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Novel Schiff base ligand and its Co(II) & Cu(II) Metal complexes: Synthesis, Physicochemical Characterization, DNA Binding, and Antifungal Screening

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Abstract: A novel Schiff base ligand 4-chloro-2-(2,5-dimethoxybenzylideneamino)-5-nitrophenol derived from 2,5-dimethoxybenzaldehyde and 2-amino-4-chloro-5-nitrophenol. Its Co(II) and Cu(II) transition metal chelates were also synthesized and physicochemically characterized by FT-IR, UV-Vis absorption spectroscopy, Thermogravimetric analysis (TGA), and Electrochemical studies. These compounds are biologically active and exhibit antifungal and DNA-binding activity. The Cu(II) metal complex gives better biological activity than the Co(II) metal complex and parent Schiff base ligand.

Keywords: Schiff base ligand, Metal chelates, Spectroscopic characterization, Antifungal, DNA binding property.

1. Introduction

Schiff base ligands and their coordinated transition metal chelates have gained considerable interest because of an enormous range of applications in various scientific platforms. These privileged ligands were investigated due to their versatile, selective, and sensitive nature toward the central metal atom. This makes them multifaceted actors in science and technology [1-5]. In Schiff base ligand, the azomethine (imine) group(-N=CH-) and other donor atoms, viz. oxygen, nitrogen, sulfur, etc., help them chelate with metal ions [6-8]. Due to chelating tendency, they possess various biological actions against microbes (such as bacteria, fungi, viruses, helminths, etc.) and specific types of tumors as anticancerous drugs. They also have many biochemical, catalytic, clinical, dying, and pharmacological properties [9-12]. Schiff base metal chelates are also used as root elements in various anticancerous and antimicrobial drugs due to their intercalating property with biochemical material like DNA.

Considering the above, we have also initiated an effort to explore further this promising area of research by designing and investigating the newly synthesized Schiff base ligand and its transition metal coordinated complexes. After an extensive literature survey, it has resulted that no work has been done with the Schiff base ligand derived from 2,5-dimethoxybenzaldehyde and 2-amino-4-chloro-5-nitrophenol along with its Copper and Cobalt metal complexes. The synthesized compound was characterized by various spectroscopic techniques and analyzed for biological potency [13].

2. Experimental

2.1. Materials and Method

Reagents such as 2,5-dimethoxybenzaldehyde, Mol. Wt. 166.17 (99 % purity); 2-amino-4-chloro-5-nitrophenol Mol. Wt. 188.57 (99.9 % purity); $CoCl_2.6H_2O$, Mol. Wt. 237.93 g/mol (99.9 % purity) and $CuCl_2.2H_2O$, Mol. Wt. of analytical grade, 170.48 g/mol(99.8 % purity), were purchased from Merck-Sigma Aldrich, India. FT-IR spectra were captured on BRUKER Alpha-II scientific FT-IR instrument, whereas LABINDIA analytical UV-3092 Spectrophotometer was used to measure UV-Vis absorption spectra of synthesized compounds. Thermal analysis of compounds done by using NETZSCH STA 449F1 instrument. Electrochemical studies were done on Metrohm Autolab B.V. (PGSTAT128N) cyclic voltammeter.

2.2. Synthesis

Synthesis of ligand [HL, (DMCA)]; 4-chloro-2-(2,5-dimethoxybenzylideneamino)-5-nitrophenol:

Methanolic solution of 2,5-dimethoxy-benzaldehyde(3.32g, 20mmol) was mixed dropwise with 2-amino-4-chloro-5-nitrophenol (3.77g, 20mmol) solution prepared in methanol(25ml) in a round bottom flask, containing just a bit of acetic acid as a motivator of reaction. The resulting reaction

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solution was continuously heated with constant stirring on a magnetic hot plate stirrer at 50-60°C for 6-8 hrs (Figure 1). A shiny yellow precipitate was separated and rinsed 2-3 times with modest amounts of methanol and diethyl ether. After that, it was recrystallized with methanol, then dried over fused CaCl2 under a vacuum.

Mol. Formula: $C_{15}H_{13}N_2O_5Cl$; Mol. Wt.: 336.73; Yield: 82%; Color: Yellowish; M.P.: > 428°C; stable; Anal. Calc.(%): C-53.50, H-3.89, N-8.32, O-23.76, Cl-10.53; Found(%): C-53.42, H-3.91, N-8.30, O-23.74, Cl-10.50;
Molar conductance (DMSO) λ_m ($Cm^2\Omega^{-1}mol^{-1}$): 17.9.

Synthesis of Coordinated Metal Complexes $[M(DMCA)_2.xH_2O].yH_2O$; (M: Co(II) and Cu(II)): The transition metal (II) coordinated chelates were synthesized by refluxing 1mM of individual metal (M: Co(II)(0.238g) and Cu(II)(0.170g)) salts in methanol with a methanolic solution of 2mM of the ligand [DMCA(HL) (0.672g)] in basic medium. The reaction combinations were continuously heated with constant stirring on a magnetic hot plate magnetic stirrer for 3-4 hrs at 50-60°C. The TLC plate detected the progress of the reaction. The colorful precipitates were separated from each reaction combination and then rinsed 2-3 times with a modest mixture of methanol and diethyl ether and recrystallized in methanol. Finally, the collected products were placed in desiccators over fused $CaCl_2$ under a vacuum environment for drying.

$[Co(DMCA)_2.2H_2O].H_2O$/DMCA-A : Mol. Formula: $C_{30}H_{28}CoN_4O_{12}Cl_2$; Mol. Wt.: 766.40 ; Yield: 76% ; Color: Wine red; M.P.: >455°C; stable; Anal. Calc.(%): C-47.01, H-3.68, N-7.31, O-25.05, Cl-9.25, Co-7.69; Found(%): C-47.12, H-3.50, N-7.30, O-25.10, Cl-9.20, Co-7.65;
Molar conductance (DMSO) λ_m ($Cm^2\Omega^{-1}mol^{-1}$): 25.4.

$[Cu(DMCA)_2.2H_2O]$ / DMCA-B: Mol. Formula: $C_{30}H_{28}CuN_4O_{12}Cl_2$; Mol. Wt.: 771.01; Yield: 81%; Color: Reddish brown; M.P.: > 455°C; stable; Anal. Calc.(%): C-46.73, H-3.66, N-7.27, O-24.90, Cl-9.20, Cu-8.24; Found(%): C-46.70, H-3.65, N-7.25, O-24.87, Cl-9.18, Cu-8.20; ;
Molar conductance (DMSO) λ_m ($Cm^2\Omega^{-1}mol^{-1}$): 29.2.

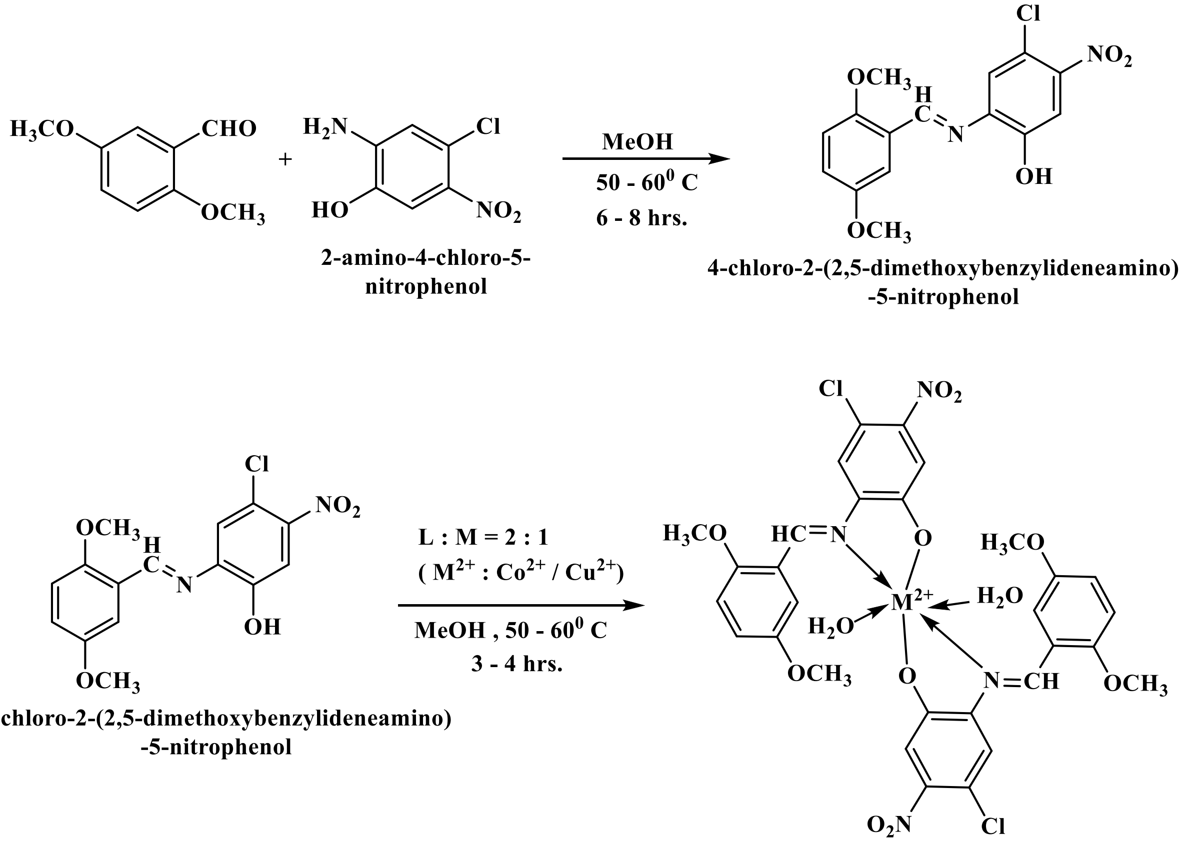


Figure 1. Projected molecular synthesis strategy and structure of DMCA(HL) and its transition metal coordinated complexes DMCA (A-B)

3. Results and Discussion

3.1. FT-IR analysis

The IR spectrum of ligand (DMCA)HL depicts some distinctive bands at 1735 cm^{-1}ν(C=N, azomethine), 1260-1300 cm^{-1} ν(C-O), and a specifically wide band at 3550-3700 cm^{-1}ν(O-H). The characteristic azomethine or imine band of Schiff base ligand moves to a lesser frequency (1724 cm^{-1} for DMCA-A and 1726 cm^{-1} for DMCA- B, this confirms that the azomethine group chelated with a metal ion. The newly appeared bands in the 515-535 cm^{-1} and 475-495 cm^{-1} depict the M-O and M-N stretching frequencies, respectively [14-17].

3.2. Electronic absorption spectroscopy

UV-Vis spectra of synthesized ligand and its metal chelates were measured in DMSO at absolute temperature (Figure 2). The absorption spectra of ligand DMCA(HL) were observed at 270 nm and 362 nm, which corresponds to π→π* transition of the aromatic ring and n→π* transition of the azomethine (C=N) group. Furthermore, these absorption bands move to remarkably lower energies (longer wavelength) in both the complexes (281 nm and 377 nm for DMCA -A(Co); 286 nm and 378 nm for DMCA- B(Cu)). These variations in electronic transitions (π→π* and n→π*) confirm the molecular environment transformation due to the binding of newly synthesized Schiff base ligand with transition metal ions [18].

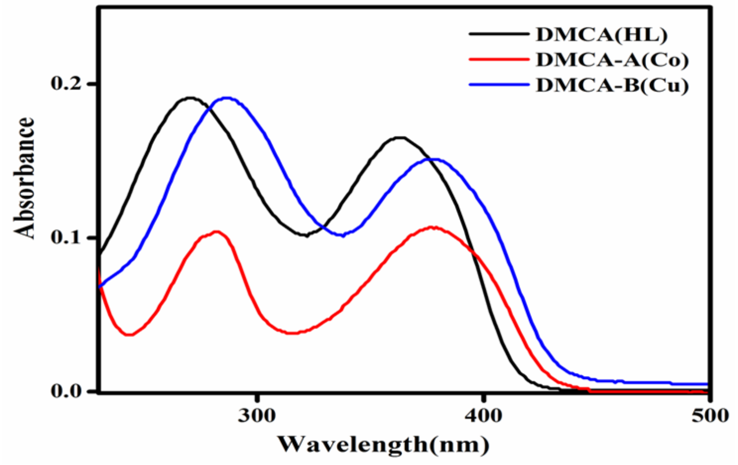


Figure 2. UV-Vis absorption spectra of DMCA(HL) and its transition metal chelates DMCA(A-B)

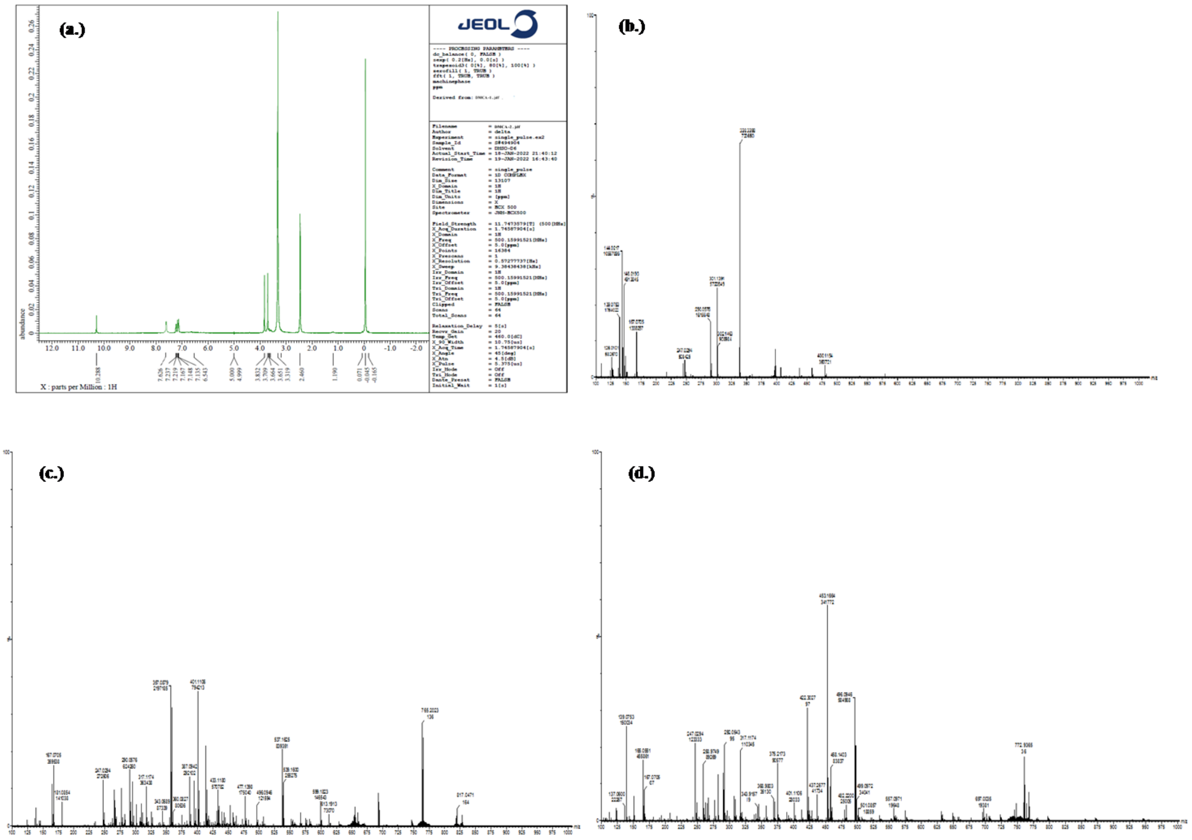


Figure 3. (a.) ^{1}HNMR of DMCA(HL), (b.) ESI-MS of DMCA(HL), (c.) ESI-MS of [Co(DMCA)$_2$.2H$_2$O].H$_2$O and (d.) ESI-MS of [Cu(DMCA)$_2$.2H$_2$O]

3.3. ^{1}HNMR and Mass spectral studies

^{1}HNMR spectral data of Schiff base ligand DMCA(HL) was recorded in DMSO-d6. ^{1}HNMR spectral data of HL attributes 10.28(s, -OH), 7.62(s, -CH=N), 7.23-6.54(m, aromatic protons) signal peaks, respectively, which supports the formation of Schiff base ligand DMCA. On the other hand, the ESI-MS spectra depict molecular ion peaks of **DMCA(HL)** and its metal-coordinated complexes **DMCA (A-B)**. By comparing the molecular formula weight with its m/z value in mass spectral data, the suggested molecular formula of each synthesized complex was verified. The assigned molecular ion peak $[M+2H]^+$ of HL was present at *m/z* 338.33 (Figure S3). Moreover, for metal complexes DMCA(A-B), the molecular ion peak $[M-H]^+$ at *m/z* 765.20 was attributed to entity $[Co(DMCA)_2.2H_2O].H_2O$, molecular ion peak $[M+H]^+$ at *m/z* 772.93 attributed to entity $[Cu(DMCA)_2.2H_2O]$.

These isotopic peaks in ESI-MS spectral data also strongly support making ligand and metal complexes. The ^{1}HNMR and ESI-MS spectral data are shown in Figure 3.

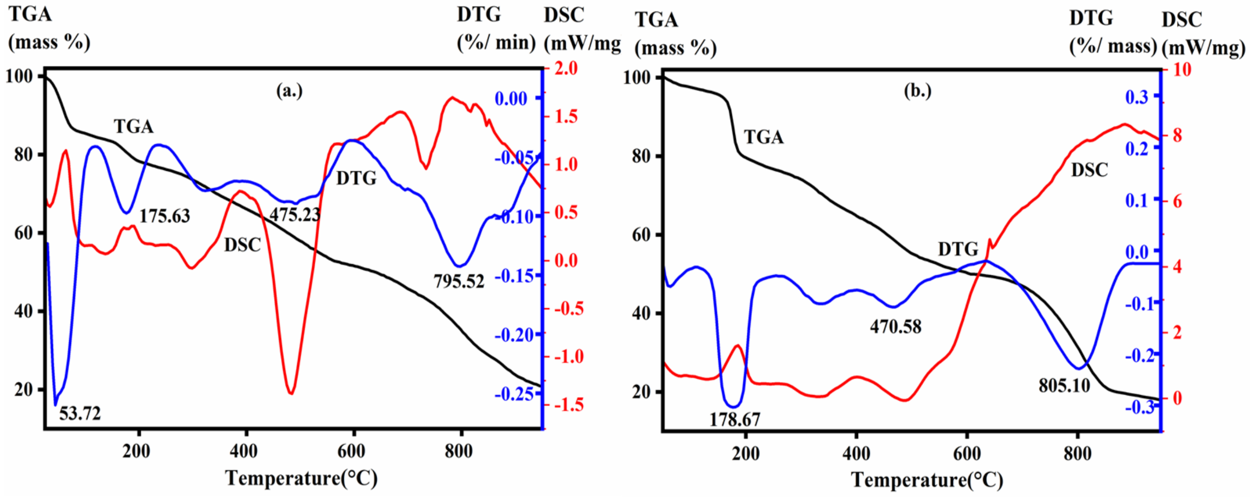


Figure 4. Thermograms of (a)$[Co(DMCA)_2.2H_2O].H_2O$ and (b) $[Cu(DMCA)_2.2H_2O]$

3.4. Thermogravimetric analysis (TGA)

In order to analyze the stability against synthesized Schiff base transition metal coordinated complexes DMCA(A-B) temperature, all the measurements were held in an N_2 atmosphere under 25 to 900°C temperature range, at a 10°C min^{-1} heating rate [19]. The TGA graphs given in Figure 4 and Table 1 conclude mass loss at different steps with temperature ranges, decomposition assignments, and final pyrolysis remaining product observed in the present analysis Table 1.

Table 1. Decomposition steps of synthesized Schiff base transition metal complexes DMCA(A-B) during Thermal analysis.

Samples	Temp. range(°C)	Wt. loss (%) Obs.	Cal.	DTG peak(°C)	Assignments	Residue Obs.%(Cal.%)
DMCA-A(Co)	25.88 - 83.04	13.64	13.04	53.72	Termination of one molecule of lattice water + two coordinated water molecules + Non-chelated part of the ligand	CoO + carbide impurities + by-product 23.31(23.86)
	88.95-203.01	7.97	7.95	175.63	Termination of a small amount of non-chelated part of the ligand	
	210.04-576.24	25.74	25.96	475.23	Termination of the remaining non-chelating part of the ligand	
	580.43-912.52	28.44	28.83	795.52	Termination of the chelating part of the ligand	
DMCA-B(Cu)	88.11-212.39	19.57	19.84	178.67	Termination of two coordinated water molecules + Non-Chelating part of the ligand	CuO + carbide impurities + by-product 19.26(19.65)
	225.59-586.95	28.36	28.79	470.58	Termination of the remaining Non-Chelating part of the ligand	
	594.15-892.48	30.99	31.64	805.10	Termination of the chelating part of the ligand	

3.5. Electrochemical studies(Cyclic Voltammetry)

The electrochemical voltammetric properties of these newly synthesized compounds were measured using the cyclic voltammetry technique, with a potential range from -1.5 to +1.5 V at 100mV s^{-1} scan. Stock solutions of free ligand DMCA(HL) and their respective metal complexes DMCA(A-B) were prepared separately in DMSO, having 0.1 M TBAP as a supporting electrolyte. The cyclic voltammogram of ligand DMCA (Epa=0.49V, Epc= -0.053V; ΔE= Epa-Epc =0.443V) shows redox behavior which is ascribed due to electronic activity of imine bond along with various functional groups attached with the Schiff base ligand [20].

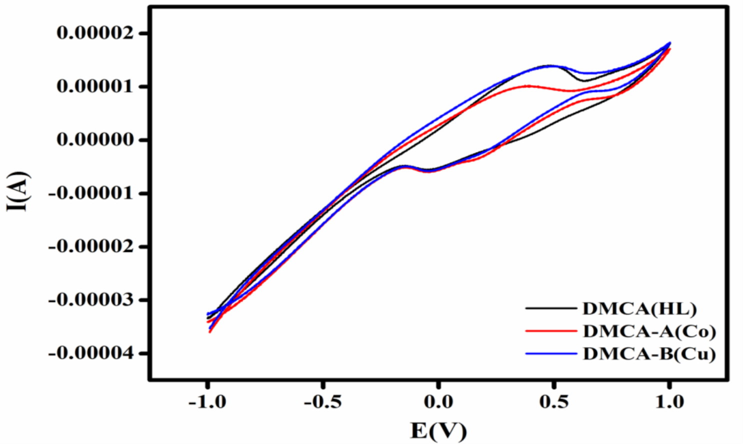


Figure 5. Cyclic Voltammogram of newly prepared Schiff base ligand DMCA(HL) and its transition metal complexes DMCA(A-B)

The cyclic voltammogram of corresponding complexes is illustrated in Figure 5. Electrochemical data were summarized in Table 2, which confirms a one-electron oxidation and quasi-reversible process ascribed to M(II)/M(I) redox system. In addition, the cathodic peak current and the square root of the scan rate ratio (Ipc /$v^{1/2}$) were almost constantly similar for all scan rates. This confirms the electrode process is diffusion-controlled.

Table 2. Cyclic Voltammetric data of synthetically created Schiff base ligand DMCA(HL) and its coordinated transition metal chelates DMCA(A-B).

Samples	Epa(V)	Epc(V)	^{a}ΔE (V)	Ia / Ic	$^{b}E_{1/2}$ (V)
DMCA(HL)	0.496	-0.053	0.443	1.25	0.27
DMCA-A(Co)	0.396	-0.043	0.353	0.92	0.21
DMCA-B(Cu)	0.508	-0.055	0.453	1.30	0.28

^{a}ΔE(V) = Epa-Epc , $^{b}E_{1/2}$ (V) = $\frac{E_{pa}+E_{pc}}{2}$

3.6. Biological Activities

3.6.1. DNA Binding

pCAMBIA DNA's (Circular DNA) stock solution was prepared by utilizing doubly distilled water with purity, having the coefficient of molar absorption (ε = 6600 M^{-1}/cm) with its λ_{max} values at 260 and 280 nm (ratio of A_{260}/A_{280} > 1.8). For spectrophotometric analysis, the concentration of all the compounds was kept steady, with the successive increase in plasmid DNA concentration (from $5x10^{-5}$ - $3x10^{-4}$M), and the most prominent band was used to calculate the alterations in absorbance. After 2 min incubation at physiological pH 7.4, the solution was ready for photometric response. The binding constants (Kb) of synthesized compounds were measured by using Wolfe - Shimer equation **(eq. 1)** :

$$\frac{[\mathbf{DNA}]}{(\boldsymbol{\varepsilon}_{\mathbf{a}}-\boldsymbol{\varepsilon}_{\mathbf{f}})} = \frac{[\mathbf{DNA}]}{(\boldsymbol{\varepsilon}_{\mathbf{a}}-\boldsymbol{\varepsilon}_{\mathbf{f}})} + \frac{\mathbf{1}}{\mathbf{K}_{\mathbf{b}}(\boldsymbol{\varepsilon}_{\mathbf{b}}-\boldsymbol{\varepsilon}_{\mathbf{f}})} \quad (1)$$

Where [DNA] is the concentration of DNA's base pairs; ε_a, ε_f, and ε_b are A_{obs} / [complex] (i.e., the apparent absorption coefficients, extinction coefficient for free compound under study, and extinction coefficient of the mix when completely bound to DNA, respectively). From the ratio of the slope to intercept of plots [DNA] / (ε_a - ε_f) vs. [DNA] binding constant, K_b was obtained [21-23].

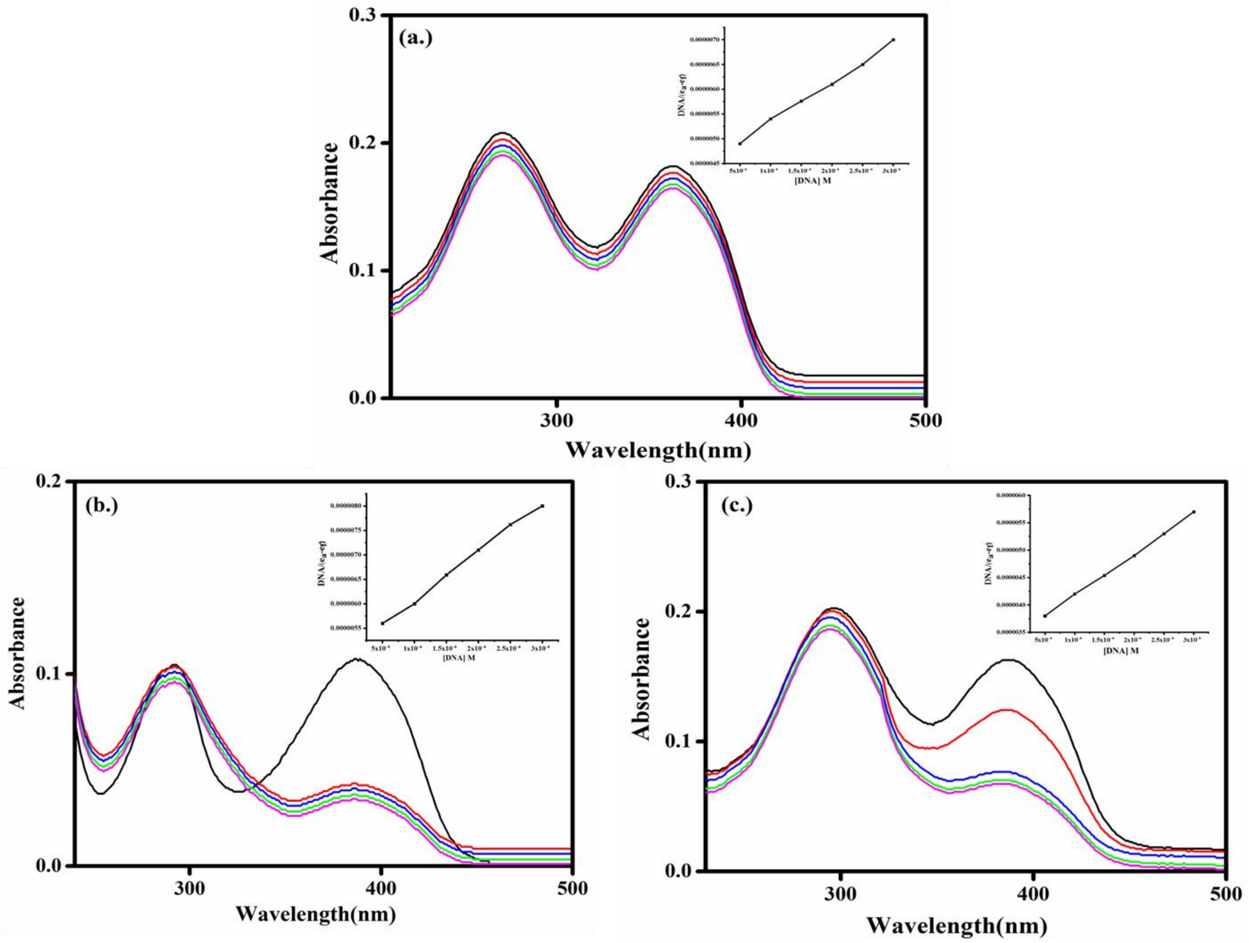


Figure 6. Electronic absorption spectral analysis of (a) DMCA(HL), (b)$[Co(DMCA)_2.2H_2O].H_2O$, (c)$[Cu(DMCA)_2.2H_2O]$ with an increasing amount of [DNA] concentration

The interactive coupling of DNA with metal chelates results in a hypochromic effect with bathochromic impact. The binding with interactive mode represents a hypochromic effect [24-26]. The DNA binding potency of all the synthesized complexes with **pCAMBIA DNA** is shown in Figure 6. In the UV region, the decrease in absorption intensity of synthesized compounds DMCA(HL), DMCA-A, and DMCA- B depicts the interaction of compounds with plasmid DNA. The binding constant of Schiff base ligand DMCA and its Co / Cu metal(II) complexes (A-B) follows the order: B($2.19x10^3$ M^{-1}) > A($1.94x10^3$ M^{-1}) > HL($1.76x10^3$ M^{-1}) (Table 3).

Table 3. Absorption spectral studies of novel synthesized Schiff base ligand DMCA(HL) and its Co / Cu metal complexes DMCA(A-B) with DNA.

Compounds	λ_{max}(nm) Free	Bound	Δλ (nm)	*Binding Constant K_b (M^{-1})
DMCA(HL)	362	363	1	1.76 x 10^3
DMCA-A(Co)	377	379	2	1.94x 10^3
DMCA-B(Cu)	378	380	2	2.19x 10^3

*K_b = Intrinsic DNA binding constant ; Error limit ±2 %

3.6.2. Antifungal activity

The fungal growth inhibition approach was utilized to calculate the inhibitory effect of the Schiff base ligand DMCA(HL) and its coordinated transition metal(II) complexes DMCA(A-B) against the two fungal species Aspergillus niger and Fusarium oxysporum [27]. In order to measure the antifungal activity, 2 % autoclaved Dextrose Agar of Potato (PDA) media was dispersed into Petri plates of 9 cm diameter. Standard solutions of the tested samples (1mg/ml) were prepared in 100% dimethyl sulfoxide (DMSO). After that, a specific volume of the stock solutions was added separately to the set of Petri plates together with PDA media to attain a final concentration of 100 µg/ml. After the solidification of media, a piece of 1.5 mm (cut from the actively growing margin) of 7 daysold-fungal cultures was placed as central inocula. Fungal growth was evaluated by measuring the diameter of the colony in cm after 7 days of incubation at an absolute 25°C temperature. The reduction percentage of growth was calculated in contrast with the controlled growth (free from complexes), using equation 2 :

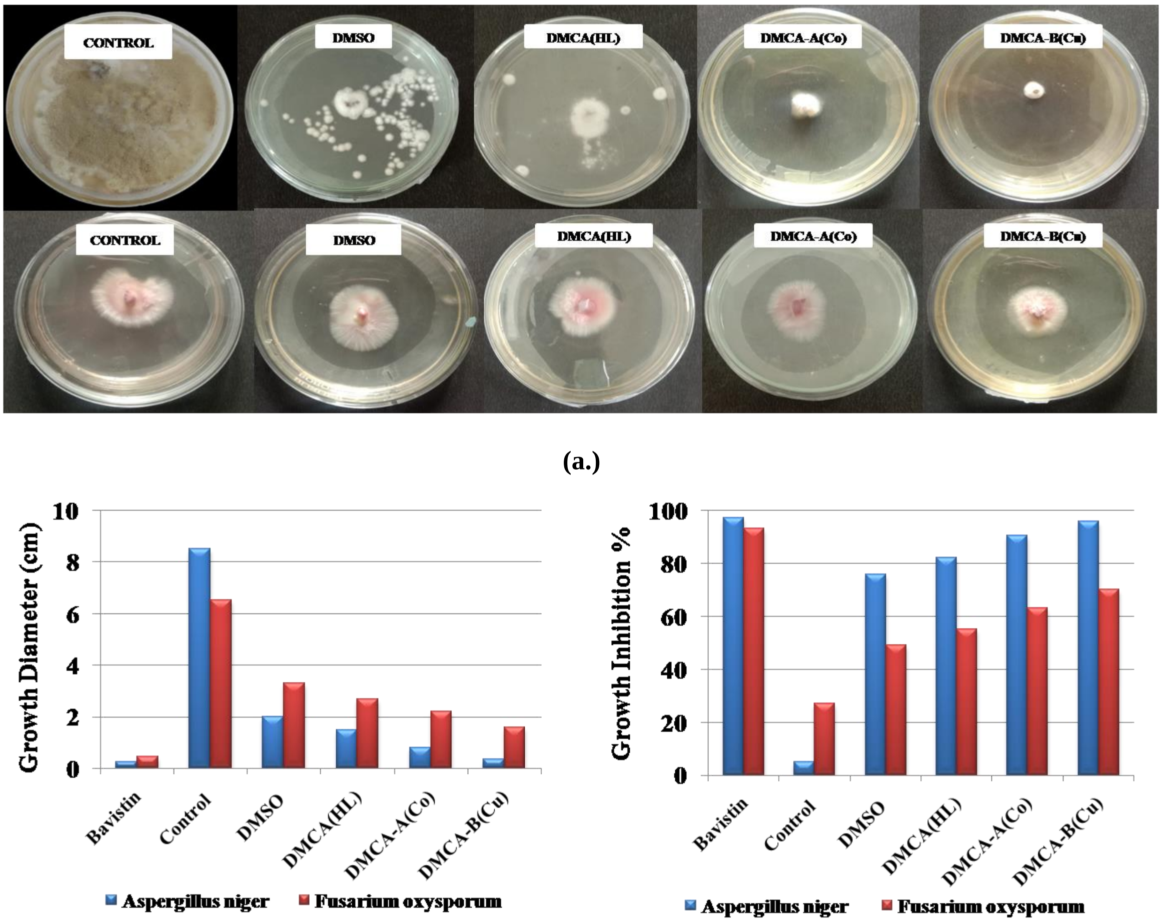
$$\%\ \text{Growth Inhibition} = \frac{D_o - D_t}{D_o} \times 100 \qquad (2)$$

D_t is the fungal growth diameter with different treatments, and D_o is the fungal growth diameter with control.

There was a decline in the fungal colony ground of various treatments and controlled treatment observation. In common, the metal complexes were more effective against fungal strains than the parent ligand [12]. It was concluded from Figure 7 (a, b) and Table 4 that the Cu (II) complex displayed good potency in contrast with Co(II) complex and ligand against the fungal growth.

Table 4. Growth inhibition % of DMCA(HL) and its metal coordinated complexes DMCA(A-B) against the two fungal species *Aspergillus niger* and *Fusarium oxysporum*.

Compounds	**Growth Inhibition %** **Aspergillus niger**	 **Fusarium oxysporum**
Bavistin	98.06	92.19
Control	5.2	28
DMSO	74	48
DMCA(HL)	82.3	55
DMCA-A(Co)	90.4	63
DMCA-B(Cu)	95.8	70



(a.)

(b.)

Figure 7. (a) Pictorial demonstration of antifungal activity and **(b)** Graphical demonstration of inhibition % Growth of Schiff base ligand DMCA(HL) and its metal complexes DMCA(A-B), against the two species of Fungi *Aspergillus niger* and *Fusarium oxysporum.*

4. Conclusion

Condensation of newly synthesized Schiff base ligand DMCA(HL) with Co(II) and Cu(II) metal ions, consequences in the creation of two new mononuclear coordinated transition metal complexes having probably the distorted octahedral molecular geometry. During experimentation, chloroform was utilized as a dissolving solvent with hexane as the topmost layer to develop a single crystal, but attempts were unsuccessful. The spectral characterization of synthesized compounds explains their structural arrangements.TGA analysis depicts the general decomposition pattern and the complexes' thermal stability. Electrochemical studies conclude that the DMCA(HL) and its metal complex DMCA(A-B) display a diffusion-controlled quasi-reversible process. The DNA binding affinities of synthesized compounds towards the pCAMBIA DNA vector were measured using UV-Vis absorption spectroscopy, and the results depict that DMCA-B(Cu) metal complex displays suitable DNA binding efficacy(against pCAMBIA DNA). The Cu(II) metal complex also exhibits potent antifungal activity against *Aspergillus niger* while average activity against *Fusarium oxysporum* in comparison to DMCA-A(Co) metal complex and parent Schiff base ligand DMCA(HL).

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Conflict of interest: We have no conflict of interest in India and Abroad.

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