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SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL EVALUATION OF 2,4-DICHLORO-6-[(4-HYDROXY-PHENYLIMINO)-METHYL]-PHENOL (DCSAP-L) AND ITS FIRST TRANSITION METAL COMPLEXES

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ABSTRACT

The rising challenge of antimicrobial resistance underscores the need for the development of novel and more potent antimicrobial agents. This research investigates synthesis, characterization, as well as biological applications of a newly synthesized compound, 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), along with its corresponding metal complexes. DCSAP-L was synthesized by reacting 3,5-Dichlorosalicylaldehyde with p-aminophenol, resulting in the formation of an imine-based ligand. Additionally, ligands reacted with copper (II) and zinc (II) chloride salts to form metal coordination complexes. The chemical structures of both DCSAP-L as well as its metal complexes were thoroughly characterized through several analytical along physico-chemical techniques, including spectroscopy. The antimicrobial activity of these compounds was evaluated *in vitro*, revealing their significant biological potential. The findings suggest that both free ligand and their metal complexes exhibit notable antibacterial and antifungal activity, highlighting their promise as potential candidates for addressing the growing issue of microbial resistance.

Keywords: 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol, DCSAP-L, Transition Metals, Spectral Studies, Biological Activities.

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INTRODUCTION

Schiff bases, first synthesized by Hugo Schiff in 1864 through the condensation of aldehydes or ketones with primary or secondary amines, are versatile ligands known for forming stable complexes with metal ions.[1-3] Schiff bases' core structure, characterized by the azomethine group (–C=N–), allows for coordination of a wide range of metals, making them valuable in numerous applications across chemistry as well as biochemistry. These compounds, with the general formula RHC=N-R', exhibit diverse reactivity and are utilized extensively in medicinal chemistry due to their broad biological activities, encompassing antimicrobial, anticancer, as well as anti-inflammatory effects.[4-5] Recent studies have highlighted the enhanced biological activities of Schiff bases substituted with hydroxyl groups, particularly in anticancer research. Moreover, Schiff base metal complexes play pivotal roles as catalysts in biochemical processes and are widely applied in fields such as polymer synthesis, dye production, and analytical chemistry.[6-7] Given their broad utility, there is an increasing interest in novel Schiff base ligands as well as their metal derivatives. This study focuses on new Schiff base synthesis, 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), as well as its coordination with transition metal chlorides, including Cu(II) and Zn(II). Metal complexes formed are characterized using various analytical as well as spectroscopic

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methods, along their antimicrobial activities are assessed through in vitro studies. This research aims to contribute to the development of Schiff base-metal complex systems with potential therapeutic applications.

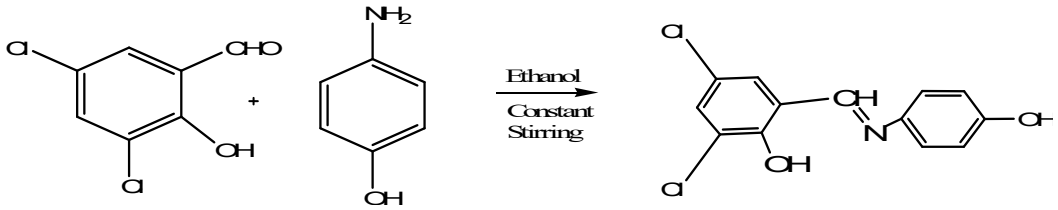
EXPERIMENTAL

Materials and Methods

Solvents and chemicals employed for synthesis were procured from Merck and used without purification. Analytical-grade reagents were employed for the preparation and recrystallization processes. The melting points of synthesized 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), as well as metal complexes, were determined using an Electro-Thermal 9100 apparatus. Elemental analysis (C, H, N) had been conducted employing an Elemental Model Ratio EL(111) analyzer at the Central Electrochemical Research Institute. Mass spectrometry was conducted using a JEOL D-300-EI spectrometer (Japan Electro-Optics Laboratory), and UV-Visible spectra were obtained with a Perkin Elmer Lambda35 spectrophotometer, covering a range of 250-800nm. Magnetic susceptibility measurements of powdered samples were recorded using a Gouy instrument. Infrared (IR) spectra of DCSAP-L and its transition metal complexes had been obtained as potassium bromide pellets with a Perkin Elmer RX^{-1} spectrophotometer in the 4000-400 cm^{11} range. ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) spectra of DCSAP-L and its diamagnetic complex had been observed on a Bruker Avance II 400MHz Fourier Transform NMR spectrometer, employing DMSO(d_6-Dimethylsulfoxide) as solvent. Thermogravimetric (TGA) and differential thermal analysis (DTA) were conducted under an inert H_2 gas atmosphere using a Perkin Elmer STA6000 instrument. Electron Spin Resonance (ESR) spectra were measured using a Micro Sed-10 Analyzer.

Synthesis of DCSAP-L

The method discussed in the literature was employed to synthesize 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L). The mixture containing 3,5-Dichlorosalicylaldehyde (1.910 g) dissolved in pure ethanol was combined with 4-aminophenol (0.965 ml) in the same amount (1:1 ratio (Scheme 1). After frequent stirring of the mixture for an hour using a magnetic stirrer, the liquid ethanol left behind was allowed to slow evaporation, which led to generate a dark orange product at ambient temperature. After washing with ethanol, the final product was filtered and allowed to dry at ambient temperature. A pure dark orange color product obtained after recrystallization from hot ethanol. Yield is 79%. m/z: 281.1; M.P. is 140°C. Analytical (%): Formed C-61.32;H-4.23;N-4.76. Calculated C-58.99;H-5.01;N-5.16.



Scheme-1: Formation of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L)

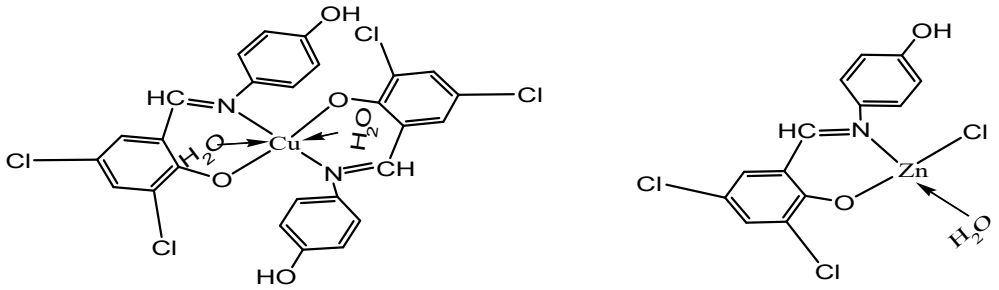
Preparation of Metal Complexes

The hot solution of Schiff base 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) (0.005mol) in alcohol (20ml) added to hot alcoholic solution(20ml) of Cu(II) and Zn(II)metal salts (0.005mol) individually and mixed well. These mixtures were allowed to reflux on a heating mantle for about 5-6 hours with Aqua's condenser to reduce the temperature in ice ice-cooled environment. The colored solid compounds were filtered, washed with enough ether, and allowed to dry in ambient air (Scheme-2).

Investigation of Antimicrobial Activities

Various concentrations of DCSAP-L and transition metal complexes (100, 200, 500, and 1000µg/ml) were kept together. Clean petri plates carrying 20ml of NA medium were seeded with 24hrs culture of bacterial strains (*E. coli (Negative)* and *S. aureus (Positive)*). Petri plates were incubated for 24 hours at 37°C. Petri plates with 20ml PDA medium were sowed with fungi strain (*A. niger).* Petri plates were incubated for 72

hours at 27°C. By estimating the inhibition zone's diameter formed around wells, anti-microbial activities were recorded. Positive control was amphotericin B. Using GraphPad Prism 6.0 software, the value points were calculated.



Scheme-2: Structures of Cu(II) and Zn(II) Complexes

RESULTS AND DISCUSSION

FT-IR Spectra

The geometry and the functional group of the ligand, 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) coordinated with the metal ion are identified by the infrared spectrum. The functional groups of both Schiff base (DCSAP-L) and transition metal complexes have their peculiar properties. The IR spectral bands of the Schiff base 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) were observed between the region of 3248.13 cm^{-1} because of the ν (OH) stretching vibration, whereas those broad bands around 3248.13-3120.24 cm^{-1} exhibited the ν (OH) stretching of metal complexes. The stretching frequency of H_2O is also observed at 3440.95-3331.32 cm^{-1} in metal complexes but is absent in ligands. Though the stretching frequency of azomethine ν (CH=N) in the ligand is 1641.01 cm^{-1}, the frequency in the metal complex is around 1620cm^{-1} cm-1.[8,9.] The frequency decrease in the metal complex is evident for the metal-ligand coordination. The band is observed in the region of 1280.73 cm-1 due to the frequency of (Ar-O).[10] Bands observed at 538.72-502.04 cm^{-1}, 447.62-422.13 cm^{-1} and 758.44-754.21cm^{-1} were due to bending frequency, which discloses the (M-O), (M-N) as well as (M-Cl) band respectively.[11,12] Those peaks are not present in 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L). Aromatic ring band's C-H stretching was obtained in the region of 3091.30-3053.99 cm^{-1}.

Proton and Carbon NMR Spectra

After dissolution in DMSO-d_6, the spectra were given to suggest the integrity of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) as well as the transition metal complex. The obtained ^{1}H NMR spectrum collates with the DCSAP-L complex and has a minor shift towards a lower field observed. Diamagnetic properties have been studied by analyzing signals emitted from ^{1}H NMR experiment spectra of ligand 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) and related Zn(II)complex. Analyzing the proton NMR spectrum of the ligand 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), signals appeared at 12.37 ppm and 9.47 ppm, denoting OH, along with a single peak at 8.61 ppm shows the imine proton (CH=N).[13-15] The signal emerged in the complex, which shifted upward relative to the similar signal in the free ligand, indicating that the metal-nitrogen bond is preserved in solution. Though the complex contains two OH groups, there is only one peak that appears in the NMR spectrum at 8.97 ppm. The absence of one peak corresponding to phenolic-OH (Hydroxyl proton) suggests that the fully deprotonated hydroxyl group was coordinated to the particular metal ion. C-H aliphatic protons signal at 2- 3 ppm is assigned. In the ^{13}C NMR spectrum of the ligand 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), peaks around the region of (116.01-155.19 ppm) show the appearance of aromatic and imine carbon atoms.[16] It was verified with various values reported in the literature. Due to the presence of a nitrogen atom, the aromatic carbons were moved downfield, whereas, C-atom bonded with the OH group showed a signal downfield from other C-atoms. In carbon NMR spectra, a signal formed at 167.70 ppm (Ar-C=N, 1C) denotes the metal complex that was identified to the azomethine carbon atom (HC=N) and a signal appears at 171.75 ppm for the phenolic carbon (Ar-C-O, 1C). The aromatic ring of ligand 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) showed signals between 116.52- 161.99 ppm (Ar-C, 8C).

Ultraviolet-Visible Spectra

The absorption peaks observed in the visible region, associated with d-d transitions, correspond to the electronic transitions occurring at specific energy levels within the metal complexes. The UV-Visible absorption spectra are crucial in determining the electronic structure and geometry of these complexes. The electronic spectra of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) and transition metal complexes were recorded within the 200-800nm range at room temperature. These spectral data provide insight into the coordination environment of metal centers and geometry of resulting complexes. Spectra for ligand (DCSAP-L) along with metal complexes were obtained in dimethyl sulfoxide (DMSO) solution, which aids in accurate analysis of the electronic transitions associated with the coordination of the metal ions.[17-20]

ESR Spectra

The quantitative analysis of spin adduct free radicals was conducted using ESR spectra by comparing the peak areas with those from stable radicals. ESR spin-trapping techniques are useful for kinetic studies, as they allow for the determination of the formation and decay rates of free radicals. Cu(II)complex's solid-state ESR spectra were obtained at ambient temperature. In this complex, the value of G was found to be G ($g_{\|}$-2.0023/g_T-2.0023), which demonstrates that the unpaired electron is primarily situated in the d_{x2-y2} orbital. This conclusion is validated by the exchange interaction term, with the value of G reinforcing the interpretation that the unpaired electron resides in a specific orbital. Tendency of $A_{\|}$ decreases with the elevation of $g_{\|}$ which induces elevation of tetrahedral distortion within Cu(II) complex's co-ordination sphere. 'f' factor($g_{\|}/A_{\|}$) recorded from ESR spectra depicts degree of distortion of Cu(II) complex, even though factor was likely to be the empirical index of tetrahedral distortive position. The value for square planar complex ranges around 110-140 cm^{-1}, which depends on the nature of coordination atoms. Due to the presence of tetrahedral distortion, the values may be larger. In Cu(II) complex, $g_{\|}/A_{\|}$ ratio is greater than 130 cm-1, which shows the presence of significant distortion along the dihedral angle from square planar geometry. Results support distorted octahedral geometry around copper complex (Fig.-1).

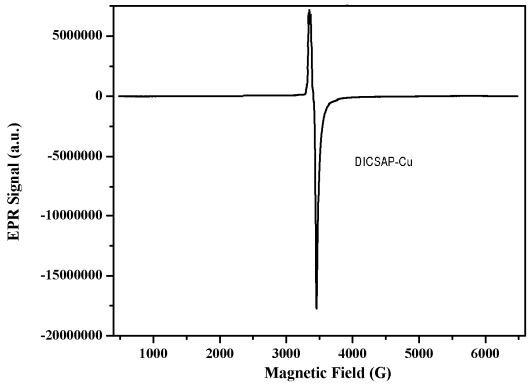


Fig.-1: Electron Spin Resonance Spectrum of Cu(II) Complex

Thermal (TG/DT) Analysis

Thermal analysis (TG) measures the changes in mass because of the increase in temperature at a controlled rate. The DTA method estimates differences between Ts (sample temperature) and Tr (temperature of a reference). A plot of Ts-Tr throughout a certain temperature range will display a succession of peaks that correspond to the temperatures at which thermal events take place. Due to some physicochemical phenomena like desorption, absorption, sublimation, etc., the results from TG experiments can be presented in various graphical ways.

Zn(II) Diamagnetic Complex

Thermal decomposition of Zn(II)complex takes place in 3 stages, as shown in the TGA curves. The first stage, above 125°C, involves a small weight loss due to adsorbed water, with the coordinated water molecules being removed around 130°C.[21] The peak of maximum mass loss is observed at 165°C. Second stage, taking place from 235-240°C, corresponds to chloride ions decomposition, with a DTG peak at 365-

370°C. The final stage encompasses decomposition of the ligand 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) between 370°C and 375°C, with the DTG peak at 525°C. The mass loss as well as residue were recorded and are shown in Fig.-2.

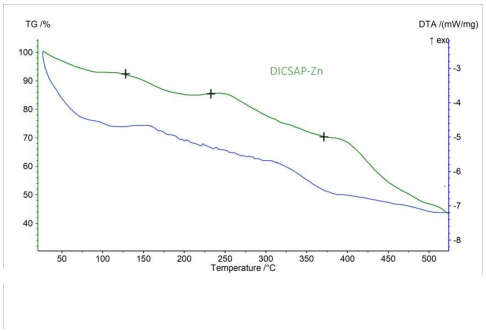


Fig.-2: Thermo Gravimetric Spectrum of Zn(II) Complex

Table-1: Elemental, Color, Molar Conductance, and Magnetic Susceptibility of DCSAP-L and Metal Complexes

COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	ABSORPTION MAXIMA (nm)	YIELD%	CALCULATED / (OBTAINED)			COLOUR	μ eff (BM)	MOLAR CONDUCTANCE	M. P. (°C)	GEOMETRY
					C	H	N					
DCSAP-L	$C_{13}H_{19}Cl_2NO_2$	281.21	305 350	8 0	55.34 (55.76)	3.22 (3.71)	4.96 (4.21)	Dark orange	-	-	140	-
Cu(II) Complex	$C_{26}H_{20}Cl_4CuN_2O_6$	660.80	410 625	7 8	47.19 (47.17)	3.05 (3.78)	4.23 (4.21)	Black	1.7	35	220	Dis.Oh
Zn(II) Complex	$C_{13}H_{10}Cl_3ZnNO_3$	398.87	300 740	7 2	39.04 (40.21)	2.52 (3.01)	3.50 (4.01)	Red	Dia	114	175	Dis. Td

Table-2: Antimicrobial Activity Data of DCSAP-L and Metal Complexes

Compounds	Zone of inhibition in(mm)(μg/ml)											
	Bacteria								Fungi			
	Staphylococcus aureus				*Escherichia coli*				*Aspergillus niger*			
	1000	500	200	100	1000	500	200	100	1000	500	200	100
DCSAP-L	15.5	12.5”	8.5	8	12	10	7.5	4.5	10.5	9.5	7.5	7.5
Cu(II) Complex	18	15	9.5	8.5	17	13	10.5	10	15.5	11	9.5	8.5
Zn(II) Complex	17	13	8.5	8	13.5	11.5	8.5	7	13.5	11	9.5	8
Standard	18	22	14	Standard	18	22	14	Standard	18	22	15	Standard
Negative control	0	0	0	Negative control	0	0	0	Negative control	0	0	0”	Negative control

Antimicrobial Activities

The antimicrobial activities of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L), as well as its metal complexes, were evaluated using the well diffusion method. Tests were conducted against *S. aureus*, *E. coli*, and *A. niger*. Nutrient agar plates were inoculated with microbial cells and treated with varying concentrations (4, 15, and 30 mg/mL) of the samples. After incubation at 37°C for 48 hours, inhibition zones were determined. Results demonstrate that metal complexes as well as ligand of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) show essential anti-microbial properties against *S. aureus*, *E. coli, and A. niger.*[22] Metal complexes were particularly effective, exhibiting stronger antimicrobial effects than the Schiff base ligand alone, against all tested organisms.

CONCLUSION

Elemental, analytical and spectral studies have been carried out for the newly synthesized Schiff base metal complexes. Metal complexes of 2,4-Dichloro-6-[(4-hydroxy-phenylimino)-methyl]-phenol (DCSAP-L) are

more proficient antimicrobial agents than their native form of metal (II) complexes. Among the experimental results, Cu(II) complex possesses higher bacterial and fungal activities than other Schiff base ligand's transition metal complexes.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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