



## STUDIES ON THE SYNTHESIS, CHARACTERIZATION, AND CYTOTOXICITY OF SOME TRANSITION METAL COMPLEXES OF 4-CHLORO-N'-[(E)-(2-HYDROXY-4- METHOXYPHENYL)METHYLIDENE]BENZOHYDRAZIDE

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### Abstract

The Schiff base 4-chloro-N'-[(E)-(2-hydroxy-4-methoxyphenyl)methylidene]benzohydrazide (HCBHV), was prepared by using 2-Hydroxy-4-methoxybenzaldehyde (vanillin) and 4-chlorobenzohydrazide then the solid complexes of Fe(II), Cu(II), Pd(II), Co(II), Mn(II), Ni(II), Zn(II), Hg(II) and Cd(II) were produced. Molar conductivity, magnetic susceptibility, X-ray diffraction, Fourier transform infrared, nuclear magnetic resonance, ultraviolet-visible, and mass spectrometry were all used to characterize these metal complexes. The metal: ligand ratio in these metal complexes was found to be 1:2 by analysis. The physicochemical investigation provides evidence for the presence of square planar geometry around Cu(II), Pd(II), tetrahedral geometry for Cd(II), Hg(II), Zn(II), and octahedral geometry around Mn(II), Co(II), Fe(II), and Ni(II) ions. The IR spectrum data shows that the ligand acts as a tridentate with an OON-donor atom sequence towards the center metal ion. Metal complexes are likely non-electrolytes because of their low molar conductance values. X-ray diffraction analysis indicates that these compounds likely adopt a monoclinic crystal structure. *S. aureus* MCC 2408, *B. subtilis* MCC 2010, *P. aeruginosa* MCC 2080, and *E. coli* MCC 2412 were used as test organisms for the HCBHV ligand and their metal complexes' antibacterial and fungicidal activities, respectively.

**Keywords:** Tridentate Schiff base, Metal complexes, Cytotoxic study, XRD, Antimicrobial study

### 1. Introduction:

The physiological activity, coordination ability, and analytical chemistry applications have all contributed to a rise in interest in the study of hydrazones [1, 2]. The donor sites of aroyl or heteroaryl Schiff bases are more numerous than those of simple hydrazone Schiff bases. This introduces a new set of characteristics for these materials. Ligands containing such hydrazones are intriguing to research because of their coordination diversity, propensity to yield stereochemistry [3] of greater coordination number, ability to behave as neutral or deprotonated ligands, and conformational

plasticity. Researchers have recently synthesized and analyzed several metal complexes of Schiff bases with N, S, and O donors [4-6]. The complexes formed from these donor ligands have been shown to have anticarcinogenic and antiviral action [7, 8], which may explain why this is the case. Antibacterial [9], antifungal [10], anticonvulsant [11], anti-inflammatory [12], analgesic [13], anti-malarial [12, 13], anti-platelet [14], anti-tuberculosis [15], and anticancer [16] bioactivities are among those demonstrated by several hydrazone derivatives. In addition to their uses as plasticizers, stabilizers, and antioxidant polymerization initiators [17], hydrazones are effective as herbicides, insecticides, nematocides, rodenticides, and plant growth regulators. Analytical chemists use hydrazone derivatives as selective metal extraction agents, while spectroscopists use them to identify certain transition metals [18].

It was predicted that the metal complexes formed when transition metal ions are bound to a strong hydrazone Schiff base ligand would exhibit greater biological activity than the metal salts or the ligand alone. Here, we present the development and characterization of 4-chloro-N'-[(E)-(2-hydroxy-4-methoxyphenyl)methylidene]benzohydrazide (HCBHV) ligand, solid complexes of Fe(II), Cu(II), Pd(II), Co(II), Mn(II), Ni(II), Zn(II), Hg(II) and Cd(II). Anti-fungal, anti-bacterial, and cytotoxic activities of the HCBHV ligand and its metal complexes were also evaluated.

## 2. Experimental:

### Materials:

All compounds were of the highest purity and analytical grade (AR). 2-Hydroxy-4-methoxybenzaldehyde (vanillin), 4-chlorobenzohydrazine, and ethanol were used to synthesize ligands. Metal sulfates in the AR grade are used for complex preparation. For the spectrum measurements, we employed spectral-grade solvents. The carbon, hydrogen, and nitrogen percentages were calculated using a Perkin Elmer (2400) CHNS analyzer. Using KBr pellets in the 4000-500  $\text{cm}^{-1}$  range, Bruker FTIR spectra were taken to record the IR spectra of the HCBHV ligand and its metal complexes. In  $\text{CDCl}_3$ , with TMS as an internal standard,  $^1\text{H-NMR}$  spectra of the HCBHV ligand were obtained. The X-ray diffraction pattern was captured on a Philips 3701 using  $\text{CuK}\alpha$  radiation ( $\lambda = 1.541 \text{ \AA}$ ) from  $20^\circ$  to  $90^\circ$ . The complexes' electronic absorption spectra were recorded using a Jasco UV-650 Spectrophotometer at room temperature; magnetic susceptibility was measured using a Guoy balance and a  $\text{Hg}[\text{Co}(\text{SCN})_4]$  standard. The conductivity of a 1 mM solution in nitrobenzene was measured using a Hitech Lab India Digital Conductivity Meter. ESR measurements were conducted on solid complexes at room temperature using a JES - FA200 ESR Spectrometer with X and Q bands.

### Synthesis of HCBHV ligand:

The HCBHV ligand was synthesized by adding 0.01 mol of 4-chlorobenzohydrazine in ethanol to a 0.01 mol solution of 2-hydroxy-4-methoxybenzaldehyde (vanillin) in ethanol, which was dissolved in 25 mL of ethanolic solution and stirred continuously. The resulting concoction was allowed to reflux for roughly 2 hours. The air cooled it to room temperature. The solid residue (yielding 80%) was washed with hot ethanol and used in further experiments.

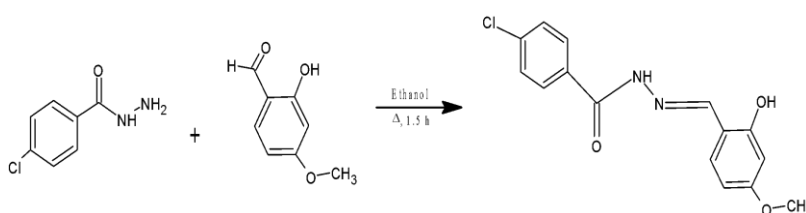


Figure 1: Synthesis of HCBHV ligand

### Preparation of complexes:

A solution of metal sulfates (0.005 mol) in 25 mL of water was added to a heated solution of the HCBHV ligand (0.01 mol) in 25 mL of ethanol while stirring continuously. The addition of 0.1N sodium hydroxide solution raised the pH of the reaction mixture to 7. The mixture was then cooled, filtered, and washed with hot ethanol, petroleum ether, and ethyl acetate, yielding a solid metal complex. The complex was dried over CaCl<sub>2</sub> in a vacuum desiccator.

#### **Antimicrobial activity:**

The antibacterial and fungicidal activities of the ligand and its complexes were evaluated using Gram-positive bacteria such as *Staphylococcus aureus* (MCC 2408), *B. subtilis* (MCC 2010), and Gram-negative bacteria such as *Escherichia coli* (MCC 2412), *Pseudomonas aeruginosa* (MCC 2080), as well as fungi such as *Candida albicans* (MCC 1439) and *S cerevisiae* (MCC 1033).

#### **Antibacterial assay:**

Each bacterium's overnight culture (0.2 mL) was diluted into 20 mL sterile nutrient broth and cultured for another 3 to 5 hours to achieve standardization. For the antibacterial test, we used a loopful of the standard cultures. Muller Hilton agar was made, and 20 mL of the sterile medium was placed into Petri dishes to set. A bacterial grass culture was grown and labeled with the help of a sterile cotton swab. A metallic borer created the wells with at least 24 mm centers. Each well received the appropriate 50  $\mu$ L of the test sample (100  $\mu$ g mL<sup>-1</sup>) in DMSO. An antibiotic reference drug was added to the other disc. At once, the plates were placed in an incubator at 37 °C for 24 hours. The diameter of the completely inhibited zones was used as a measure of activity. Antibiotics like streptomycin were used to measure growth inhibition [19-21].

#### **Antifungal activity:**

This investigation employed *Candida albicans* (MCC 1439) and *Saccharomyces cerevisiae* (MCC 1033) as fungal test strains. Traditional drug *fluconazole* (FLZ) was prepared in water, whilst the ligand and its synthesized complexes were prepared in DMSO [22-24].

#### **Cytotoxic study:**

In a shallow rectangular plastic plate (22x32 cm), *brine shrimp* eggs (*Artemia salina* Leach) were incubated in an artificial seawater solution from a commercial salt mixture and double-distilled water. The plastic dish was divided into two halves using a perforated instrument. The bigger, darker chamber held approximately 50 mg of eggs, while the smaller, lighter chamber was exposed to natural light. The *nauplii* were pipetted from the lighted side two days later. We dissolved 20 mg of the test compound for the sample in 2 ml of DMF. Nine vials were filled with stock solutions containing 500, 50, and 5  $\mu$ g/mL (three duplicates of each dilution were used for each test sample, and the LD<sub>50</sub> represents the mean of three values), and one was kept as a control containing only 2 mL of DMF. The solvent was allowed to evaporate overnight. After two days, the shrimp larvae (1 mL each vial) and 10 adult shrimp (30 shrimp/dilution) were ready to be placed in salt water. The volume was then increased to 5 mL per vial with seawater. After 24 hours, the survivors were counted [25]. The LD<sub>50</sub> values [26] were determined using data from the Finney computer tool.

### **RESULTS AND DISCUSSION:**

All complexes have a characteristic color, can be stored at room temperature without deterioration, and are readily soluble in the organic solvents: nitrobenzene, chloroform, DMF, and DMSO. Element analysis revealed that all complexes had a metal: ligand stoichiometry of 1:2. **Table 1** provides molar conductance values and microanalytical data. The volumetric analysis assessed the metal concentrations in complexes [27]. All the complexes are low-conductance, demonstrating that they are not electrolytic [28]. Studies using magnetic measurements have suggested that the Zn(II), Cd(II), Pd(II), and Hg(II) complexes are diamagnetic, while the Mn(II), Cu(II), Fe(II), Co(II), and Ni(II) complexes are paramagnetic.

**Table 1:** Physical characterization, analytical, magnetic moment, and molar conductance data of compounds

Comp	Color	MW	% Yield	MP/ DP	Element Content						Cond $\Omega^{-1}\text{cm}^2 \text{mol}^{-1}$	MM (B.M)
					M	C	H	N	O	Cl		
HCBHV	White	290.70	74.92	184	-	59.12	4.30	9.19	15.75	11.63	-	-
Fe(CBHV) <sub>2</sub>	Blue	635.24	66.43	203	8.79	52.89	3.78	8.82	10.0	10.08	7.58	5.11
Co(CBHV) <sub>2</sub>	Brown	640.39	76.89	203	9.21	52.47	3.75	8.75	9.99	11.10	10.09	5.14
Ni(CBHV) <sub>2</sub>	Green	640.09	69.59	203	9.17	52.49	3.75	8.75	10.00	11.10	0.59	3.12
Pd(CBHV) <sub>2</sub>	Green	687.40	81.09	209	15.42	48.88	3.49	8.15	9.31	8.25	0.83	-
Cu(CBHV) <sub>2</sub>	Green	644.95	77.92	208	9.0	52.10	3.72	8.68	9.92	11.00	0.332	1.92
Zn(CBHV) <sub>2</sub>	Yellow	646.79	71.55	199	10.11	51.92	3.71	8.66	9.90	11.00	6.38	-
Cd(CBHV) <sub>2</sub>	Yellow	693.81	74.89	207	16.20	48.43	3.46	8.67	13.80	9.00	3.12	-
Hg(CBHV) <sub>2</sub>	Green	781.4	80.89	206	25.67	43.00	3.07	7.17	12.29	8.09	0.73	-
Mn(CBHV) <sub>2</sub>	Brown	634.34	80.80	196	8.66	52.97	3.78	8.63	10.11	11.20	10.68	5.23

#### <sup>1</sup>H NMR spectra of HCBHV ligand:

The synthesized ligand's <sup>1</sup>H-NMR spectrum was recorded in CDCl<sub>3</sub> to be examined. It exhibits the following signals at  $\delta$ 3.87 (*s*, 3H, -OCH<sub>3</sub>),  $\delta$ 7.71-7.14 (*dd*, 1H, Ar-H),  $\delta$ 7.36-7.31 (*dd*, 1H, Ar-H),  $\delta$ 7.62-7.64 (*ddd*, 2H, Ar-H),  $\delta$ 7.96-7.98 (*ddd*, 2H, Ar-H),  $\delta$ 6.88-6.90 (*dd*, 1H, Ar-H),  $\delta$ 8.38 (*s*, 1H, N=C-H),  $\delta$ 9.62 (*s*, 1H, -NH-) and  $\delta$ 11.79 (*s*, 1H, Ar-OH).

#### Mass spectra of the HCBHV ligand:

The HCBHV ligand mass spectrum data has been recorded, revealing that the molecular ion peak (M<sup>+</sup>) occurs at  $m/z = 305$ . This is denoted by the peaks that correspond to their respective molecular masses.

#### FT(IR) spectra:

Four distinct bands at 3277, 3450, 1642, and 1590 cm<sup>-1</sup> can be identified in the FT-IR spectrum of the free HCBHV ligand. These bands correspond to the (-N-H) and free (-O-H) stretching modes of the phenolic moiety, the carbonyl (-C=O), and the azomethine (-C=N) stretching modes. Deprotonation of the bound (-OH) group during complexation and subsequent coordination of the oxygen to a metal ion is evidenced by the absence of broadband in the 3400-3500 cm<sup>-1</sup> region in the spectra of the metal complexes [29]. As a result of the coordination of the oxygen of the carbonyl group with the metal ion, the (C=O) band shifts to a lower wavenumber when compared to the unbound HCBHV ligand. When the nitrogen of the azomethine group is coordinated with the metal ion, the  $\nu$ (C=N) band shifts to a lower wavenumber compared to the free HCBHV ligand, which is supported by the red shift in the (N-N) stretching frequency from 961 cm<sup>-1</sup> to 968 cm<sup>-1</sup> regions [30-32]. New bands, attributed to (M-O) and (M-N) vibrations, were seen in the spectra of metal chelates around 570-624 and 511-601 cm<sup>-1</sup> [33]. Therefore, the phenolic oxygen, carbonyl oxygen, and azomethine nitrogen are responsible for the coordination in the HCBHV ligand molecule.

**Table 2:** FT(IR) spectral data of HCBHV ligand and its metal complexes

Comp	-OH (C2)	-NH-	-C-H=	>C=O	>C=N-	Phenolic C-O	N→M	O-M
HCBHV	3450	3277	2837	1642	1590	1281	-	-
Fe(CBHV) <sub>2</sub>	-	3275	3088	1590	1555	1282	623	525
Co(CBHV) <sub>2</sub>	-	3275	3088	1590	1555	1282	623	527
Ni(CBHV) <sub>2</sub>	-	3276	3088	1592	1556	1282	624	527
Pd(CBHV) <sub>2</sub>	-	3387	3093	1591	1553	1281	622	525
Cu(CBHV) <sub>2</sub>	-	3274	3087	1590	1554	1281	622	601
Zn(CBHV) <sub>2</sub>	-	3375	3075	1612	1527	1281	612	529

<b>Cd(CBHV)<sub>2</sub></b>	-	3270	3075	1590	1555	1281	607	526
<b>Hg(CBHV)<sub>2</sub></b>	-	3258	3033	1631	1534	1303	608	511
<b>Mn(CBHV)<sub>2</sub></b>	-	3258	2923	1604	1552	1296	570	535

### Electronic absorption spectra and magnetic measurements:

**Table 3** exhibits the compounds' magnetic and electronic absorption spectra. Bands at 502, 480, and 380nm can be attributed to the  ${}^2B_{1g} \rightarrow {}^2A_{1g}$  transition and charge transfer in the electronic spectra of the Cu(II) complex in DMF. It has been hypothesized that the Cu(II) complex has a square planar geometry [34, 35] based on electronic spectrum data and an observed magnetic moment of 1.92 B.M. There are three bands observed in the spectra of the Co(II) complex, located at 900, 662, and 405nm and corresponding to the  ${}^4T_{1g} \rightarrow {}^4T_{2g}$  (F),  ${}^4T_{1g} \rightarrow {}^4A_{2g}$  (F), and charge transfer transitions, respectively. A high spin octahedral shape is suggested by these transitions and the reported magnetic moment of 5.14 B.M. [35, 36]. The octahedral geometry of the Mn(II) complex is supported by the observation of a magnetic moment of 5.23 B.M. and spectral bands at 558 and 390, which can be attributed to  ${}^6A_{1g} \rightarrow {}^4T_{1g}$ , and charge transfer, respectively [34, 37]. Considering a magnetic moment value of 5.65 B.M., the octahedral structure of the Fe (II) complex was confirmed, and a single peak at 518 nm was assigned to the  ${}^5T_{2g} \rightarrow {}^5E_g$  transition [38]. There was a magnetic moment of 3.12 B.M. in the Ni(II) complex. Spectra of the Ni(II) complex show three transitions that can be attributed to  ${}^3T_{2g} \rightarrow {}^3T_{2g}$ ,  ${}^3T_{2g} \rightarrow {}^3T_{1g}$ (F), and  ${}^3T_{2g} \rightarrow {}^3T_{1g}$ (P) at 972, 618, and 524 nm, respectively [39-40]. An octahedrally distorted environment was indicated. Transitions at 404 and 307 nm [41] were seen in the electronic spectra of the Pd(II) complex. A square planar geometry was attributed to these changes [37]. However, the *d-d* band is not present in the Zn(II), Cd(II), and Hg(II) complexes because the  $d^{10}$  transition is filled in these complexes [42-43].

**Table 3:** Electronic spectral data of HCBHV ligand and its metal complexes

Compound	$\lambda$ nm	Transition
<b>HCBHV</b>	353	$n \rightarrow \pi^*$
	280	$\pi \rightarrow \pi^*$
<b>Mn(CBHV)<sub>2</sub></b>	558	${}^6A_{1g} \rightarrow {}^4T_{1g}$ ( <sup>4</sup> P)
	390	${}^6A_{1g} \rightarrow {}^4E_g$ ( <sup>4</sup> D)
<b>Fe(CBHV)<sub>2</sub></b>	625	${}^5T_{2g} \rightarrow {}^5E_g$
	435	L $\rightarrow$ M charge transfer
<b>Co(CBHV)<sub>2</sub></b>	900	${}^4T_{1g}$ (F) $\rightarrow$ ${}^4T_{2g}$ (F) ( $\nu_1$ )
	662	${}^4T_{1g}$ (F) $\rightarrow$ ${}^4T_{2g}$ (P) ( $\nu_2$ )
	975	${}^3A_{2g}$ (F) $\rightarrow$ ${}^3T_{2g}$ (F) ( $\nu_1$ )
<b>Ni(CBHV)<sub>2</sub></b>	753	${}^3A_{2g}$ (F) $\rightarrow$ ${}^3T_{1g}$ (F) ( $\nu_2$ )
	404, 307	L $\rightarrow$ M charge transfer
<b>Cu(CBHV)<sub>2</sub></b>	502	${}^2B_{1g} \rightarrow {}^2A_{1g}$ ( $\nu_1$ )
<b>Zn(CBHV)<sub>2</sub></b>	353, 278	L $\rightarrow$ M charge transfer
<b>Cd(CBHV)<sub>2</sub></b>	351, 270	L $\rightarrow$ M charge transfer
<b>Hg(CBHV)<sub>2</sub></b>	354, 277	L $\rightarrow$ M charge transfer

### ESR spectrum of Cu(II) complex:

The following values are obtained from the ESR spectra of copper (II) complex measured in a polycrystalline sample at room temperature:  $g_{||} = 2.061$ ,  $g_{\perp} = 2.142$ . The trend,  $g_{\perp} < g_{||}$ , suggested that the electron is delocalized in the  $d_{z^2}$  orbital of the Cu(II) ground state, and the spectrum is typical of axial (compressed octahedral) symmetry. The parameter G, calculated as  $G = (g_{||} - 2)/(g_{\perp} - 2)$ , is found to be substantially smaller than 4, indicating significant interaction in the solid state [44].

### X-ray diffraction study:

A sample Co(II) metal complex's X-ray diffractogram was scanned in the 5-100° range at 1.54 Å. The diffractogram and related data show each peak's inter-planar spacing (*d*-values), relative intensity, and 2θ values. The Co(II) complex diffractogram displayed twelve reflections with maxima

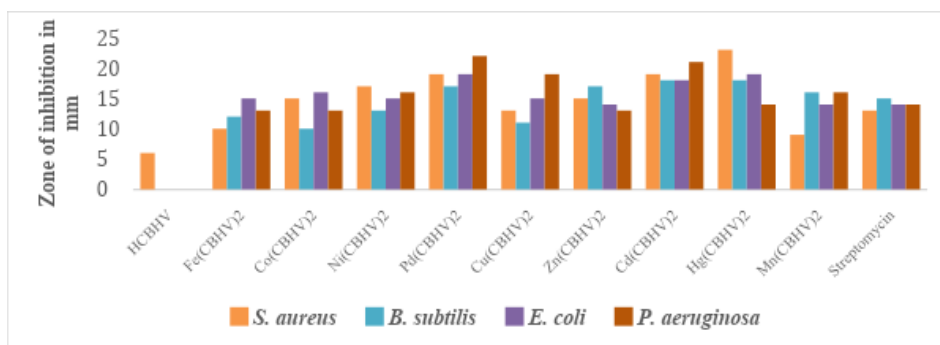
at  $2\theta$  ( $30.35^\circ$ ), corresponding to a  $d$  value of  $2.93\text{\AA}$ . A computer algorithm has been used to index the complex's X-ray diffraction pattern concerning prominent peaks with relative intensities larger than 10% [45]. The indexing technique produces volume, unit cell characteristics, and Miller indices (hkl). Lattice constants  $a = 8.30\text{\AA}$ ,  $b = 3.14\text{\AA}$ ,  $c = 6.70\text{\AA}$ , and unit cell volume  $V = 165.29(\text{\AA})^3$  were obtained from the Co(II) complex unit cell. The conditions such as  $a \neq b \neq c$  and  $\alpha = \gamma = 90^\circ \neq \beta$  necessary for the compounds to be monoclinic were examined and determined to be satisfactory in conjunction with these cell characteristics of the Co(II) complex. The metal complex has a monoclinic crystal structure according to the aforementioned values. Based on these investigations, the theoretical density was calculated and found to be  $2.30\text{ g cm}^{-3}$  for the Co(II) complex. The experimental density value of the complex was obtained using the specific gravity method [46] and was found to be  $2.31\text{ g cm}^{-3}$  for the Co(II) complex. A comparison of experimental and theoretical density reveals good agreement within the bounds of experimental error.

### Antimicrobial activity:

Using the disc diffusion method, the HCBHV ligand and its metal complexes were evaluated in vitro for their antibacterial efficacy against bacteria, including *Staphylococcus aureus* (MCC 2408), *B. subtilis* (MCC 2010), and Gram-negative bacteria such as *Escherichia coli* (MCC 2412), *Pseudomonas aeruginosa* (MCC 2080), as well as fungi such as *Candida albicans* (MCC 1439) and *S cerevisiae* (MCC 1033) [37]. The substances were evaluated in DMSO at a  $100\text{ g/mL}$  concentration and contrasted with streptomycin, a well-known antibiotic (**Table 4**). Compounds were tested in vitro for fungicidal activity using sabouraud dextrose agar (SDA) medium and the mycelia dry weight technique [48] against *Candida albicans* (MCC 1439) and *Saccharomyces cerevisiae* (MCC 1033). The substances were examined at  $100\text{ }\mu\text{g/mL}$  in DMF and contrasted with the control (**Table 5**). It is evident from **Tables 4** and **5** that metal chelates have a higher level of inhibition than ligands and metal salts. Regarding the relative activity of the free ligand and its complexes, the results agree with earlier studies [48-49]. Under the same experimental settings, the metal chelates had more antibacterial activity than the corresponding free ligand and control against the same microbe. The increased lipophilic character of the metal ions in complexes causes the higher activity of metal chelates [50]. The influence of metal ions on the regular process causes the activity to rise with concentration.

**Table 4:** Antibacterial studies of HCBHV ligand and its metal complexes

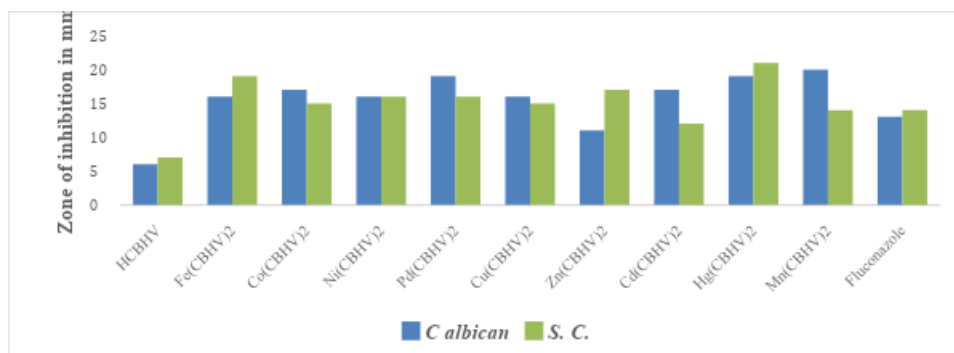
Compound	Antibacterial Activity (zone of inhibition) (mm)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
HCBHV	6	0	0	0
Fe(CBHV) <sub>2</sub>	10	12	15	13
Co(CBHV) <sub>2</sub>	15	10	16	13
Ni(CBHV) <sub>2</sub>	17	13	15	16
Pd(CBHV) <sub>2</sub>	19	17	19	22
Cu(CBHV) <sub>2</sub>	13	11	15	19
Zn(CBHV) <sub>2</sub>	15	17	14	13
Cd(CBHV) <sub>2</sub>	19	18	18	21
Hg(CBHV) <sub>2</sub>	23	18	19	14
Mn(CBHV) <sub>2</sub>	9	16	14	16
Streptomycin	13	15	14	14



**Figure 2:** Antibacterial activity of HCBHV ligand and its metal complexes

**Table 5:** Antifungal studies of HCBHV ligand and its metal complexes

Compound	Antifungal Activity (zone of inhibition) (mm)	
	<i>C. albican</i>	<i>S. cerevisiae</i>
<b>HCBHV</b>	6	7
<b>Fe(CBHV)<sub>2</sub></b>	16	19
<b>Co(CBHV)<sub>2</sub></b>	17	15
<b>Ni(CBHV)<sub>2</sub></b>	16	16
<b>Pd(CBHV)<sub>2</sub></b>	19	16
<b>Cu(CBHV)<sub>2</sub></b>	16	15
<b>Zn(CBHV)<sub>2</sub></b>	11	17
<b>Cd(CBHV)<sub>2</sub></b>	17	12
<b>Hg(CBHV)<sub>2</sub></b>	19	21
<b>Mn(CBHV)<sub>2</sub></b>	20	14
<b>Fluconazole</b>	13	14



**Figure 3:** Antifungal activity of HCBHV ligand and its metal complexes

### Cytotoxic study:

**Table 6** summarizes the compounds' conclusions from toxicity tests against newly hatched nauplii of *Artemia salina* and their capacity to scavenge free radicals. All synthetic complexes showed some toxicity, with the Mn(II) complex showing the highest level. Based on its effective antibacterial action, this data might be interpreted similarly certain way. It would be logical to infer that Pd(II) exhibits its toxicity in this way since the nauplii coexist with some bacterial strains.

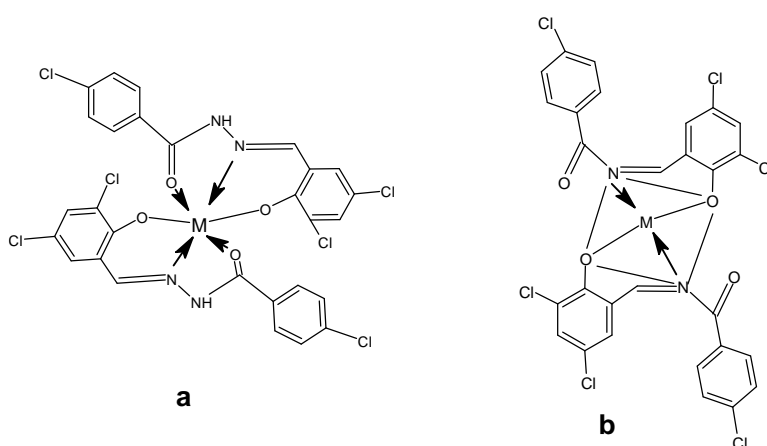
**Table 6:** Brine shrimp bioassay of HCBHV and their metal complexes

Compound	LD <sub>50</sub> (M)
<b>HCBHV</b>	-
<b>Fe(CBHV)<sub>2</sub></b>	$>2.178 \times 10^{-4}$

<b>Co(CBHV)<sub>2</sub></b>	$>3.45 \times 10^{-4}$
<b>Ni(CBHV)<sub>2</sub></b>	$>6.11 \times 10^{-4}$
<b>Pd(CBHV)<sub>2</sub></b>	$>1.78 \times 10^{-4}$
<b>Cu(CBHV)<sub>2</sub></b>	$>4.57 \times 10^{-4}$
<b>Zn(CBHV)<sub>2</sub></b>	$>8.49 \times 10^{-4}$
<b>Cd(CBHV)<sub>2</sub></b>	$>4.57 \times 10^{-4}$
<b>Hg(CBHV)<sub>2</sub></b>	$>5.91 \times 10^{-4}$
<b>Mn(CBHV)<sub>2</sub></b>	$>7.05 \times 10^{-4}$

### Conclusion:

Based on the preceding discussion, we propose an octahedral geometry for the Co(II), Mn(II), Fe(II), and Ni(II) complexes, tetrahedral geometry for Hg(II), Cd(II) and Zn(II) complexes and a square-planar geometry for the Pd(II) and Cu(II) complexes. The physicochemical and spectroscopic information presented thus far suggests that the ligand is monobasic, OON tridentate, and forms its coordination compounds with oxygen from the carbonyl and phenol groups and the nitrogen atom of the azomethine group (**Figure 4**). The complexes show increased antibacterial activity compared to the free ligand and are physiologically active. The XRD analysis indicates the Co(II) complex has a monoclinic crystal structure. Spectral studies are used to identify complex structures:



Where M = **a**) Fe(II), Mn(II), Co(II), Ni(II) and Cu(II) **b**) Pd(II), Cd(II), Hg(II) and Zn(II)

**Figure 4:** Structures of proposed metal complexes

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