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Research article

# Antimicrobial and antioxidant studies of Schiff base, 2-(2-hydroxy-2-methyl-1-phenylpropylidene) hydrazine carbothioamide and its mixed ligand cd (II) complexes

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## ABSTRACT

New mononuclear mixed ligand Cadmium(II) complexes of the type [Cd(L)(diimine)] (1,2) [where L = 2-(2-hydroxy-2-methyl-1-phenylpropylidene)hydrazinecarbothioamide; diimine = 2,2'-bipyridine (1), 1,10-phenanthroline (2)] have been synthesized and characterized by spectroscopic techniques such as FT-IR, UV-Visible, and $^{1}H$ and $^{13}C$NMR Spectroscopy. From the investigations of spectral data, it is evident that the heterocyclic bases (2,2'-bipyridine and 1,10-Phenanthroline) act as neutral bidentate ligand coordinating to the metal ion through two nitrogen donor atoms addition to azomethane nitrogen, thiolate Sulphur, and hydroxyl oxygen in the Schiff base ligand. The synthesized Schiff base metal chelates have been screened for their anti-microbial activities using the agar well diffusion method against different selected types of bacteria and fungi in addition to antioxidant activity. The prepared Schiff base ligand and its metal complexes exhibited good antimicrobial and antioxidant activities. The antibacterial and antifungal efficacy of Complex-1 was higher than that of all the prepared compounds. In the case of antioxidant activity, Complex-2 has stronger scavenging activity among them.

**Keywords:** Cd (II) metal complexes, 2,2'-Bipyridine, 1,10-Phenanthroline, Antimicrobial activities.

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## INTRODUCTION

Because of the pharmacological properties of both the ligands and the complexes, the synthesis of transition metal complexes with thiosemicarbazone ligands has received a lot of attention [1]. Thiosemicarbazone ligands typically coordinate to metals via oxygen, nitrogen and Sulphur donor atoms in their (N, S) bidentate form or (N, N, S or O, N, S) tridentate form, resulting in metal complexes with varying molecular geometries [2]. Such complexes are especially important because of their potential biological benefits, such as anticancer [3], fungicidal [4], antibacterial [5], antiviral [6], antifungal [7,8], antitumor [9], and other biological activities [10], particularly with the first row of transition metal complexes. Free ions are more hazardous than their metal complexes due to the lack of bioavailability of the metal. Taking this into account, researchers have been looking at the biological functions of new cadmium Schiff base metal complexes [11-16]. These have also been proved as effective antibacterial agents due to the presence of active biological strains [17]. With this perspective, the current research focused on cadmium based mixed ligand metal complexes.

Because of their structural diversity and wide range of applications, mixed-ligand complexes receive a lot of attention in coordination chemistry. Mixed-ligand compounds also have a wide range of biological applications [18].

In the present work, 2-(2-hydroxy-2-methyl-1-phenylpropylidene) hydrazine carbothioamide and heterocyclic bases like 2, 2'-bipyridine and 1,10-phenanthrolinehave been used as co-ligands for the synthesis of cadmium (II) complexes was carried out. Synthesis, structures, spectroscopy and antimicrobial aspects of complexes are investigated.

## MATERIALS AND METHOD

### Chemicals

The starting materials such as 2-Hydroxy-2-methylpropiophenone, thiosemicarbazides and heterocyclic bases like 2,2'-bipyridine, and 1,10-Phenanthroline were acquired from Sigma Aldrich, India. Cd (II) salt in the form of $CdCl_2 \cdot 2H_2O$ was purchased from S. D. Fine chemicals. The organic solvents like acetonitrile, methanol, ethanol, dichloromethane was purchased from Merck, which were not subjected to any further purification process.

**Synthesis of the ligand and their Cd (II) complexes**

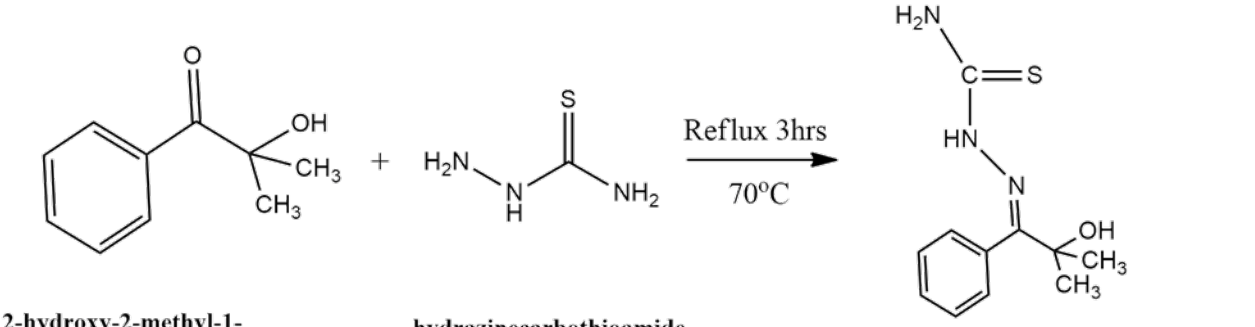
Synthesis of Schiff base ligand, 2-(2-hydroxy-2-methyl-1-phenylpropylidene) hydrazine carbothioamide

The Schiff base ligand was synthesized as per the reported literatureprotocol [19,20]. To the solution of 2-hydroxy-2-methyl propiophenone (0.01 mol) in methanol, thiosemicarbazone (0.01 mol) was added slowly. The above mixture was refluxed for 3 hours with constant stirring at a temperature of 70ºC. Then the reaction mixture was evaporated at room temperature. Colorless compound was separated out. It was washed with methanol. Purity was checked by Thin Layer Chromatography (TLC).

Synthesis of the mixed ligand Cadmium (II) Complexes (1,2)

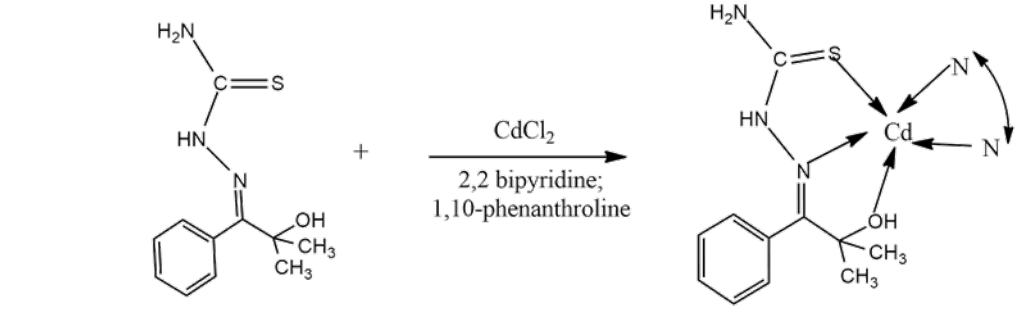
Schiff base ligand (L) (0.001mol) was dissolved in 20 ml of methanol followed by methanolic $CdCl_2$ (0.001 mol) was added. The mixture was refluxed for 1 h. with constant stirring. The yellow-colored solution was formed. To the resultant reaction mixture, heterocyclic base (2,2'-bipyridine) (0.001 mol) which is dissolved in a mixture of dichloromethane and methanol was added slowly. The mixture was refluxed for 6 hours with constant stirring. The lemon-yellow colored precipitate was formed after that time period which was then filtered, and evaporated using Rota evaporator. The yellow color precipitate was obtained. The precipitate was washed several times with cold ethanol and dried under vacuum pump over anhydrous $CaCl_2$. The schematic representation for synthesis of Schiff base ligand L and Cd (II) metal complexes are presented in figure 1 and figure 2. The same procedure was followed for the Schiff base mixed ligand complex with 1,10-Phenanthroline.

**Figure 1:** Schematic preparation of Schiff base ligand

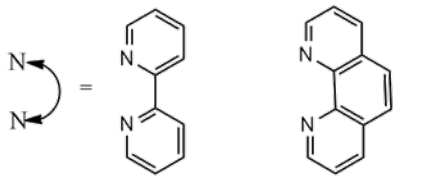



**2-hydroxy-2-methyl-1-phenylpropan-1-one** **hydrazinecarbothioamide** **2-(2-hydroxy-2-methyl-1-phenylpropylidene)hydrazinecarbothioamide**

Figure 2: Schematic preparation of mixed ligand Cd (II) metal complexes




**2-(2-hydroxy-2-methyl-1-phenylpropylidene)hydrazinecarbothioamide**



**2,2'-bipyridine** **1,10-phenanthroline**

(1) (2)

**ANTIMICROBIAL ASSAYS**

**Preparation of media**

The antibacterial activity of ligand and their metal chelates is assessed using the agar well diffusion method. These assays are used to create a dosage response curve for a specific bacterial strain versus a specific test chemical. Two Gram positive bacteria strains, Bacillus subtilis and Staphylococcus aureus and two Gram negative bacteria strains, Escherichia coli and Klebsiella pneumoniae are tested for antibacterial activity. Three fungal strains, Aspergillus

Niger, Aspergillus flavus, and Rhizopus oryzae were used to test antifungal activity. Antibacterial and antifungal reference medications were Streptomycin and Fluconazole, respectively.

**Antioxidant activity**

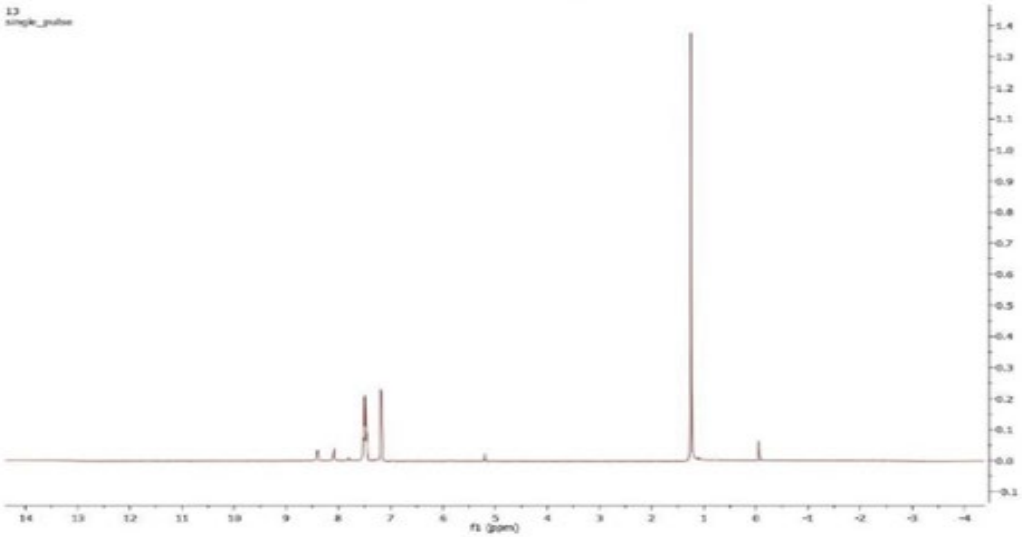
The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical technique was used to examine the antioxidant and free radical scavenging properties of the produced compounds in vitro. In methanol, a 0.1 M DPPH solution was prepared. The crude extract was then dissolved in 10%Dimethylsulfoxide (DMSO) at various concentrations (20 - 100 g $mL^{-1}$) and 500 µL of the DPPH solution was combined with 1 mL of the crude extract. These solutions were mixed together and incubated in the dark for about 30 minutes at room temperature. At 517 nm, the absorbance of ascorbic acid was compared to that of a blank with no scavenger activity.

## RESULTS AND DISCUSSION

**$^{1}$H NMR spectra**

The $^{1}$H NMR spectra (Model: Bruker 400) of the prepared Schiff base was recorded using TMS as an internal standard in DMSO-d6 as a solvent for ligand. This was shown in figure 3. The peak around 1.0 ppm can assign to the six methyl hydrogens in the compound. The alcoholic hydrogen was seen at 5.2 ppm. The peaks from 7.2 ppm to 7.7 ppm can assign to aromatic hydrogens. The -NH group and -$NH_2$ group hydrogen atoms of the semi thiocarbazide fragment were seen at 8.0 ppm and 8.3 ppm respectively. The $^{1}$H NMR spectra of compound clearly insights into formation of the imine group between ketone and amine to give Schiff base ligand.

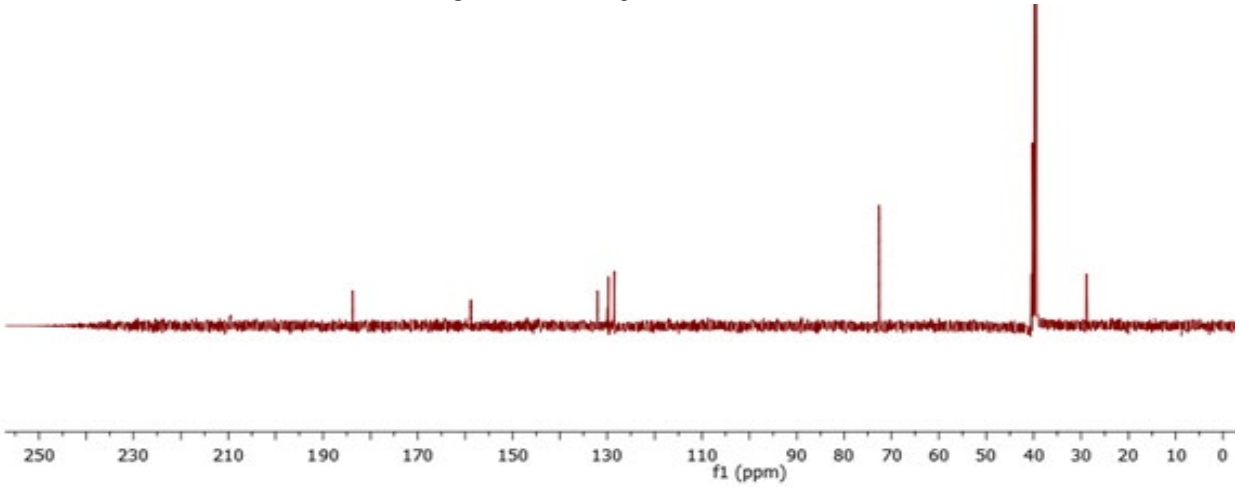
**Figure 3:** $^{1}$H NMR Spectra of Schiff base



**$^{13}$C NMR spectra**

The $^{13}$C NMR spectra (Model: Bruker 400) of the prepared Schiff base was recorded using TMS as an internal standard in DMSO-d6 as a solvent for ligand. The spectral image was shown in figure 4.$^{13}$C NMR spectra is an important tool to elucidate the structure of the prepared Schiff base ligand. The two methyl carbons attached to tertiary carbon atom was seen at 28 ppm. The tertiary carbon that adjacent to imine group was seen at 72 ppm. The aromatic carbon atoms are seen in the range of 128 ppm to 134 ppm. The most importantly, the peak at 158 ppm was given to imine carbon atom. The -C=S group carbon atom was assigned to the peak at 183 ppm. From the Carbon NMR spectra, it was concluded that the formation of Schiff base complex was successful using the starting ketone and amine.

**Figure 4:** $^{13}$C NMR Spectra of Schiff base



**FT-IR analysis**

Infrared spectra's were recorded using Bruker alpha spectrometer in the wave number range of 4000 – 400 $cm^{-1}$.In the uncoordinated ligand, an important strong band appears at 1666 $cm^{-1}$ attributing to free azomethine group which confirms the formation of Schiff base by condensation of ketone and amine, but in metal complexes a negative shift up to 1658 $cm^{-1}$ suggests coordination of the imine nitrogen to metal centers. This may occur due to decrease in bond strength of imine bond and simultaneous increase in bond strength between azomethine nitrogen and metal center. The $-NH_2$ stretching frequency was clearly seen around 3200 $cm^{-1}$ to 3300 $cm^{-1}$andsometimes coupled with -OH and -NH stretching's. The aliphatic -C-H stretching was seen around 2900 $cm^{-1}$. The aromatic -C=C- stretching was seen at 1490 $cm^{-1}$. The -C=S stretching was seen at 1389 $cm^{-1}$. In the case of metal complexes, the metal complexes show absorption peaks in the region 420 - 427 $cm^{-1}$ corresponding to M-N and 655 - 658 $cm^{-1}$for M-O vibrations confirming the bond formation between azomethine nitrogen, hydroxyl oxygen and metal ion. Another absorption band in the range of 3300 - 3550 $cm^{-1}$ in complexes marks the presence of coordinated or lattice water. This is presented in Table 1 and the FT-IR Spectra of Schiff base ligand is shown in figure 5.

**Figure 5:** FT-IR Spectrum of Schiff base ligand

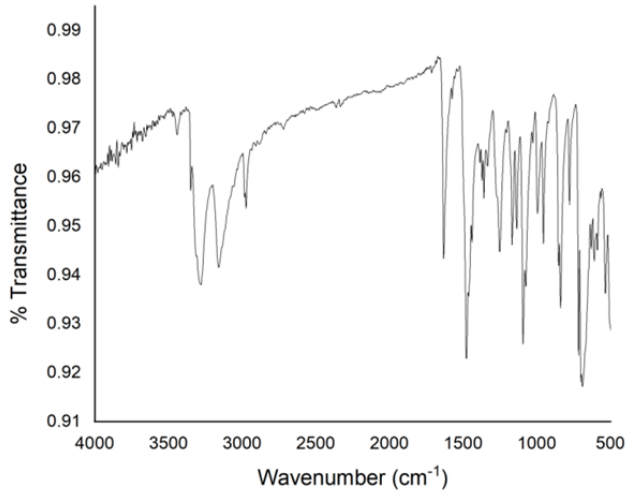


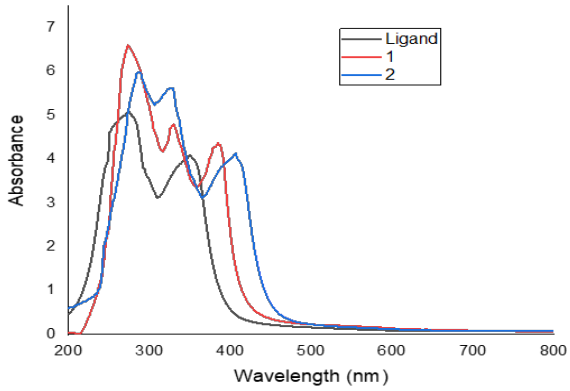

**Table 1:** FT-IR Spectral data for Ligand and its Cd (II) Metal Complexes

| Compound | υ (C=N) | υ (N-N) | υ (C=S) | υ (Cd-N) | Bands due to heterocyclic bases |
|---|---|---|---|---|---|
| L | 1666 | 1099 | 1389, 871 | -- | -- |
| 1 | 1658 | 1089 | 1375, 863 | 427 | 1499, 657 |
| 2 | 1656 | 1088 | 1379, 864 | 425 | 1498, 656 |

**Electronic spectra**

UV–Visible spectra were recorded in the range of 200 – 800 nm on Cary 5000 version spectrophotometer at room temperature using solutions in Dimethylformamide (DMF). The electronic absorption spectra of Cd (II) complexes recorded in DMF solution is shown in figure 6. The bands observed indicate π→π* transitions, confirming metal center binding with the Schiff base, 1,10-phenanthroline / 2,2'-bipyridine. The ligand's electronic spectrum shows bands at 274 nm and 347 nm that corresponds toπ→π* transitions and n→π* transitions of the azomethine and thioamide functions, respectively. Because of the coordination of the ligand with metal ions, these transitions were shifted to higher or lower frequencies [21]. The shift of the π→π* bands to longer wavelength regions in complexes is caused by the weakening of the C═S bond and the enhancement of the conjugation system during complexation [22]. There were no detectable absorptions above 500 nm in DMF solution, indicating the absence of d–d bands, which is consistent with the $d^{10}$ configuration of the Cd (II) ion.

**Figure 6:** Electronic Spectrum of ligand and its complexes (1,2)




**Biological activity**

The antibacterial activity of synthesized compounds was tested in-vitro using the agar-well diffusion method against a variety of harmful bacteria and fungus strains.

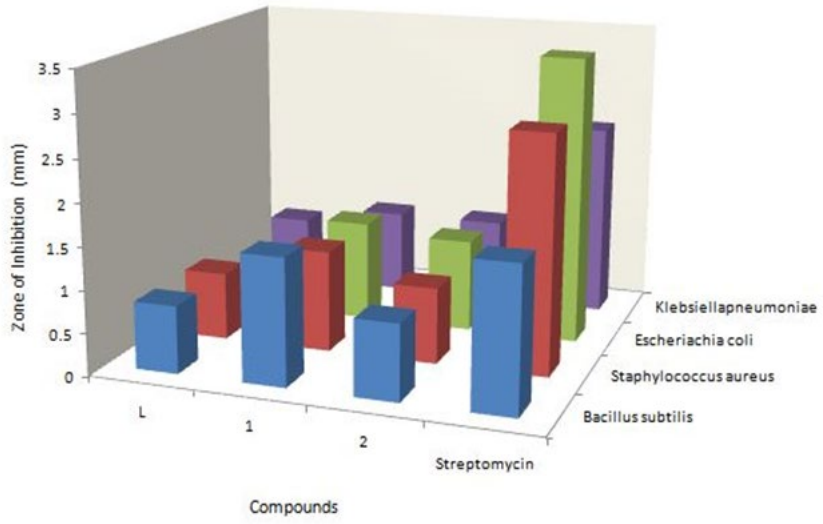
Antibacterial Activity

For both bacteria, the ligand antibacterial activity was shown to be weaker than that of the standard reference antibiotic (Streptomycin), however the Cd (II) complexes were found to be more effective and reached good activity when compared to the standard drug. This indicates, the formation of complexes with ligands and metal ions was happened. The antibacterial activity of complex-1 was higher than that of remaining complex and the Schiff base ligand. Because of the chelation interaction between Schiff bases and ligand complexes, complex-1 demonstrated good activity for both positive and negative bacteria and is shown in Table 2 and figure 7. Lipophilicity, which controls the rate at which molecules enter the cell, is similarly affected by coordination.

**Table 2:** Antibacterial activity of compounds [Zone of inhibition (mm)]

| Compounds | Gram's (+) | | Gram's (-) | |
|---|---|---|---|---|
| | Bacillus subtilis | Staphylococcus aureus | Escherichia coli | Klebsiella pneumoniae |
| L | 0.8 | 0.8 | 0 | 0.8 |
| 1 | 1.5 | 1.2 | 1.2 | 1.0 |
| 2 | 0.9 | 0.9 | 1.1 | 1.0 |
| Standard Drug (Streptomycin) | 1.7 | 2.8 | 3.4 | 2.3 |

**Figure 7:** Zone of inhibition values for antibacterial activity



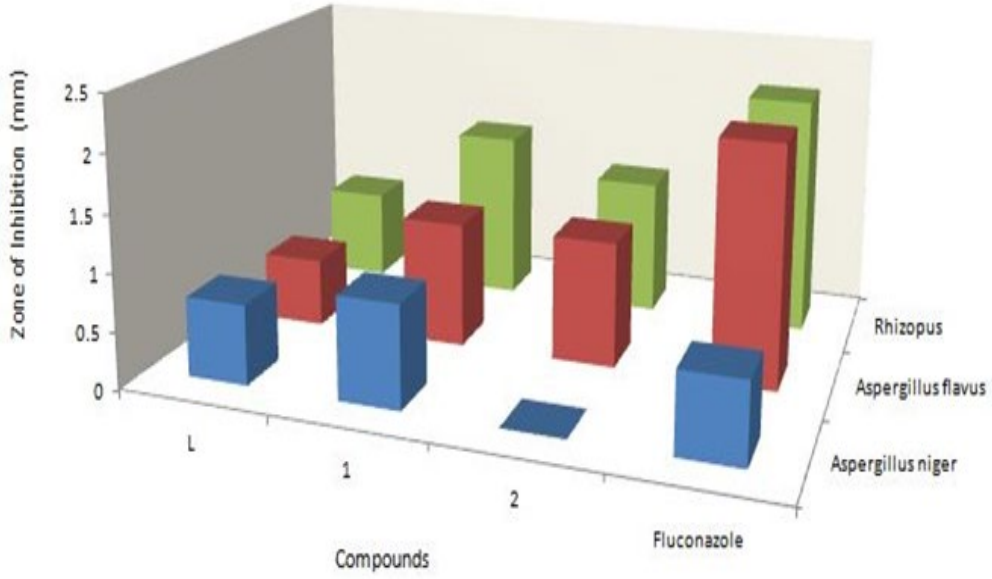

**Antifungal activity**

Fluconazole was considered as the standard drug for evaluating antifungal activities of the ligand and its metal complexes. Complex-1 had moderate antifungal efficacy against three different fungal strains (Aspergillus Niger, Aspergillus flavus, Rhizopus oryzae). Complex-2, on the other hand, shows lesser efficacy against Aspergillus flavus and Rhizopus oryzae. Complex-2 is not reactive for Aspergillus Niger. Henceforth, Complex-1 shown better antifungal efficacy. Table 3 and Figure8 depicts inhibition zone values of the complexes and the ligand in comparison with the standard drug (Fluconazole).

**Table 3:** Antifungal activity of compounds [Zone of inhibition (mm): Paper Disc Method]

| Compounds | Aspergillus Niger | Aspergillus flavus | Rhizopus oryzae |
|---|---|---|---|
| L | 0.7 | 0.6 | 0.8 |
| 1 | 0.9 | 1.1 | 1.5 |
| 2 | 0 | 1.2 | 1.1 |
| Standard Drug (Fluconazole) | 0.7 | 2.1 | 2.1 |

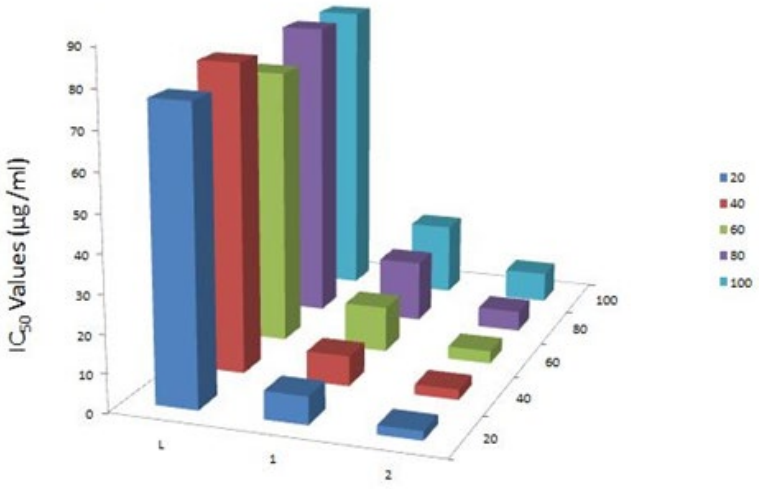
**Figure 8:** Zone of inhibition values for antifungal activity




**Antioxidant activity**

Using the DPPH assays method, the ability of the newly synthesized Schiff bases ligands and their complexes to scavenge free radicals may be assessed, and the findings are shown in Figure 9. Because of its stability and simplicity, the DPPH radical is frequently used to evaluate antioxidant activity quickly. The creation of stable 1,1-diphenyl-2-picrylhydrazine resulted in a decrease in the intensity of the DPPH band, demonstrating the ability of metal complexes and Ascorbic acid (used as a standard) to scavenge free radicals [23]. Increased antioxidant activity is indicated by a decrease in absorbance and a decrease in the $IC_{50}$value [24,25]. The $IC_{50}$ values of the test compounds were observed to be in the order L > 1 > 2, indicating that complex-2 has a stronger scavenging activity, while complex 1 has a moderate scavenging activity.

**Figure 9:** $IC_{50}$ values of Schiff base ligand and Cd (II) metal complexes (1,2)




## CONCLUSION

In this paper, reported the synthesis of two Schiff base mixed ligand Cd (II) metal complexes namely [Cd(L)(diimine)] (1, 2) [where L = 2-(2-hydroxy-2-methyl-1-phenylpropylidene) hydrazine carbothioamide; diimine = 2,2'-bipyridine (1), 1,10-phenanthroline (2)]. The complexes were elucidated by the use of various techniques. The antibacterial properties of Schiff base and its metal complexes were investigated against a varied range of Gram-positive and Gram-negative bacterial strains. Also, the antifungal and antioxidant assays were studied. The complex-1 shown better biological activities for antibacterial and antifungal, whereas complex-2 shown good DPPH scavenging activity.

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