble	Sparingly Soluble	Insoluble
DMSO	Soluble	Soluble	Soluble	Sparingly Soluble	Sparingly Soluble	Soluble	Soluble	Soluble	Soluble	Soluble

Metal complexes absorb radiation and the transitions obtained are attributed to the excitation of the metal ion electrons [13]. The results of the UV-Visible spectroscopic studies revealed absorptions at 257,332nm, 250,340nm and 252,335nm for the Mn (II), Fe (II), and Pb (II) complexes respectively. The absorptions are attributed to d-d transitions in the metal ions. The electronic spectra of the Co(II),Cu(II) and Ni(II) complex as shown in table(III). The complexes Co(II) and Cu(II) show sharp peaks of high intensity at 815 and 812 nm and Ni(II) complex has two bands at 330 and 250nm.It is evident that both complexes shift to higher wavelengths indicating the chromophoric group of the ligand is involved in bonding with the metal ions in the complexes [14]. The absorptions are attributed to d-d transitions in the metal ion in the electronic spectra of the Zn(II) and Cd(II) complex shown in the table above. For Zn(II) complex, a single band was observed. The complex showed a sharp peak of high intensity at 450 nm. For Cd(II) complex, a single band was also observed. Cd(II) complex showed a single sharp peak of high intensity at 720 nm. When compared with those of the free ligands, we observe that the peaks at 720 and 450 nm all shift to higher wavelengths indicating the chromophoric group of the ligands is involved in bonding is involved in bonding with the metal ions in the metal ions in the complexes [15, 16].

The FTIR data obtained showed absorption peaks at 1588 cm⁻¹, and 971 cm⁻¹, which are attributed to the v(C-N), and v(C-S) absorptions respective in the ligands. These absorptions were found to occur at higher wave numbers in the synthesized metal complexes (1600-1636) cm⁻¹ for the v(C-N) absorptions, and (942-978) cm⁻¹ for the v(C-S) absorptions respectively (Table III). The single band at the region, (942-978) cm⁻¹ suggests a bidentate coordination mode between the dithiocarbamate ligands and the metals [17]. The v(C=C), v(C-O) absorption peaks for the aromatic ring, and the phenolic moiety were found at 1491 cm⁻¹,1482 cm⁻¹ for the schiff base and dithiocarbamate ligand, and 1278 cm⁻¹ for the dithiocarbamate ligand. However N(C=C) absorptions were observed at lower wave numbers in the metal complexes 1478 cm⁻¹(Mn²⁺), 1462 cm⁻¹(Fe²⁺), and 1465 cm⁻¹ (Pb²⁺). The phenolic v(C-O) absorptions were obtained at 1332 cm⁻¹(Mn²⁺), 1330 cm⁻¹(Fe²⁺), and 1336cm⁻³(Pb²⁺) respectively [6]. The bands shown at 3319,1582 and 1616cm-1 were assigned to N-H vibration of amides. The bands corresponding to the v(C-S) vibration show absorption at 1081,1038,1093 and 1084cm⁻¹ in the ligand and Co(II), Cu(II) and Ni(II) complexes

respectively. The shift in the absorption band of the free ligand to those observed in the metal complexes is a result of the coordination between the metal ions and the two sulphur atoms of the (ligand) N-phenyl dithiocarbamate which serve as bidentate sites for the ligand, Furthermore, the shift observed at the absorption bands 3319,1582 1616cm-1 representing the N-H vibrations in the free ligand and the metal complexes respectively is an indication of the coordination that stretches between the nitrogen atoms of the Schiff base ligand to the central metal ions in the respective complex.

	Uv- visible (max (nm)			IR SPECTUM CM- ¹				
COMPLEXES		V(M=O)	V(C-N)	(C-S)	V(C=C)	V(C-N)	V (M-S)	N-H
[Mn(II)Anti-phDTC]	332,251	447	1600	1055	1478	1332	942	1602
[Pb (II) Anti-phDTC]	340,250	425	1636	1062	1465	1336	952	1578
Fe(II) Anti-phDTC]	335,252	438	1616	1089	1462	1330	978	1579
Cu(II) Anti-phDTC]	815	450,446	3319	1038	1642	1153	97	3319
Co(II) Anti-phDTC]	812	458,470	1583	1093	1491	1353	931	1582
Ni(II) Anti-phDTC]	330	456	1604	1084	1464	1352	920	1604
	250							
Zn(II) Anti-phDTC]	450	433	1592	1085	1484	1278	995	1608
Cd(II) Anti-phDTC]	720	466	1580	1090	1487	1337	942	1581
Alanine(Ligand)	375,335,251	-	1578	1065	1491	1353	-	1588
phDTC] (Ligand)	370,340,250	-	1588	1081	1482	1278	911	-

Table-III.	Spectrosco	pic Studies	of Metal M	lixed Ligand	Complexes
				6	

From the FTIR data table III, the bands shown at $1602 - 1588 \text{ cm}^{-1}$ on careful comparism were assigned to v(N-H) vibration of primary or secondary amines or amides. The bands corresponding to the v(C-S) vibration show absorption at 1065, 1081 and 1090 1085,1084,1093,1038,1089,1062 and 1055 cm⁻¹ in the ligand and metal complexes respectively. This shift in the absorption band of the free ligand to those observed in the complexes is a result of the coordination between the ligand and the metal ion at the two sulfur atoms of the *N*-phenyl dithiocarbamate ligand which serve as bidentate sites for the ligand. In the same way, the shift observed at the absorption bands 1602, 1578, 1579, 3319, 1582, 1604, 1608, 1581 and 1588 cm⁻¹ representing the N–H vibrations in the free ligand and the metal complexes respectively is an indication of the coordination that stretches between the nitrogen atoms of the Schiff base ligand to the central metal ion in the respective complex [14, 17].

Anti bacterial activites of the metal complexes were performed on following bacteria and the results obtained were shown in table IV. The selected pathogens include *Escherichia coli, Salmonella typhia, Strephylococcus aureus, Streptococcus pneumonia ,Shigella flexneri, Vibro cholera, Klebseilla pneumonia ,Proteus vulgaris,* and *Salmonella typhii.* The zone of inhibition (mm) was compared with the standard drug (ampicillin) which was used as a positive control agent. DMSO (Dimethylsulphuroxide) was used as negative control which gave no activity against all test microorganism/DMSO.Results obtained from the antibacterial tests, showing that the synthesized mixed ligand showed good activity against the selected bacteria, *Escherichia coli, Straphylococcus aureus and Streptococcus pneumonia* when compared with the standard drug. (Table IV).The ligands and their metal complexes show positive activities against selected bacteria at 30mg/ml. The highest zone of inhibition of 22mm and 30mm were observed for the Nickel (II) complex against *Klebseilla pneumonii, Streptococcus pneumonia.* The lowest MIC values of 7.5mm was observed with Ni (II) complex against *Shigella flexneri*, Co (II) mixed ligand complexes were proactive against all the selected bacteria, the highest zone of inhibition of 31 mm is observed for the Zn(II) complex against *Klebseilla pneumonia*. The lowest MIC value of 7.5 mg/ml was observed with Zn (II) complex against *Klebseilla pneumonia*.

Organisms	MnII	Pb(II)	Fe(II)	Cu(II)	Co(II)	Ni(II)	Zn(II)	Cd(II)	Anil	PhDTC	Amp	Mycoti	DMSO
Escherichia coli	19	19	16	24	21	22	21	17	12	10	17	12	Nil
Proteus valgaris	31	23	17	26	19	16	19	23	9	12	17	14	Nil
Salmonella typhii	20	22	16	15	14	22.5	14	13	12	13	10	11	Nil
Shigella flexneri	27	22	25	24	21	7.5	27	19	10	11	14	13	Nil
Staphylococcus aureus	18	17	15	20	16	14	16	21	12	10	13	10	NIL
Bacillus subtilis	18	17	14	12	14	12	19	24	9	10	10	11	Nil
Strepto coccus	26	19	24	10	14	30	26	12	9	10	10	15	Nil
pneumonia													
Psendomonas	14	12	12	18	14	10	15	10	10	9	11	7	Nil
aeruginosa													
Vibro chlolerae	24	18	16	29	14	16	29	26	8.0	10	7	-	Nil
Klebseilla pneumonia	29	26	24	31	21	22.5	31	2.2	10	12	16	19	Nil

Table-IV. Zone of inhibition in (mm) of the metal complex against various bacteria isolated at 25mg/ml concentration.

KEY: anil =Aniline

PhDTC = Phenyldithiocarbamate

Mycol = Mycotin

DMSO = Dimethylsulphuroxide

Metal chelates have more antibacterial activity than uncomplexed ligand because of chelation in that the polarity of the metal ion is reduced to a greater extent with donor groups, due to the overlap of the ligand orbital and

 $Amp \ = Amphicillin$

partial sharing of the positive charge of the metal ion with the donor groups further increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophility of the complexes [18, 19].

Fungal like Aspergillus flavus, Aspergillus fumigates, Fasuarium Solani, Rhizopus Mucor mocedo, Monilia Americana, Trichoderma viride, Saccharomycodis ludwigi, Aspergillus niger and Candida albicaus were selected micro-organisms to assess the potency of the metal complexes or compounds. The disc dilution method was used to assess the ability of the compounds prepared to inhibit the growth of the fungi species. The zone of inhibition (mm) was compared with the standard drug (mycotine) which also acted as the positive control agent. DMSO was used as negative control and it gave no activity on all the fungi species. The metal complexes of Pb(II), Cu II Zn II and Cd II showed good anti fungi activities against the selected fungi species while the rest metal complexes have shown moderate inhibitions between 12mm - 25mm against the selected organisms within the lowest MIC values of 12.5mg/ml.

The Cu (II) complex show more antifungal activity against the selected pathogens than Ni (II) complex. The highest zone of inhibition of 37.3 mm can be seen with Cd(II) complex against *Aspergillus funmigatus* and the lowest zone of inhibition with *Trichaderma viride* which recorded 10mm within the lowest MIC values of 12.5 mg/ml concentration. The Cd(II) and Zn(II) complexes show moderate antimicrobial activity against the selected pathogens. The detailed results as shown in table V.

Organisms	Mn(II)	Pb(II)	Fe(II)	Cu(II)	Co(II)	Ni(II)	Zn(II)	Cd(II)	(Al	(Ph DTC)
Aspergillus Flavus	12.5	12.5	10.8	13.5	12.4	12.0	12.5	12.5	10.0	8.5
Aspergillus funmigatus	12.5	37.5	25.0	12.5	12.0	11.0	37.5	37.5	8.0	9.5
Fusarium solani	12.5	12.5	12.5	29.4	22.0	13.0	12.5	12.5	7.8	10.5
Rhizopus stolon	12.5	12.5	10.6	24.0	13.5	25.0	25.0	12.5	NIL	NIL
Mucor mucedo	12.5	12.5	25.0	28.0	21.0	11.0	12.5	12.5	10.5	9.5
Monilia Americona	12.5	12.5	12.5	17.0	20.0	17.0	12.8	25.0	7.5	10.5
Trichoderma Viride	10.4	37.5	27.5	15.3	10.0	12.0	10.0	9.8	8.5	9.5
Saccharo mycodis ludwigii	12.5	25.0	25.0	12.7	15.0	14.0	12.5	12.5	7.8	8.0
Aspergillus niger	12.5	12.5	12.5	24.0	13.0	12.0	37.5	37.5	10.5	7.5
Candida albicaus	12.5	25.0	25.0	28.0	22.0	18.0	12.5	12.5	10.0	9.5

Table-V. Antifungal Activity of Metal Complexes at the Minimum Inhibitory Concentration of 12.5 mg/ml

The ligands showed potential activities which varied from high to moderate (Table V). The metal complexes show positive activities against selected fungi isolates at 12.5 mg/ml concentrations than the parent ligands.

5. Conclusion

The results obtained so far have indicated that metal complexes are non-polar in nature, d-d transitions were observed for metal complexes in the visible regions. Metal complexes were found to have coordinated through sulphur atom of the alanine Schiff base and nitrogen via oxygen atoms of the N-Phenyl dithiocarbamate ligands while metal complexes were found to be potent against the selected fungi and bacteria.

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