

# METAL COMPLEX BASED ON CHITOSAN SCHIFF BASE AS POTENT ANTIMICROBIAL AND ANTIOXIDANTS

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## ABSTRACT

In the present work new Schiff base ligand have synthesized and were complexed with Copper, Cobalt and Manganese (II) metal ions. The Schiff base ligand was synthesized by condensation of chitosan with 6-methoxy-2-naphthaldehyde. The ligand and their metal complexes have been characterized by C, H, N analysis and UV-Visible Spectroscopy and IR spectra for tentative structure proposal. Thermal analysis by TGA/DTA confirms the stability of metal complexes. The ligand and their metal complexes were tested for their antimicrobial, antioxidant and antifungal activity against gram positive and gram negative which showed that the metal complexes show more potent activities than Schiff base.

*Key Words:* Schiff base, Metal complex, UV-Visible Spectroscopy and IR, biological activity.

  
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## 1. INTRODUCTION

Chitosan is biocompatible, biodegradable and non-toxic biomolecule used in various medicinal applications such as antimicrobial and wound healing, antioxidant biomaterials [1, 2]. It is also used as effective chelating agent due to its ability to bind with cholesterol, fats, proteins and metal ions. The use of natural compounds and medicinal plants dates back to ancient times, chitosan possess multiple properties in different fields, could be a stimulating starting point for the discovery of new pharmacological - antimicrobial platforms. Chitosan in different forms such as solutions, films and composites has been reported as antimicrobial agents against wide range of organisms such as yeast, algae, bacteria and fungi both in *in vivo* and *in vitro* interactions [3]. Allan and Hardwiger reported the broad-spectrum antibacterial activity of chitosan material [4,5], together with the great commercial potential, the antimicrobial property of chitosan and its derivatives has attracted great attention from researchers. With this in mind, we investigated the effect of chitosan Schiff (CSB) base for their antimicrobial and antioxidant effects.

Chitosan is usually derived by the random N-deacetylation of chitin under alkaline conditions [6]. The inherent reactive amino groups of chitosan permit certain chemical modifications which lead to several important applications [7].

Schiff bases (SB) are important intermediates for the synthesis of various bioactive compounds. SB molecules allow probable positions for bio-chemically active compounds that are related to intermolecular hydrogen bonding and proton transfer equilibria. The transition metal complexes of Schiff base act as a significant moiety for various applications because it has various coordination sites containing nitrogen, Sulphur or oxygen as ligand atoms to bind

with different metal centers and ease the synthesis of metal complexes [8].

SB have been well documented in a broad range of biological activities [9], including antimalarial, antiproliferative [10], analgesic, anti-inflammatory [11], antiviral, antipyretic, antifungal [12] and antibacterial [13] properties. From all these observations, it seems to be the azomethine group ( $>C=N-$ ) is crucial for their biological activities. CSB have been mostly investigated as the antibacterial agents than as the antifungal agents. Antimicrobial activities of CSBs are illustrated in various physical forms such as powder form, gels, thin films [14-17].

Also it's observed from the literature that CSBs exhibit better antimicrobial property than the base molecule chitosan [18]. The high affinity for the chelation of the SB towards the transition metal ions is used in preparing the complexes [19]. Also, in the literature various easier synthetic pathways have been reported for the synthesis of Chitosan based Schiff bases [20-21].

Antibacterial materials could be classified into two groups: inorganic and organic materials. Inorganics are metals, metal oxides and metal phosphates [22]. Metal oxides are significantly important in various fields and they are considered as safe materials to human beings and animals [23]. Organic compounds such as phenols, halogenated compounds and quaternary ammonium salts are reported to be good antibacterial. Recent years natural materials, such as chitosan (CTS) and chitin as antibacterial materials have been focused effectively [24]. Antioxidants studies on metal and metalloid and chitosan molecules have been reported well in the literature [25- 27].

The present work highlights the hybrid characteristics of metal and chitosan moiety in the form of SB. In the present work novel CSBs base was synthesized by condensation of chitosan with 6-methoxy - 2 - naphthaldehyde and then were complexed with Cu, Co, Mn. The

prepared compounds have been characterized by Infrared spectroscopy which determines the formation of corresponding functional group and Thermal analysis (TGA/DTA) which determines the stability of the complexes.

Synthesis of CSB and its metal complexes as drugs and its characterization need considerable effort to get a compound of interest. Beside all the limitations and side effects transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics. The present work highlights the hybrid characteristics of metal and chitosan moiety in the form of SB.

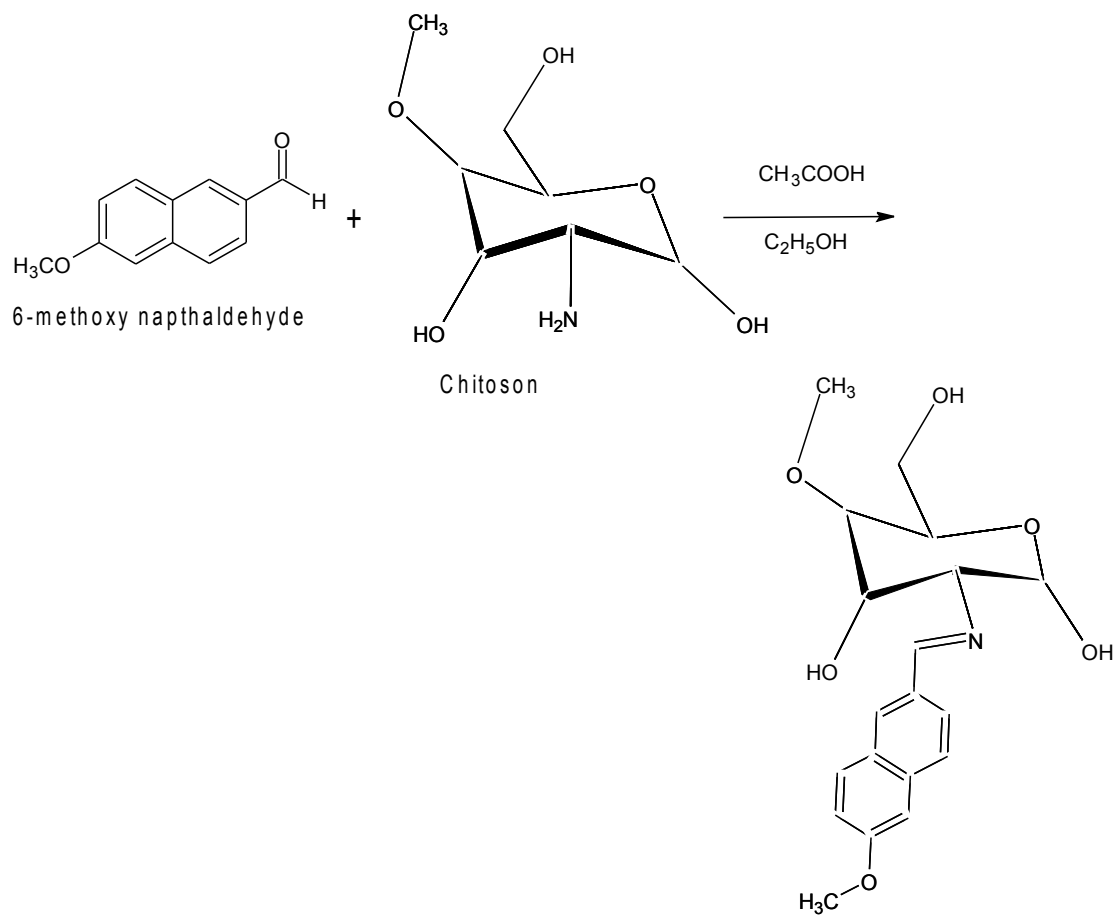
Concerning the biological activity, the synthesized SB and its metal complexes were screened for in-vitro antioxidant study by DPPH scavenging assay, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (*E coli*) and antifungal activity against *Aspergillus flavus*, *Chrysosporium Keratinophilum*, and *Candida albicans*. The studies have shown that metal complex have better biological activity than compared to CSB.

## 2. MATERIALS AND METHODS

All the reagents used namely, Chitosan, 6-methoxy-2-naphthaldehyde, cobalt chloride, manganese chloride, copper sulphate, acetic acid, DMSO, ethanol are of synthetic grade. Elemental microanalysis of the compounds, C, H and N were performed using Shimadzu elemental vario EL III model elemental analyzer. Melting points of the ligand and its complexes were recorded using melting point apparatus. UV-Visible spectra were recorded in PC based double beam spectrometer 2202 in DMF solution. The IR spectra were obtained in a KBr disk using a BIO-RAD FTS 135 spectrometer. Thermal analyses were done by TGA and DTA.

## 2.1 Synthesis of CSB

The CSB (ligand) was prepared by mixing about 4g (0.01M) of purified Chitosan dissolved in acetic acid and ethanol, the mixture was stirred for about 30 minutes to get a homogeneous mixture. The chosen aldehyde i.e. 6-methoxy-2-naphthaldehyde of 0.5g (0.01M) dissolved in ethanol and added to the above mixture and stirred for 48 hours. The solution was washed with excess ethanol. The obtained yellow colored product was filtered, dried and then recrystallized to get pure crude product. The prepared compound was checked for its purity by TLC using glass plates percolated with silica gel 60GF254 and suitable solvent system as mobile phase. Proposed reaction represented in **scheme 2.1**.



Scheme 2.1 Synthesis of CSB

## 2.2 Synthesis of metal complex

About 0.2g of CSB dissolved in DMSO and then mixed with alcoholic solution of metal salts i.e. Copper sulphate, Cobalt chloride and manganese chloride. The resulting solution was refluxed for 8-10 hours to reduce the concentrated to half of its initial volume. The obtained complexes were filtered, washed with DMSO and dried. Physical properties and IR analysis of all the synthesized ligand complexes are summarized in **Table 1 and Table 2**.

## 2.3 Assay of biological activity

Newly synthesized Schiff base and its metal complexes were screened for their antibacterial activity against four bacterial strains, namely *Staphylococcus aureus* and *Escherichia coli* by well plate method [27-29]. Also, the compounds were screened for antifungal activity [30-31] against *Aspergillus Flavus*, *Chrysosporium Keratinophilum* and *Candida Albicans*. Ketoconazole and Amphotericin B were used as the standard drug. The mechanism of the antimicrobial activity of Chitosan was different for Gram- positive and Gram-negative. Also the DPPH scavenging assay was reported for all the complexes prepared.

### 2.3.1 Antibacterial activity

The *in vitro* antibacterial activity of newly synthesized compounds was determined by well plate method in Muller Hinton Agar [28-29]. The newly synthesized compounds were tested for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Ciprofloxacin was used as the standard drug. The test compounds were dissolved in dimethyl sulfoxide at concentrations of 1 mg/ml. All bacterial strains were maintained on nutrient agar medium at  $\pm 37^{\circ}\text{C}$ . The cultures were inoculated in fresh 10 ml nutrient broth to yield an

initial suspension of approximately 10-100 cfu/ml. All broths were then incubated statically at the aforementioned temperature for microorganisms, for 18-24 hours so that all cells were in the stationary phase. The bacterial suspensions were diluted ten fold in distilled water, and 0.1 ml from the appropriate dilution was spread plated on nutrient agar in order to give a population of approximately  $10^6$  cfu/plate. The wells were dug in each petri plate by sterilized cork borer. The compounds were dissolved in Dimethyl sulfoxide and appropriate dilutions were made. The same procedure was repeated for different micro - organisms. Each experiment was carried out in triplicate. After the inoculation of organism and compound, the Petri plates were incubated for 18 hours at 37°C. After the incubation, the inhibition zone was measured and the values for dimethyl sulfoxide were subtracted to get the actual values. The results are summarized in **Table 4**.

### 2.3.2 Antifungal activity

The newly synthesized compounds were also screened for their antifungal activity [30-31] against *Aspergillus Flavus*, *Chrysosporium Keratinophilum* and *Candida albicans*. The compounds were dissolved in Dimethyl sulfoxide and antifungal activity was determined by well plate method at concentration of 1 mg/ml. The required amounts of each fungal strain were removed from the stock and suspended in 5ml of distilled water with 2 drops of Tween 80. This suspension was uniformly spread on Petri plates containing Potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25°C for 3 days. The plates were then examined for the presence of zone of inhibition and the results were recorded. Ketoconazole was used as a positive control at a concentration of 1 mg/ml. Antifungal activity of the CSB is based

on its chains crossing the cell membrane inhibiting the cell from the inside. Activity of Chitosan for fungus is assumed to be fungi static but not fungicidal. The results are summarized in **Table 4**.

### 2.3.3 Antioxidant studies

Antioxidants scavenge free radicals from damaging the body cells. They also inhibit the Oxidation that forms free radicals. Usually free radicals are produced during break down of food or exposed to tobacco / smoke. Antioxidants play important role in treating heart diseases, cancer and other diseases. Synthetic antioxidants preferred over natural antioxidant due to its effectiveness as well as cost. The synthetic antioxidant may be Schiff Base which acts as scavenge for free radical. Therefore CSB and their metallic complexes shows higher antioxidants capacity.

The newly synthesized Chitosan based Schiff bases and its metal complexes were evaluated for antioxidant study by DPPH scavenging assay according to the method of Brand-Williams *et al.* [32]. The antioxidant screening revealed that, some of the tested compounds showed good free radical scavenging capacity on comparison with the standard Butylated Hydroxytoluene (BHT).

Free radical scavenging activity of the test compounds were carried out based on the scavenging activity of stable DPPH. 100 mg/mL of each test sample and standard BHT was taken in different test tubes and the volume was adjusted to 1mL using MeOH. Freshly prepared 3mL of 0.1 mM DPPH solution was mixed and vortexed thoroughly and left in dark for 30 min. The absorbance of stable DPPH radical was measured at 517 nm. The DPPH control (containing no sample) was

prepared using the same procedure. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation of DPPH radical scavenging activity.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{(\text{Abs Control})} * 100$$

Where Abs Control is the absorbance of DPPH radical + methanol; Abs Sample is the absorbance of DPPH radical + test sample/standard BHT. The antioxidant study results are tabulated in **Table 5**

### 3. RESULT AND DISCUSSIONS

#### 3.1 Physical data of synthesized CSB and Metal complex

The physical properties and the micro analytical data of the CSB ligand and metal complexes were summarized in the **Table 1**. The color change from the CSB to metal complexes implies the formation of new coordination complexes. The molecular formula of ligand and metal complexes are proposed based on the result of micro analytical tool in combination with spectral techniques.

#### 3.2 Elemental analysis and UV-visible spectroscopy

The structure of the products were confirmed by elemental analysis, which shown that the difference between the found values and calculated values of carbon, hydrogen and nitrogen elements are situated within the range which confirmed the correctness of the suggested structures of the prepared compounds (**Figure 1, 2, 3**). The absorption band at 410nm was observed in the UV- Vis spectrum of the free Schiff base. Upon complexation the absorption band was shifted to 540nm, 510nm, 530nm, respectively due to the formation of metal complexes like Co, Cu, Mn.

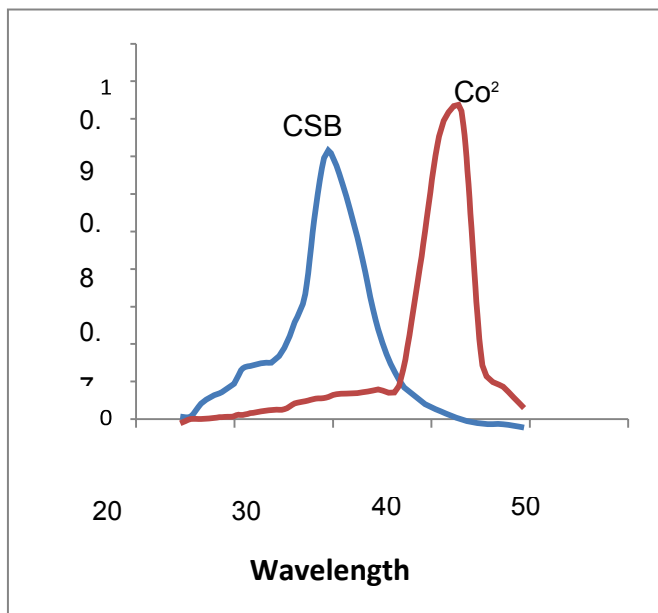


Figure 1: UV-Visible spectra of CSB and Co (II) Complex

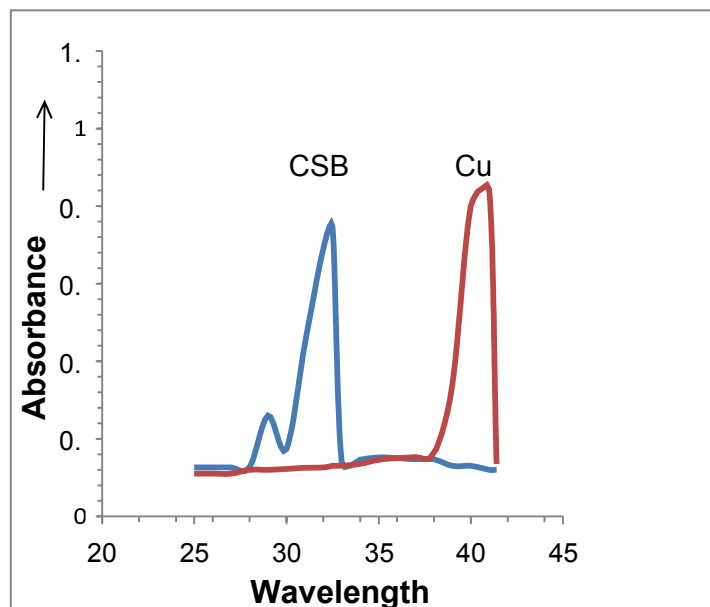


Figure 2: UV-Visible spectra of CSB and Cu (II) Complex

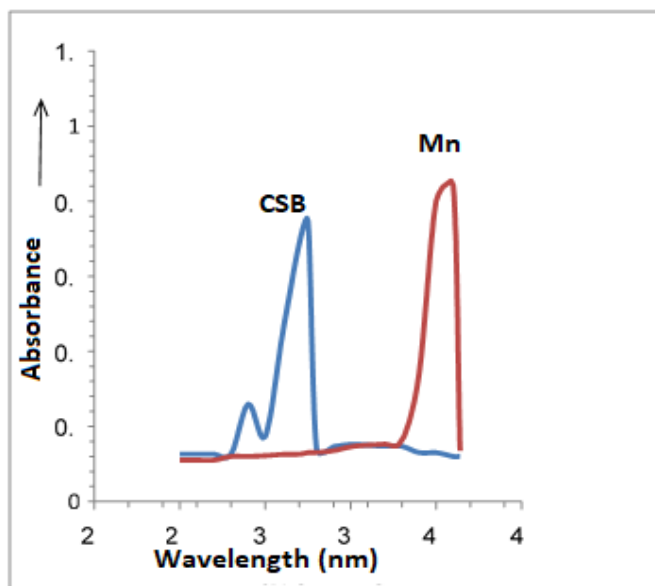
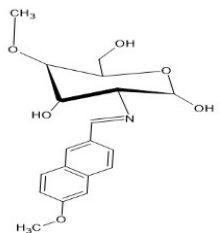
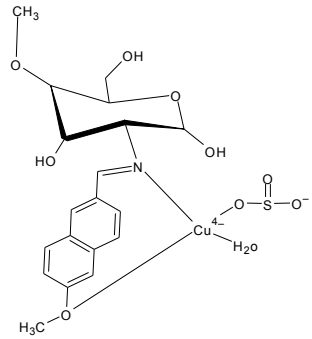
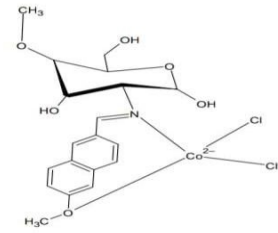
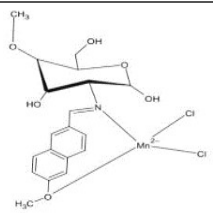


Figure 3: UV-Visible spectra of CSB and Mn (II) Complex

Compound	Molecular formula	Molecular weight	Color	Melting point (°C)	Elemental analysis Cal (found) %			
					C	H	N	M
CSB	C <sub>19</sub> H <sub>23</sub> NO <sub>6</sub> 	361.389	Yellow	223- 225	63.15	6.41	3.88	-
Cu-CSB	Cu-C <sub>19</sub> H <sub>25</sub> NO <sub>10</sub> S 	523.016	Dark green	288- 290	43.63	4.82	2.68	12.15
Co-CSB	Co-C <sub>19</sub> H <sub>23</sub> NO <sub>6</sub> Cl <sub>2</sub> 	491.229	Dark Blue	268- 270	47.35	5.36	2.76	11.62
Mn-CSB	Mn-C <sub>19</sub> H <sub>23</sub> NO <sub>6</sub> Cl <sub>2</sub>	487.234	Purple	298 - 300	47.73	5.41	2.78	10.92

								
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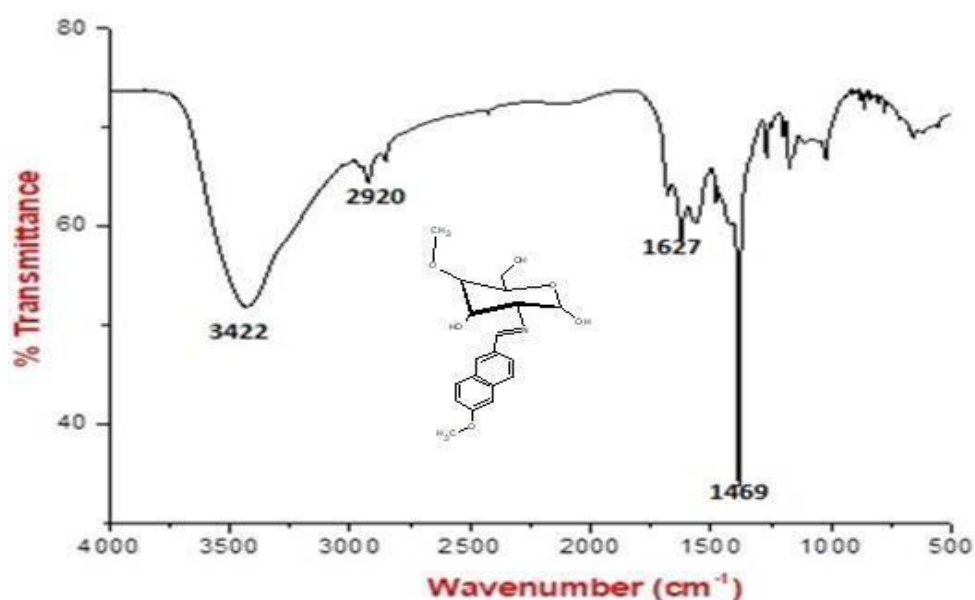
**Table 1:** Physical data of synthesized compounds

### 3.3 Infrared spectroscopy (IR)

The presences of characteristic bands of CSB Ligand and its metal complexes were confirmed by IR spectral analysis.

#### 3.3.1 IR spectra of CSB

The spectra of CSB (Ligand) **Figure 4** exhibited bands in the region  $1627\text{ cm}^{-1}$  which were due to the stretching frequency of C=N group and a broad band was observed at  $3422\text{ cm}^{-1}$ , which is due to the O-H stretching frequency of aromatic group. The characteristic bands observed around  $2920\text{ cm}^{-1}$  and  $1469\text{ cm}^{-1}$  were assigned to C-H bond and C=C bond which attributes to aromatic compounds.



**Figure 4:** FT-IR spectra of CSB (L)

The Change in the frequency and intensity of the bands observed in FT-IR spectra of polymeric Schiff base metal complexes such as Copper (II), Cobalt (II) and Manganese (II) indicates the formation of coordination bond with ligand. Obtained IR frequency of CSB ligand and their metal complexes were listed in **Table 2**

Compound	OH, NH cm-1	C-H cm-1	C=N cm-1	CH <sub>2</sub> -CH <sub>2</sub> cm-1	C-O cm-1	M-N cm-1	M-O cm-1
CSB	3422	2920	1627	1469	1264		
Cu-CSB	3409	2855	1629	1462	1172	563	470
Co-CSB	3432	2976	1639	1440	1192	440	640
Mn-CSB	3433	2923	1635	1443	1111	496	600

**Table 2:** IR result of CSB and its metal complexes

### 3.3.2 IR spectra of Cu-C<sub>19</sub>H<sub>25</sub>NO<sub>10</sub>S

The IR spectra of Cu complex shown in **Figure 5** reveals that, the positional shift have been observed from 1627cm<sup>-1</sup> to 1629cm<sup>-1</sup> for the band C=N complex. The band in the region 1462 cm<sup>-1</sup> were assigned for presence of C=C group. In addition, the formation of new bands observed at 624 cm<sup>-1</sup>, 563 cm<sup>-1</sup> and 470cm<sup>-1</sup> corresponds to Cu-N and Cu-O justifies the formation of metal to ligand bonds and thus metal complex.

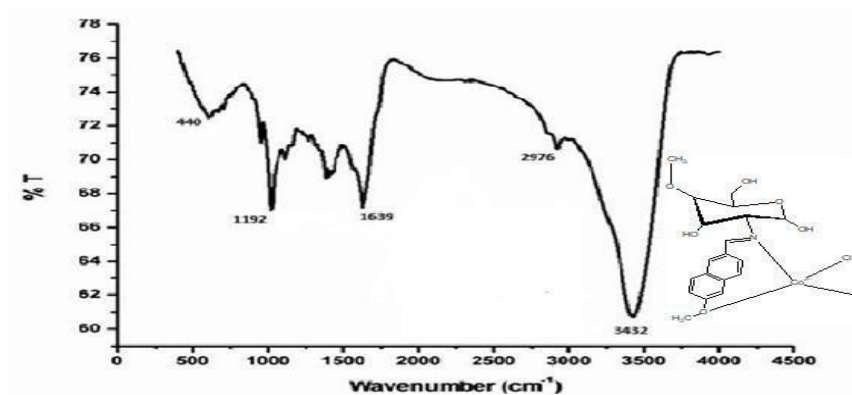


Figure 5: FT-IR spectra of CSB -Co Complex

### 3.3.3 IR spectra of Co-C<sub>19</sub>H<sub>23</sub>NO<sub>6</sub>Cl<sub>2</sub>

The IR spectra of Co complex shown in **Figure 6** reveals that, the positional shift have been observed from 1627cm<sup>-1</sup> to 1639cm<sup>-1</sup> for the bands C=N in the complex. The band in the region 440cm<sup>-1</sup> and 640cm<sup>-1</sup> were assigned for the presence of Co-N and Co-O groups.

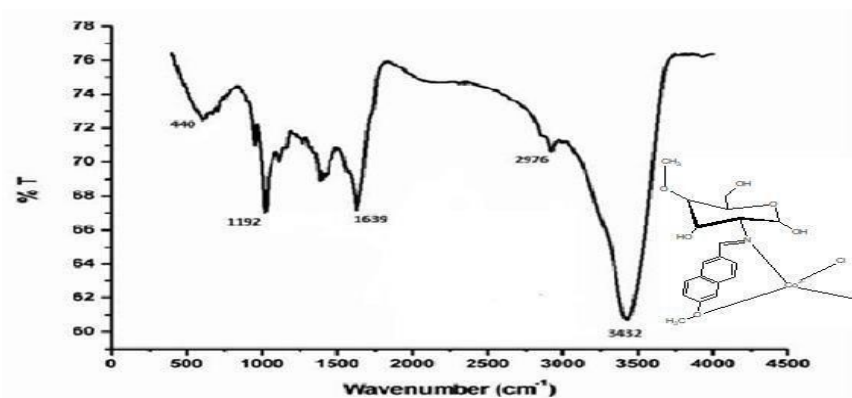
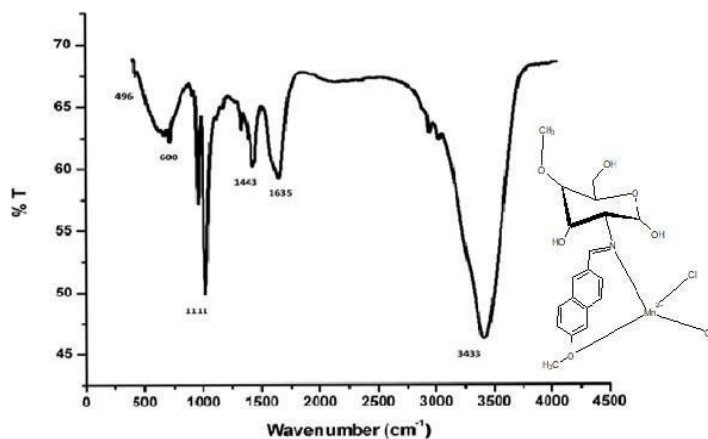


Figure 6: FT-IR spectra of CSB -Co Complex

### 3.3.4 IR spectra of Mn-C<sub>19</sub>H<sub>23</sub>NO<sub>6</sub>Cl<sub>2</sub>

The spectra of Mn complex shown in **Figure 7** reveals that, the positional shift have been observed from  $1627\text{cm}^{-1}$  to  $1635\text{cm}^{-1}$  for the band C=N in the complex. The band in the region  $496\text{cm}^{-1}$  and  $600\text{cm}^{-1}$  which were assigned for Mn-N and Mn-O groups.



**Figure 7:** FT-IR spectra of CSB -Mn Complex

### 3.4 Thermal analysis

#### Thermogravimetric analysis (TGA)

In TGA physical and chemical properties of the synthesized material are measured as a function of constant increasing in temperature [33]. This provides the information about physical characters such as absorption, sublimation, vapourization, and also the chemical phenomenon that are desolvation, oxidation and reduction. TGA gives the material characterization through the analysis of decomposition pattern with respect to temperature and it is possible to predict the stability of the synthesized compounds with respect to its rate of decomposition.

#### Differential thermal analysis (DTA)

DTA is a thermoanalytic technique, which is similar to differential scanning calorimetry (DSC). In DTA the sample will be analyzed in the presence of inert reference made to study and made to undergo identical thermal cycles. The temperature difference between

sample and the reference will be recorded. This difference in temperature is plotted against time or temperature.

Changes in the sample are either exothermic or endothermic which can be detected relative to inert reference. DTA curves provide the data on transformation which are based on glass transition, crystalline, melting point, and sublimation. This method determines the measure of decomposition in various atmospheric conditions.

#### **Thermal analysis of Schiff base metal complex:**

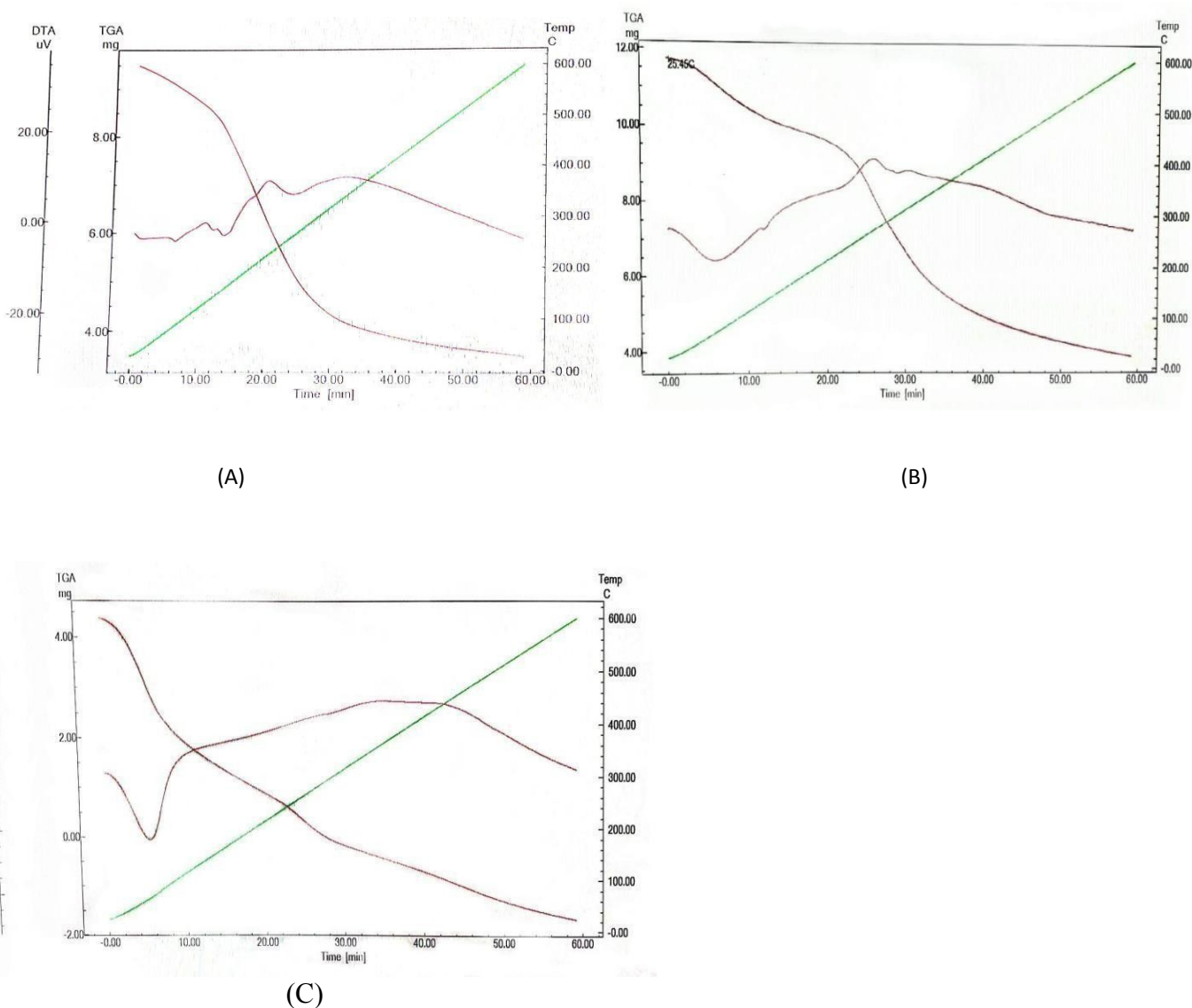
**Cu-C<sub>19</sub>H<sub>25</sub>NO<sub>10</sub>S** - The TGA curve of Cu complex as shown in **Figure 8(A)** was performed in range 30-600°C at the rate of 10°C/min in nitrogen atmosphere. It shows one step degradation which is extended from 30-600°C which can be assigned to weight loss of about 75% due to loss of two co-ordinate H<sub>2</sub>O molecule, SO<sub>2</sub> and ligand moiety. The final product may be CuO with few residual carbon atoms.

The DTA degradation peak involves two stages of decomposition first it precedes by an endothermic peak at 121°C and 242°C which corresponds to release of H<sub>2</sub>O molecule and corresponding fragments.

**Co-C<sub>19</sub>H<sub>23</sub>NO<sub>6</sub>Cl<sub>2</sub>** - The TGA curve of Co complex as shown in **Figure 8(B)** exhibits two step degradation. The first step is around 80 -200°C which is related to loss of H<sub>2</sub>O molecule and the second step is around 260 – 600°C this corresponds to release of CO<sub>2</sub>, Cl gas and polymer backbone. The final product may be Co-O and carbon residue. The DTA curve shows strong endothermic peak around 46°C with loss of H<sub>2</sub>O molecule then followed by an exothermic peak at 268°C where the other fragments of chitosan gets decomposed.

**Mn-C<sub>19</sub>H<sub>23</sub>NO<sub>6</sub>Cl<sub>2</sub>** - The TGA curve of Mn complex shown in **Figure 8(C)** exhibit three steps degradation. The first step decomposition is seen in 30 – 1000°C

range with the weight loss of about 20-25% respectively, which corresponds to the elimination of H<sub>2</sub>O molecule. The thermal degradation of chitosan ligand occurs at 110-2900c with weight loss of about 30-48%. The third stage of decomposition ranges from 300-6000c with loss of 90% mass which finally gives Mn-O and carbon residue. DTA curve of Mn complex shows a sharp endothermic peak at low temperature which may corresponds to release of molecule and other fragments may be CO<sub>2</sub>, Cl respectively.



**Figure 8:** TGA and DTA curves of (A) Cu-CSB, (B) Co-CSB, (C) Mn-CSB

complexes.

### 3.5 Study of biological activity

#### 3.5.1 Anti - microbial studies of Schiff base metal complexes.

The newly synthesized Schiff base and its metal complexes were screened for their antibacterial activity against four bacterial strains, namely *Staphylococcus aureus* and *Escherichia coli* by well plate method [28-29]. Four different pathogenic organisms were used for antibacterial activity. The *in vitro* antibacterial activity of the newly synthesized compounds was determined by well plate method. Ciprofloxacin was used as the standard drug. The results are summarized in **Table 3**

Comp. No.	Zone of inhibition (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
Schiff base	12±0.09	09±0.11
Cu - Complex	14±0.10	10±0.12
Co- Complex	12±0.13	12±0.09
Mn- Complex	13±0.12	11±0.21
<b>Ciprofloxacin (Std.)</b>	19±0.23	18±0.22

**Table 3:** Antibacterial data of Schiff base metal complexes

#### 3.5.2 Antifungal studies of Schiff base metal complex:

The newly synthesized compounds were also screened for their antifungal activity [30-31] against *Aspergillus niger* and *Candida albicans*. The antifungal activity was determined by well plate method at concentration of 1 mg/ml. Ketoconazole and Amphotericin B was used as the standard. The results are summarized in **Table 4**

Comp. No.	Zone of inhibition (mm)		
	<i>Chrysosporium keratinophilum</i>	<i>Aspergillus flavus</i>	<i>Candida albicans</i>
Schiff base	14±0.23	12±0.23	9±0.06
Cu - Complex	10± 0.13	11±0.21	9±0.03
Co- Complex	16±0.11	11±0.10	8±0.09
Mn- Complex	12±0.12	15±0.13	11±0.16
Ketoconazole (Std.)	23±0.32	-	-
Amphotericin B (Std.)	-	20±0.20	21±0.13

**Table 4:** Antifungal activity data of Schiff base metal complexes

### 3.5.3 Antioxidant activity of CSB and their metal complexes:

The antioxidant screening revealed that some of the tested compounds showed good free radical scavenging capacity on comparison with the standard Butylated Hydroxytoluene (BHT). The DPPH scavenging activity for tested compounds showed activity ranging from 69.8% to 59.6%, whereas standard drug BHT showed 90.4% inhibition Table 5. In the present study metal complexes of displayed significant radical scavenging activity relatively compared to the Schiff base. The antioxidant study results are tabulated in Table 5

Compound	Schiff Base	Cu - Complex	Co - Complex	Mn - Complex	BHT
DPPH Assay in %	59.6	66.2	73.8	69.8	90.4

**Table 5:** DPPH radical assay of Schiff base and its metal complexes:

## 4 CONCLUSIONS

In the present work new CSB and their metal complexes were synthesized using chitosan and 6-methoxy-2-naphthaldehyde and then complexed with Co(II), Cu(II), Mn(II). The newly synthesized CSB and their metal complexes were characterized by elemental analysis, UV, Visible and IR spectroscopy. Stability of the complexes was tested by subjecting the sample for thermal analysis. The IR spectra reveals that there is a formation of imine bond which is the characteristic peak of SB and the metal coordinate peak was observed upon complexation.

The synthesized SB and their transition metal complexes exhibit good biological applications. In specific metal complexes has shown higher activity than free ligands. Among transition metal complexes the activity of Co complex was higher than the other metal complexes against *Chrysosporium keratinophilum* and exhibited good scavenging activity. Also, the copper complex showed better inhibition against *S. aureus*. It is clear from the above studies that the metal complexes exhibited better activity compared to chitosan molecule.

Schiff base complexes are considered most important stereo chemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety. Results obtained from biological activity reveals that, synthesized metal complexes show bright path towards Pharmaceutical sciences.

## 5 ACKNOWLEDGMENTS

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