

Mixed Ligand Complexes Containing (2-Hydroxy-4-Methoxyphenyl) (Phenyl) Methanone and 2-Aminophenol: Synthesis and DNA Cleavage

P. Subbaraj, A. Ramu, N. Raman, J. Dharmaraja

Abstract – A bidentate NO type Schiff base ligand (HA) has been obtained by the condensation reaction of 2-hydroxy-4-methoxy benzophenone with aniline. Using this Schiff base as main ligand (HA) and 2-aminophenol as co-ligand (B), few novel mixed ligand complexes of composition MAB [M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] have been synthesized. The resulting Schiff base (HA) and the mixed ligand complexes have been structurally characterized by UV-Vis., IR, ¹H-NMR, FAB-Mass, EPR, XRD, SEM and magnetic studies. XRD and SEM reveal that all the complexes are microcrystalline nature with uniform morphology. In-vitro biological activities of free Schiff base (HA) and the mixed ligand complexes (1-5) were screened against few pathogenic bacterial and fungal strains by well diffusion technique. All the complexes show moderate potent activities than Schiff base ligand. Furthermore, the oxidative DNA cleavage activities of free Schiff base and the mixed ligand complexes have been done by agarose gel electrophoresis method in peroxide medium.

Keywords—Mixed ligand complexes, Powder XRD, SEM, Biological, DNA activity

I. INTRODUCTION

Bioactive donor site of N, S / O atoms in organic ligand moieties were widely used in the development of metal based drugs, analytical, industrial, medicinal, agricultural, biological and clinical areas. The poly functional donor site containing Schiff base moieties were extensively used in the field of analytical, biocidal, agrochemical, enzyme models, catalysis, food, chemical and dye industries [1, 2].

The azomethine (-CH=N-) group containing Schiff base ligands and their metal complexes have been extensively studied because of their interesting catalytic [3] as well as biological activities such as antimicrobial, anticancer, antineoplastic, antiviral, antitumour, anti-HIV, antimalarial, antibiotics, anti-inflammatory and diuretic agents in chemotherapeutical and agricultural areas [4-8].

Moreover, they have been used as biological models, oxygen carriers and drugs [9]. 2-aminophenol (*o*-aminophenol) is widely used in oxidative hair dye formulations. Mixed ligand complexes have been found to act as an active catalyst in reactions of industrial importance including hydrogenation, hydroformation, and oxidative hydrolysis of olefins and carboxylation of methanol. These complexes have also shown catalytic activity in various oxidation reactions of environmental and biological importance [10, 11].

In recent years, a number of binding and cleavage studies of transition metal(II) complexes with DNA have been carried out. It is used to develop new reagents in the treatment of several diseases in clinical and therapeutic approaches [12]. As part of our systematic work on mixed ligand complexes [13-15], we now report the synthesis, characterization, biological activities and DNA studies of mixed ligand complexes containing transition metal complexes [M(II) = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] with Schiff base (derived from 2-hydroxy-4-methoxybenzophenone with aniline) and 2-aminophenol. Various physio-chemical, analytical and spectral studies have been used to understand the coordination environment of Schiff base ligand and the mixed ligand complexes. *In vitro* antimicrobial activity of Schiff base ligand (HA) and the complexes have also been studied. In addition, the oxidative DNA cleavage activities of these complexes were tested against *pUC-19* DNA using gel electrophoresis in the presence of H₂O₂ medium and this method is also useful to explore the new models for DNA interaction.

II. EXPERIMENTAL METHOD

A. Materials and Methods

2-hydroxy-4-methoxybenzophenone (extra pure), aniline and 2-aminophenol were purchased from Sigma Aldrich, S.D. Fine and Merck products respectively. Metal chloride salts were obtained from Merck. Solvents for the physical measurements were of analytical grade and were purified according to literature methods. DNA was purchased from Genei (Bangalore, India). Agarose (molecular biology grade) and ethidium bromide (EB) were obtained from Sigma (USA).

B. Physical measurements and Analytical techniques

Melting point (mp) of all the mixed ligand complexes was determined on Gallenkamp apparatus in open glass capillaries and is uncorrected.

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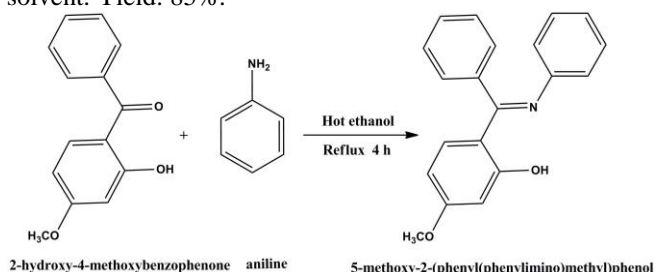
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Micro analytical data were performed on Carlo Ebra 1108, Heraeus, CHNS analyzer. Metal contents of the mixed ligand complexes were estimated gravimetrically by the standard procedure. Molar conductance of the mixed ligand complexes (1×10^{-3} M) was measured using an Elico CM 180 conductivity bridge by using 0.01 M KCl solution as calibrant. Fast atomic bombardment mass spectra (FAB-MS) were recorded using a VGZAB-HS spectrometer using 3-nitrobenzylalcohol as a matrix. Magnetic susceptibility measurements were carried out on a Gouy's balance at room temperature using mercuric tetra(thiocyanato)cobaltate(II) as the calibrant. Diamagnetic corrections were applied in compliance with Pascal's constant. Electronic absorption spectra were recorded with a Shimadzu UV-1601 double beam spectrophotometer in the 200–1100 nm range. Vibrational spectra were recorded using KBr pellets on Perkin-Elmer 783 series spectrophotometer, in the 400–4000 cm^{-1} range. ^1H NMR spectra of the ligand and the diamagnetic Zn(II) complex were carried out in DMSO- d_6 at room temperature using TMS as internal standard on a Bruker Advance DRX 300 FT-NMR spectrometer. X-band EPR spectra of Cu(II) complex at room temperature and liquid nitrogen conditions in DMSO medium were recorded on a Varian E-112 ESR spectrometer using DPPH as internal standard. Thermal stabilities of the complexes were recorded in dynamic nitrogen atmosphere (flow rate 20 ml / min) with a heating rate of 10 $^\circ\text{C}$ / min using a Perkin Elmer (TGS-2 model) thermal analyzer. Powder X-ray diffraction (XRD) patterns were recorded with a Bruker AXS D8 advance powder X-ray diffractometer (X-ray source: Cu, Wavelength 1.5406 A°). Si(Li)PSD was used as detector. Scanning Electron Micrography with Energy Dispersive Spectrometry associated (SEM/EDS) using JSM-5610 scanning electron microscope was used for morphological evaluation.

C. General procedure for synthesis of Schiff base ligand (HA) and Mixed ligand complexes (MAB)

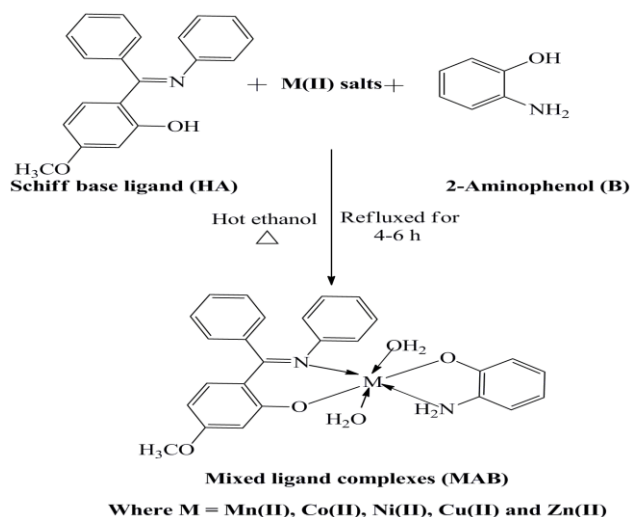
Hot ethanolic solution (20 mL) of 2-hydroxy-4-methoxybenzophenone (2.28 g, 10 mmol) was added drop wise into an ethanolic solution (20 mL) of aniline (0.93 g, 10 mmol). The resultant mixture was refluxed for 4 h under stirring (Scheme I). The resultant hot solution was reduced to 1/3 of its original volume and kept aside at room temperature for evaporation. The yellow crystalline solid product was separated and recrystallized from the same solvent. Yield: 85%.



Scheme I: Synthesis of Schiff base ligand (HA)

To 10 mL of ethanolic solution of Schiff base ligand (0.304 g, 1 mmol) was added drop wise to the ethanolic solution (10 ml) of metal(II) chloride (1 mmol) and stirred for 2 h. To this solution, add 10 mL of hot ethanolic solution

of 2-aminophenol (0.110 g, 1 mmol) and refluxed for 4 - 6 h (Scheme II). The resulting solution were reduced to 1/3 of its original volume by water bath and kept aside. On standing, the mixed ligand complexes were obtained and collected by vacuum filtration, washed several times with cold water, ethanol and anhydrous ether. The mixed ligand complexes were obtained as powder and dried in air and stored in vacuo over anhydrous CaCl_2 at room temperature. The yield of the isolated complexes is found to be 50–65%.



Scheme II: Synthesis of mixed ligand complexes (MAB)

D. In vitro biological activities

In vitro biological activities of Schiff base ligand and the complexes in DMSO medium were tested against three Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus*, two Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* using Muller Hinton agar nutrient and three fungi namely *Aspergillus niger*, *Enterobacter species* and *Candida albicans* using potato dextrose agar as the medium by well diffusion technique [16]. About 2–8 h, older microorganism species inoculums containing approximately 10^4 – 10^6 colony forming units (CFU) per ml were used in these analyses. All the analyses were made in three replicate for each and the detailed procedure for measuring the zone of inhibition described as earlier [13 - 15]. The results were recorded as zone of inhibition in mm and compared with the commercially available ampicillin and nystatin, used as antibacterial and antifungal control respectively. All the analyses were made in three replicate for each and the results were compared with control.

E. DNA cleavage studies

Stock solution of *pUC-19* DNA concentration per nucleotide [C(p)] in this buffer medium was measured from its absorption intensity at 260 nm after 1:100 dilutions. Stock solutions were kept at 4 $^\circ\text{C}$ and used after not more than four days. Doubly distilled H_2O was used to prepare the buffer. DNA cleavage activities of Schiff base ligand and the complexes were monitored by agarose gel electrophoresis with DNA. Experiments were performed under aerobic conditions with oxidant (H_2O_2) by incubation at 310 K for 2 h as follows: *pUC-19* DNA 30 μM , 50 μM of each complex solution, 50

μM of H_2O_2 in 0.05 M Tris-HCl buffer ($\text{pH} = 7.2$) containing 50 mM NaCl solution were generally taken. After incubation, 1 μL of loading buffer (bromophenol blue in H_2O) was added to each tube, the solution was mixed thoroughly and then loaded on 1 % agarose gel media. Samples were examined through electrophoresis at a constant voltage (50 V) for 2 h in Tris-acetic acid-EDTA buffer. After electrophoresis, the gel was stained for 30 min by immersing it in 1 $\mu\text{g} / \text{cm}^3$ ethidium bromide solution. Oxidative cleavage was visualized by viewing the gel under UV light and then photographed.

III. RESULTS AND DISCUSSION

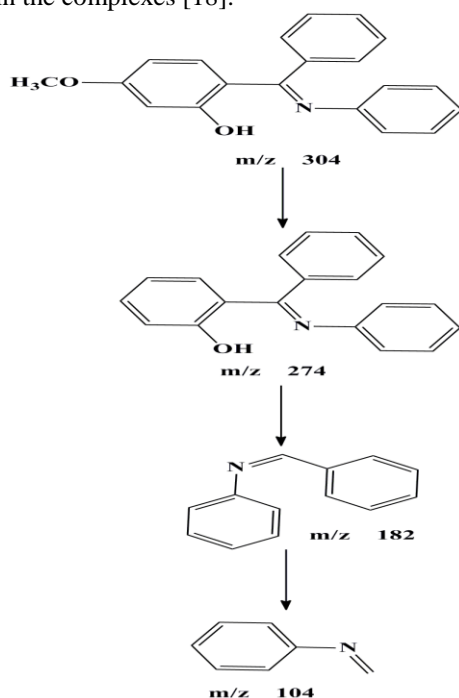
The synthesized Schiff base ligand (HA) and the complexes are air stable, non-hygroscopic and insoluble in water. The ligand was soluble in organic solvents and the mixed ligand complexes are soluble in DMF & DMSO. The synthesized ligand and their complexes were characterized with the help of several analytical and spectral techniques.

A. Elemental analysis and conductivity measurements

From the micro elemental analysis data of free Schiff base and the complexes, it is found out that these values are well agreed with the calculated values. The observed low molar conductance values of Schiff base ($8.5 \Omega^{-1} \text{mol}^{-1} \text{cm}^2$) and their complexes ($12.2 - 16.9 \Omega^{-1} \text{mol}^{-1} \text{cm}^2$) show non-electrolytic nature [17] with no counter ions.

B. FAB-Mass spectra

FAB-Mass spectra of Schiff base and the complexes provide a vital clue for elucidating the structure of the compounds. The stoichiometric composition of ligand and the complexes was calculated from the comparison of mass spectra of ligand and the complexes. The intensity of the peak is the mirror image of stability and abundance of ions present in the complexes [18].



Scheme III: FAB-Mass cleavage pattern of Schiff base

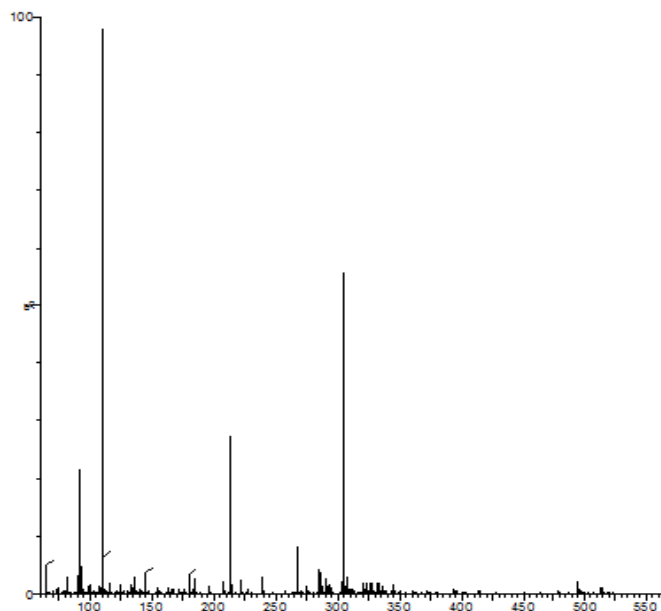


Fig. 1: Mass spectrum of the Schiff base ligand (HA)

The molecular ion peak for the Schiff base (HA) and its fragmentation pattern along with m/z values are observed at 304 with $[M+1]$ pattern (Fig. 1) and the most intense peaks (m/z) at 274, 182 and 104 corresponding to the fragments of $[\text{C}_{19}\text{H}_{15}\text{N}_1\text{O}_2]$, $[\text{C}_{13}\text{H}_{13}\text{N}]$ and $[\text{C}_7\text{H}_7\text{N}]$ moieties respectively (Scheme III) which confirm the proposed structure of the Schiff base ligand (HA). The copper complex shows the molecular ion peak at 513 m/z . Thus, the FAB spectral results, confirming the stoichiometry composition present in free ligand (HA) and the complexes, reinforce the conclusion drawn from the elemental analysis data.

C. IR studies

On comparison of vibrational spectra of free Schiff base (HA) with the mixed ligand MAB type complexes to find out the way of chelation of ligand to the central metal atom. The Schiff base ligand shows strong bands around at 1602 and 1240 cm^{-1} which indicate the presence of azomethine ($\nu_{\text{CH}=\text{N}}$) and phenolic-OH (ν_{OH}) group respectively. In the mixed ligand complexes both the peaks are shifted towards lower frequency at 1575 – 1588 and 1221 – 1230 cm^{-1} respectively *i.e.*, azomethine-N and deprotonated phenolic-O atoms indicating both are involved in the chelation [19]. This is further confirmed from the appearance of weak to medium intensity bands in far IR region at 425 – 462 and 546 – 580 cm^{-1} which correspond to $\nu_{(\text{M}-\text{O})}$ and $\nu_{(\text{M}-\text{N})}$ modes respectively [19]. The co-ligand of 2-aminophenol (B) exhibits two characteristic bands, at 3375 cm^{-1} for $\nu(\text{NH}_2)$ and 1268 cm^{-1} for $\nu(\text{OH})$ vibrations. On metal chelation, the phenolic-OH frequency of 2-aminophenol is shifted towards lower frequency region (1260 cm^{-1}). This indicates that the ligand coordinates to the metal(II) ion in a bidentate manner *via* amine-N and deprotonated phenolic-O atoms respectively. Both the ligands (Schiff base and 2-aminophenol) form a stable 5,6 membered chelate rings around the central metal ions. All the mixed ligand complexes show an additional peak in the regions 3200 – 3500, 835 – 855 and 710 - 732 cm^{-1} due to ν_{OH} stretching, rocking and wagging modes of coordinated water molecules respectively.

Furthermore, the strong evidence for the presence of coordinated water molecules is also supported from TGA/DTA analysis of the complexes.

D. Electronic spectra coupled with magnetic values

Electronic spectra of Schiff base (HA) and the complexes were recorded (10^{-3} M) in DMSO solution at room temperature. The electronic spectrum of Schiff base ligand exhibits three bands at 40815, 34580 and 29420 cm^{-1} which can be attributed to $\pi \rightarrow \pi^*$ transition of benzene moieties and intra molecular charge transfer transition of $n \rightarrow \pi^*$ respectively [20, 21]. The Mn(II) mixed ligand complex shows weak absorption bands at 24175, 20385 and 13700 cm^{-1} which correspond to ${}^6A_{1g} \rightarrow {}^4T_{1g}({}^4G)$, ${}^6A_{1g} \rightarrow {}^4T_{2g}({}^4G)$ and ${}^6A_{1g} \rightarrow {}^4A_{1g}$, ${}^4E_g({}^4G)$ transitions [21] respectively and these transitions confirm the octahedral environment around the Mn(II) ion which is further supported from the observed magnetic susceptibility value (5.52 BM) [20]. Co(II) complex shows a three absorption bands around 9850, 14625 and 19260 cm^{-1} which are assigned to ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{2g}(\text{F})$, ${}^4T_{1g}(\text{F}) \rightarrow {}^4A_{2g}(\text{F})$ and ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$ transitions [21] respectively. The magnetic susceptibility value of Co(II) complex is 5.72 BM which corresponds to octahedral geometry. The Ni(II) complex shows four absorption bands around 10070, 16740, 25884 and 34790 cm^{-1} respectively. The transitions of these bands are assigned as ${}^3A_{2g}(\text{F}) \rightarrow {}^3T_{2g}(\text{F})$, ${}^3A_{2g}(\text{F}) \rightarrow {}^3T_{1g}(\text{F})$, ${}^3A_{2g}(\text{F}) \rightarrow {}^3T_{1g}(\text{P})$ and LMCT transitions respectively with distorted octahedral geometry around the Ni(II) ion. Cu(II) complex shows a broad band which is centred at 14726 cm^{-1} indicating distorted octahedral geometry of the complex with ${}^2E_g \rightarrow {}^2T_{2g}$ transition. The observed μ_{eff} value (1.85 BM) further substantiates the complex environment. Zn(II) complex show a broad band in UV region at 26204 cm^{-1} which corresponds to octahedral geometry and does not show any d-d transition in the visible region.

D. NMR Spectral studies

The proton NMR spectra of Schiff base ligand (HA) and the diamagnetic zinc(II) mixed ligand complex have been recorded in DMSO- d_6 using TMS (tetramethylsilane) as internal standard. The NMR spectra of ligand support the conclusions derived from the IR and FAB-Mass spectral studies. The Schiff base ligand (HA) shows a multiplet signals around 6.4 – 7.6 ppm due to the presence of aromatic phenyl moiety. Also the signal at 8.85 ppm corresponds to the azomethine (-HC=N-) group in the Schiff base (Fig. 2).

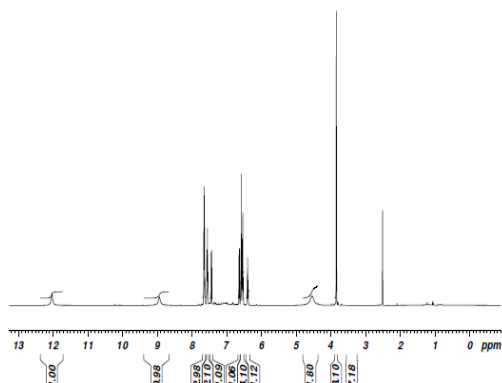


Fig. 2: Proton NMR spectrum of Schiff base (HA)

The signal at 12 ppm in the spectrum of ligand (HA) corresponds to phenolic-OH group (Fig. 2) and the absence of this signal in the spectrum of Zn(II) complex suggests that deprotonated phenolic-O groups are involved in chelation. The downfield shifting of azomethine (-HC=N-) group in Schiff base from 8.85 ppm to 8.7 ppm in the Zn(II) complex indicates that sp^2 hybridized azomethine group is also involved in the complexation. In Zn(II) complex, few peaks have been appeared in between 4.72 – 4.85 ppm due to the presence of coordinated water molecules in the complex.

E. EPR Spectra

The EPR spectra of Cu(II) complex were recorded at room temperature (300 K) and liquid nitrogen temperature (77 K). The g-tensor values were computed from the spectrum using tetracyanoethylene (TCNE) free radical as 'g' marker. From the observed spectrum, the Cu(II) complex follows the g-tensor values as: g_{\parallel} (2.25) > g_{\perp} (2.03) > g_e (2.0027) which indicate that the unpaired electron is localized in $d_{x^2-y^2}$ orbital of the Cu(II) ion with $3d^9$ configuration [22] with considerable covalent nature [23] present in Cu-L. The observed g_{avg} value of this complex is equal to 2.07 and deviated from g_e (2.0027) value due to the covalence property. These values also give evidence for the elemental and vibrational studies of metal chelating environment. Absence of any half field signal at 1600 G corresponding to $\Delta M_s = \pm 2$ transitions, ruling out any magnetic exchange *i.e.*, Cu-Cu interactions in the complex.

F. Thermogravimetric analysis

The thermogram analysis (TGA/DTA) of the complexes is helpful to confirm the following: (i) thermal stability of the compounds, (ii) presence of water molecule inside or outside the coordination sphere and (iii) decomposition temperature [24]. In this study, the heating rate at 10 $^{\circ}\text{C}/\text{min}$ under N_2 atmosphere was studied. Enhancing the temperature upto 180 $^{\circ}\text{C}$, a steady weight loss of about 5-6% of Ni(II) complex confirms the presence of coordinated water molecule present in the complex (Fig. 3). By increasing the temperature a sudden weight loss obtained at 350 and 417 $^{\circ}\text{C}$ indicates that evaporation of the organic moieties and followed by formation of stable metal oxide at 736 $^{\circ}\text{C}$.

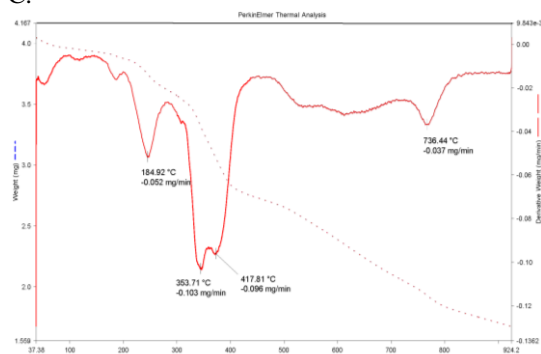


Fig. 3: Thermogram of Ni(II) mixed ligand complex

G. Powder X-ray diffraction and SEM morphology analyses

The powder XRD (PXRD) and scanning electron microscopy (SEM) analysis were useful to determine the particle size and morphology of the sample. Powder XRD spectrum of Schiff base ligand (HA) was compared with the spectrum of Mn(II) complex. The spectra of Schiff base and the Mn(II) mixed ligand complex exhibit a slight crystalline nature with sharp peaks due to the presence of coordinated water molecule. Few new peaks were appeared in the spectrum of complex compared to the spectrum of ligand (HA) which indicate the formation of metal chelates [25]. The particle size and structure of mixed ligand complex has been shown from SEM pictograph (Fig. 4). From the pictograph, the complex has uniform morphology and the particle size of the Mn(II) complex is 10 μm with broken brick like shape. This leads to believe that we are dealing with homogeneous phase material.

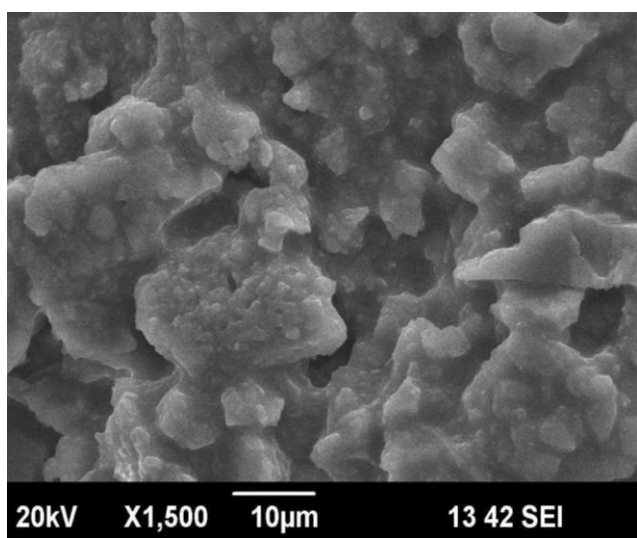


Fig. 4: SEM pictograph of Mn(II) mixed ligand complex

H. Biological activities

In-vitro biological activity of Schiff base ligand (HA) and the mixed ligand complexes was tested against few bacterial and fungal strains by well diffusion method using agar as nutrient. Commercially available standard drugs ampicillin (antibacterial control) and nystatin (antifungal control) were used as control. All the mixed ligand complexes show remarkable biological activities against different microorganisms (Fig. 5). The higher inhibition zones of the Schiff base ligand and the mixed complexes can also be explained on the basis of Overtone's concept and Tweedy's chelation theory [26].

Also it increases the delocalization of π - electrons over the whole chelate ring resulting in high lipid solubility within the chelate ring system. From the results, all the mixed ligand complexes show more significant antibacterial and less pronounced antifungal activities. Moreover, the biological activities of the mixed ligand complexes are found to be increased as the stability of the complexes increased. The activity of Schiff base ligand (HA) and the mixed ligand complexes follows the order as:

Control > CuAB > MnAB > CoAB ≈ NiAB > ZnAB > Schiff base ligand

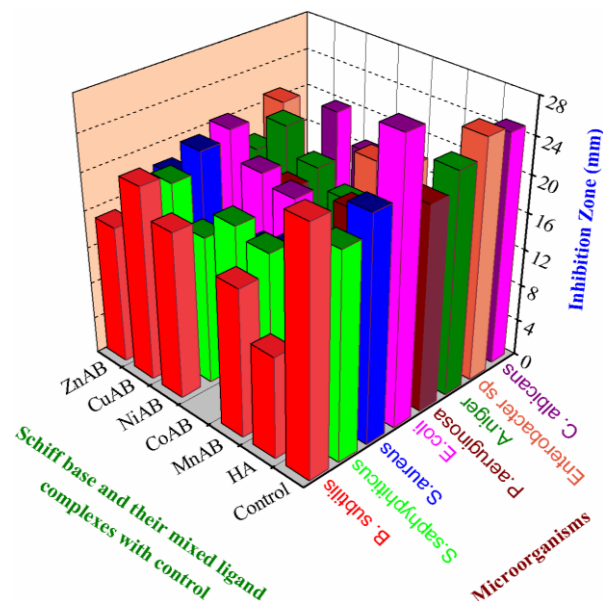


Fig. 5: Biological activities of mixed ligand complexes

I. Oxidative cleavage studies

The cleavage efficiency of mixed ligand complexes with *pUC-19* DNA was investigated by gel electrophoresis method in the presence of an oxidant (H₂O₂) medium. Control experiment using *pUC-19* DNA alone does not show any significant cleavage even on longer exposure time. In general, the oxidative cleavage is proposed to account of *pUC-19* DNA cleavage caused by the M(II) ions reacting with the H₂O₂ to produce the diffusible hydroxyl radicals or molecular oxygen or peroxy derivative generated from the co-reactant H₂O₂ [27]. Cleavage is inhibited by free radical via the abstraction of a hydrogen atom from sugar units (at C4' position) and predicts the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed and finally cleaves *pUC-19* DNA. From the pictograph, the result shows that three out of five complexes have the ability to cleave the *pUC-19* DNA in the presence of oxidant.

IV. CONCLUSION

In the present study, NO type of bidentate Schiff base ligand (HA) has been derived from 2-hydroxy-4-methoxybenzophenone and aniline. The MAB type of mixed ligand complexes were also synthesized from Schiff base (HA) and 2-aminophenol with M(II) ions [where M(II) = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)]. The synthesized Schiff base and their mixed ligand complexes were structurally characterized by using various physico-chemical and spectral studies. From the spectral studies the geometry of mixed ligand complexes has been explored and found to have distorted octahedral environment with mononuclear nature. The *in-vitro* biological activities of the mixed ligand complexes show much potent activities towards different microorganisms than the Schiff base.

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