Section A-Research paper

Fabrication and Characterisation of 1-[(4,5-dihydro-furan-2-yl)-morpholin-4-yl-methyl]-pyrrole-2,5-dione and its cobalt (II) complex

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Abstract

A novel mannich base of an imide and its cobalt (II) complex was synthesized and characterized. Their structural features have been established based on the data obtained from the FT-IR, UV-visible, Mass, and NMR spectra of synthesized compounds which are recorded and discussed here. The antioxidant property of the ligand and its complexes were studied using the scavenging nature of the DPPH free radical. To determine the antimicrobial property of the synthesized ligand and its Co(II) complex, it was tested for its potency against microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans*, employing the agar-well diffusion method.

Keywords: Mannich base; metal complexes; geometry; antioxidant property, DPPH, antimicrobial activity.

1. Introduction

Heterocycles are a very important and unique class of compounds for the synthesis of more than half of all known organic compounds that have a wide range of physical, chemical, and biological properties due to their broad spectrum of reactivity and stability[1]. As the structural subunits of the heterocycles exist in many natural products like vitamins, antibiotics, hormones, alkaloids, and antibiotics, they can be used in pharmaceuticals, agrochemicals, dyes, and many others[2]. In addition to naturally occurring compounds, a large number of synthetic heterocyclic

compounds with important physiological and pharmacological properties are also known[3]. These compounds can be altered in any desired manner to yield potent and selective drugs[4]. Moreover, compounds having heterocyclic moieties display enhanced solubility and salt-formation properties that enable their oral absorption and bioavailability[5]. Among heterocyclic compounds, nitrogen-containing heterocycles like mannich bases are the core structures of numerous biologically active compounds and it also exhibits numerous applications in chemistry, biology, and other sciences[6]. They are the building blocks of life due to their wide occurrence in nature and play a central role in the chemical reactions that occur in all organisms[7]. Moreover, heterocyclic compounds with nitrogen would play an important role in coordination chemistry[8]. These days, the development of drug resistance is a major problem, and to overcome this situation, it is necessary to synthesize new classes of compounds. Mannich bases containing bridged N-atom exhibit pronounced biological activities[9]. The end product of the Mannich reaction[10, 11] is the Mannich bases which are beta-amino ketones carrying compounds. Mannich reaction is a nucleophilic addition reaction that involves the condensation of a compound with active hydrogen(s) with an amine (primary or secondary) and formaldehyde (any aldehyde)[12]. These Mannich bases may be used as a bioactive lead for the synthesis of important pharmacophores which may further be used for the preparation of different potential agents of high medicinal value which possess aminoalkyl chains. Examples of clinically useful Mannich bases that consist of an aminoalkyl chain are a cocaine, fluoxetine, atropine, ethacrynic acid, trihexyphenidyl, procyclidine, ranitidine, biperidene[13-15,] and so forth. Mannich bases are known to play a vital role in the development of synthetic pharmaceutical chemistry. Earlier studies have confirmed that the Mannich bases are highly reactive and it is easy to convert it to other useful compounds as well. For example, it is reduced to form physiologically active amino alcohols[16]. Mannich bases are known to possess potent activities antiinflammatory[17,18], anticancer[19,20], antifilarial[21], antibacterial[22,23], antifungal[23,24], anticonvulsant[25], anthelmintic[26], antitubercular[27,28], analgesic[29], anti-HIV, antimalarial[30], antipsychotic[31], antiviral[32], activities and so forth.

In the present work, we have designed a novel Mannich base from Maleimide, Furfuraldehyde, and Morpholine. Maleimide compounds specifically have shown antifungal and antibacterial properties, ability to inhibit Protein Kinase C (PKC), antitumor property, and analgesic activity[33]. Also, we know that the morpholine ring is a versatile and readily accessible synthetic building block, it is easily introduced as an amine reagent or can be built according to a variety of available synthetic methodologies[34]. We further know that Furans are an important heterocyclic class that possesses a variety of bioactivities. The furan moiety is considered to be a common structural motif in many natural products, and many research groups have reported the synthesis of substituted furans for biological assay [35]. Since all the reactants of the one-pot Mannich reaction are biologically active, it is expected that the novel Mannich base would also be highly biologically active. Its structure was confirmed with the usual UV- Vis, FT-IR, NMR, and Mass spectral analysis. Further, a metal coordination complex was also prepared from the

Cobalt(II) chloride salt, for whom the structure was predicted, and the biological activity like the anti-oxidant capabilities and the anti-microbial properties were analyzed and compared with that of the free ligand.

2. Objectives of the study

With a detailed survey of the research papers, we can understand that only a few studies have been made on the synthesis of the Mannich bases with maleimide, hence the present investigation has been undertaken.

The present work aims at

i. Synthesis of N-(morpholinofurfuryl)maleimide and its cobalt(II) complex.
ii. Characterization of the organic ligand and the metal complex
iii. Screening of Anti-oxidant activity
iv. Screening of Anti-microbial activity

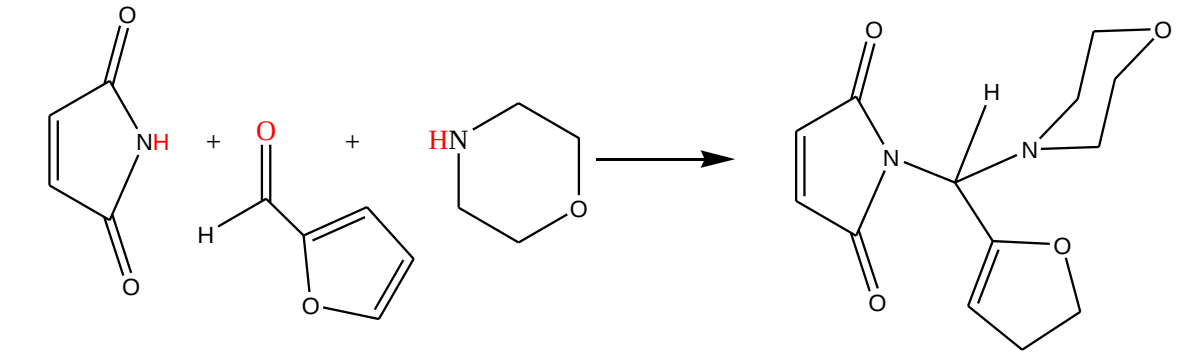
3. Research Methodology

3.1. Materials and Instrumentation

Furfuraldehyde and Morpholine were purchased from Merck and Aldrich chemicals. Maleimide was purchased from Alfa Esser. All the solvents used were dried and freshly distilled before use. All the other chemicals and reagents used for the biological studies were of high quality in biological grade. Melting point determination was performed with an electrical instrument and is uncorrected. Infrared spectra were recorded in KBr pellets with PERKIN ELMER Spectrum TWO spectrometer in the range 4000 cm^{-1} to 400 cm^{-1}. The ^{1}H-NMR and ^{13}-NMR were recorded on a high-resolution 500 MHz NMR spectrometer. Electronic spectroscopy was recorded with a PERKIN ELMER Lamda 365 Spectrophotometer in the range 190 nm to 1100 nm.

3.2. Preparation of N-(morpholinofurfuryl)maleimide (MOFM)

Synthesis of N-(morpholinofurfuryl)maleimide was a Mannich condensation reaction between morpholine, furfuraldehyde, and maleimide in a 1:1:1 mol ratio. About 0.48 g of Maleimide (AR grade)(0.005 M) was first mixed with 0.5629 g of formaldehyde (0.005 M) with constant stirring. Now 0.44 g of Morpholine (AR grade)(0.005 M) was also added into the mixture at room temperature drop by drop and the stirring was continued for about 30 to 40 minutes. An oily mass was obtained. Now added petroleum ether and stirred vigorously. Allowed to settle. Decant off the solvent. The black-colored precipitate obtained was filtered and dried. It was then recrystallized from hot ethanol. The percentage yield of the compound was 73%. MOFM is a black solid and its melting point was found to be 107-110˚c. It is insoluble in water whereas completely miscible in organic solvents like DMF, DMSO, methanol, $CHCl_3$, etc.



Pyrrole-2,5-dione Furan-2-carbaldehyde Morpholine 1-[(4,5-Dihydro-furan-2-yl)-morpholin-4-yl-methyl]-pyrrole-2,5-dione

3.3.Preparation of the Metal (II) complexes

The synthesized organic ligand in ethanol (0.262 g, 0.001mol) and the metal chloride solution $CoCl_2.6H_2O$ of the same molarity in ethanol are stirred continuously in a hot plate separately. Now, the ligand solution is added dropwise into the cobalt hexahydrate solution. The mixture of the solution is stirred for one and half an hour and then it is refluxed at 50˚c for 3 hours. The resultant solution is kept undisturbed and the complex is formed in two to three days. It is washed with ethanol and dried. The complex is recrystallized from hot ethanol.

3.4. Antioxidant activity (DPPH assay)

The free radical scavenging activity of the substances was measured with the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of the extract (Krings & Berger, 2001). The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for a few minutes. The absorbance is noted for different concentrations of the ligand or its cobalt (II) complex. The antioxidant activity was expressed as

$$\%\, Inhibition = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \times 100\ \%$$

IC_{50}, the amount of sample extracted into 1 ml solution necessary to decrease by 50 % is also calculated from the plot of % inhibition vs concentration.

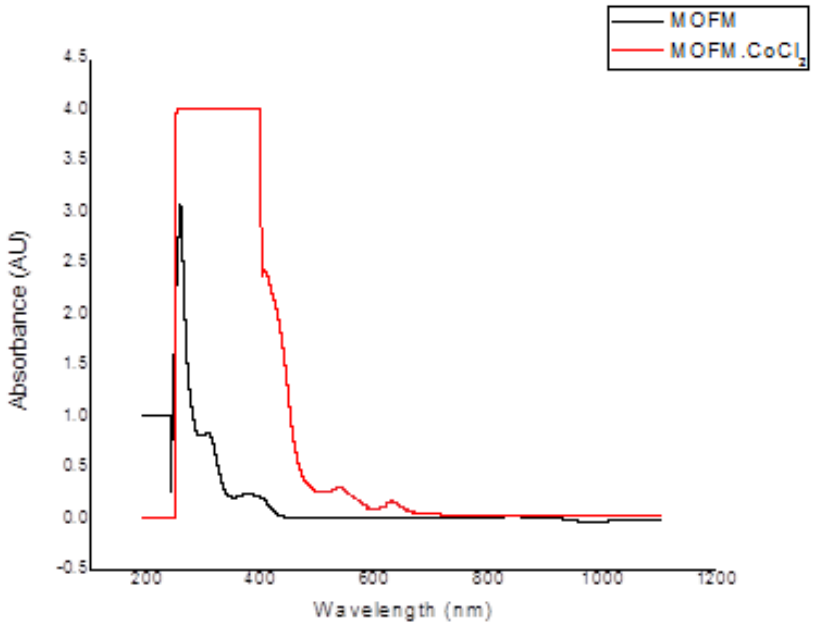
3.5. Antimicrobial Screening

Antimicrobial screening of the organic ligand (MOFM) and its cobalt (II) complex was tested against various bacteria like *Escherichia coli* (E.Coli), *Staphylococcus Aureus* (S. aureus), and various fungi such as *Aspergillus niger* (A.niger), *Candida albicans* (C.albicans). Here tetracyclin and fluconazole are used as standard drugs for antibacterial and antifungal studies respectively. The antimicrobial screening was carried out by earlier reported procedures[36,37].

4. Analysis and discussion

The Mannich base ligand, MOFM was synthesized by the Mannich reaction of maleimide, furfuraldehyde, morpholine in the ethanolic medium in a single-step process. Furthermore, the cobalt (II) complex was also prepared. The resulting MOFM compound and its cobalt (II) complex existed as a stable compound at room temperature in atmospheric conditions. From the spectral analyses, the formation of the compounds was confirmed.

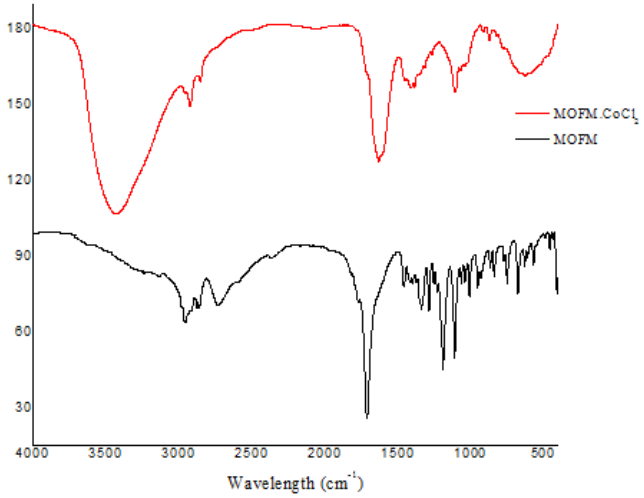
4.1.UV- Visible spectra



The electronic absorption spectra of the MOFM and its cobalt (II) complex (MOFM.$CoCl_2$) were recorded in the region 200 - 900 nm. The electronic spectra of the free MOFM display an intense absorption band at 258.80 nm, 305.90 nm, and 376.45 nm. The first two bands correspond to the $\pi - \pi^*$ and $n - \pi^*$ transitions of the maleimide moiety respectively and the latter one may be due to the $\pi - \pi^*$ transition of the furfuryl group. In MOFM.$CoCl_2$, the two bands appeared at 520 nm and 650 nm which are due to the $^2B_{1g} \rightarrow {}^2E_{1g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transition indicating that Co complex with square planar geometry[38].

4.2.FT-IR Spectral study

The FT-IR spectra of the MOFM and MOFM.$CoCl_2$ is discussed as follows.



Comparison of the absorption frequencies (cm^{-1})

Maleimide	Furfuraldehyde	Morpholine	MOFM	Peak Assignments
3442, 3203	-	3334, 3302	-	υ_{N-H} stretching
3069	2841	-	2965	$\upsilon_{=C-H}$ stretching
-	-	2949		υ_{C-H} aliphatic stretching
1801, 1763, 1772, 1711	1702	-	1703	$\upsilon_{C=O}$ stretching
-	-	1463, 1375	1418 - 1464	υ_{CH2} scissoring
1357	1395-1365	1348	1399 - 1338	υ_{C-H} rocking
1302, 1296	-	1201, 1142	1286, 1253	υ_{C-N-C} stretching
1146	-	1142	1110	υ_{C-N} stretching
-	1013	1097	1065, 1040, 1010	υ_{C-O-C} stretching
681	-	-	677	cis disubstituted alkene
631	632	-	631	$\upsilon_{=C-H}$ bending

Our reactants are Morpholine, furfuraldehyde, and Maleimide. On the completion of the reaction and when the Mannich base N-(morpholinofurfuryl) maleimide (MOFM) is formed the NH stretching vibrations of maleimide and morpholine in the range of 3442-3002 cm^{-1} disappeared. This confirms the linkage between the maleimide and the morpholine moieties.

The important bands of the organic ligand and its complex are tabulated

Sample	$\upsilon_{C=O}$	υ_{C-N-C}
MOFM	1703	1286, 1253
$MOFM.CoCl_2$	1680	1265

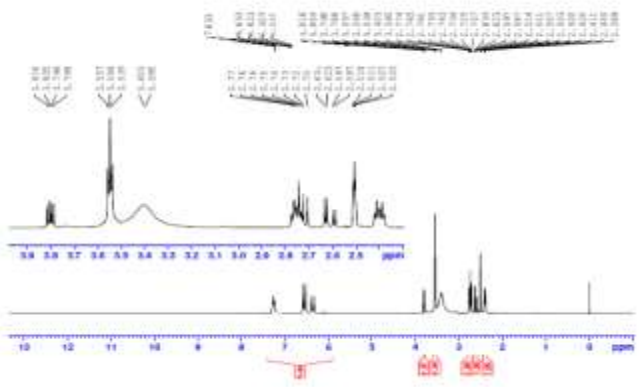
The $\upsilon_{C=O}$ bands of the free ligand appear mainly in 1772, 1703 cm^{-1}. There are two C=O groups in the ligand. The coordination of both the carbonyl oxygen atoms of the maleimide moiety to the same metal center is not sterically possible and hence it is ruled out. Therefore one of the two carbonyl oxygen atoms is coordinated with the metal (II) ions. This is confirmed by the lowering of the band at 1703 cm^{-1} due to the carbonyl group stretching of MOFM to 1680 cm^{-1} in MOFM.$CoCl_2$. Also, the nitrogen of the morpholine group has participated in the band formation which is proved with the help of the decrease in the C-N-C frequency from 1286, and 1253 cm^{-1} in the MOFM to 1265 cm^{-1} in MOFM.$CoCl_2$ complex. Based on this evidence, it is concluded that MOFM acts as a bidentate ligand.

4.3. NMR Spectroscopy

4.3.1. ^{1}H - NMR Spectral Study of MOFM

The NMR study for the newly synthesized MOFM sample was analyzed with DMSO as the solvent. Its spectra are given in the figure, and their results are tabulated together with that of morpholine, furfuraldehyde, and maleimide in a table

^{1}H -NMR spectrum of MOFM



The ^{1}H - NMR spectrum of MOFM shows several signals. With the help of these signals, we can predict the structure of MOFM.

Comparison of the Chemical shift on maleimide, furfuraldehyde, morpholine, and MOFM and their assignments

Maleimide (ppm)	Furfuraldehyde (ppm)	Morpholine (ppm)	MOFM (ppm)	Assignments
-	9.666	-	-	Aldehyde proton of furfuraldehyde
8.5		2.59	-	N-H protons

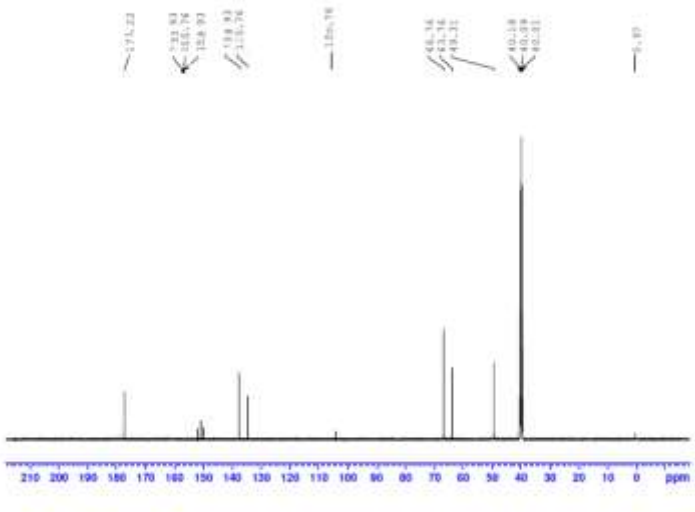
6.728	7.734, 7.299, 6.632		6.031, 6.053, 6.623, 6.633, 7.633	HC=CH protons
		2.860	2.63 - 2.500	$N(CH_2)_2$ protons
		3.669	3.82 - 3.56	$O(CH_2)_2$ protons
			2.389	-N-C-H

The signal due to the aldehyde of furfuraldehyde at 9.666 ppm is absent in the novel MOFM compound also there is a new signal at 2.389 which is due to the proton which is α to nitrogen of maleimide moiety and morpholine moiety. This says that the product formation is confirmed.

4.3.2. ^{13}C - NMR Spectral Study of the organic ligand (MOFM)

The ^{13}C NMR spectrum was taken for the newly prepared MOFM in DMSO. The spectrum is as under.

^{13}C -NMR spectrum of MOFM



The spectral information is compared with that of the maleimide, furfuraldehyde, and morpholine and tabulated.

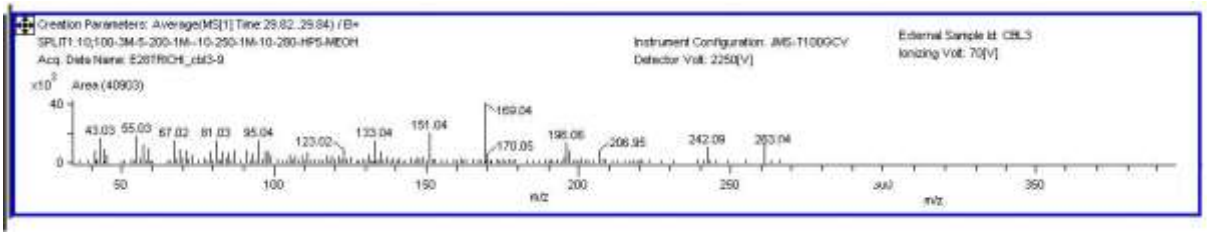
Comparison of the Chemical shift on maleimide, furfuraldehyde, morpholine, and MOFM and their assignments

Maleimide (ppm)	**Furfuraldehyde (ppm)**	**Morpholine (ppm)**	**MOFM (ppm)**	**Assignment**
171.36	177.93		171.22	Carbonyl carbons of
135.17	153.06, 148.31,		153.93, 155.76,	Olefinic carbons
		68.16	66.76	$O(CH_2)_2$ carbons of the

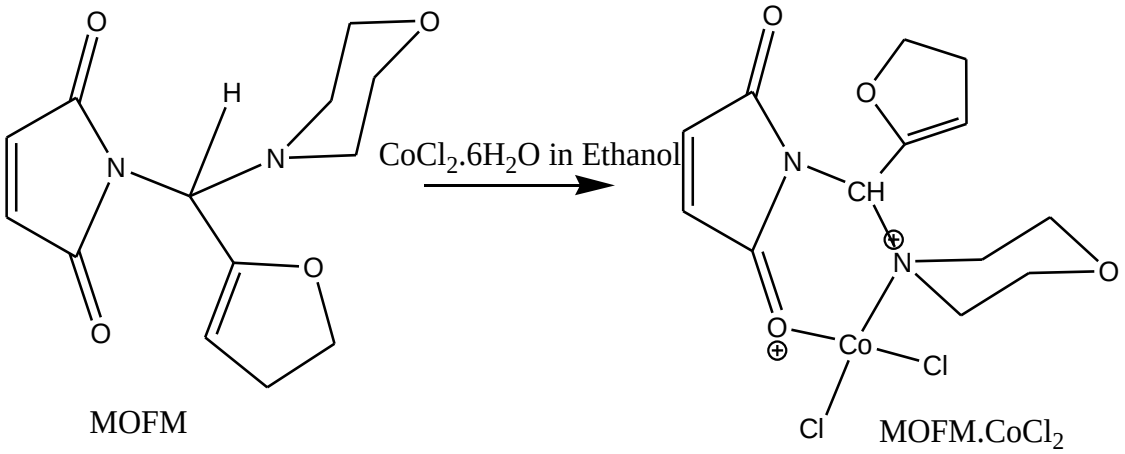
		46.61	49.31, 40.18,	$N(CH_2)_2$ carbons of the
			63.76	Aliphatic sp^3 carbon

The ^{13}C-NMR spectrum of MOFM shows signals at δ 171.22 ppm due to the carbonyl carbons of the maleimide moiety while that of the furfuraldehyde is absent. Also, a new signal is observed at 63.76 which is due to aliphatic sp^3 carbon between the maleimide and morpholine moiety. This shows that both the maleimide group and the morpholine group is connected through the furfuraldehyde with the loss of water molecule.

4.4.Mass Spectroscopy



The mass spectrum of the ligand shows the molecular ion peak at m/z 263.04, calculated being 262. Hence the structure of the novel organic ligand and the metal complex is found to be

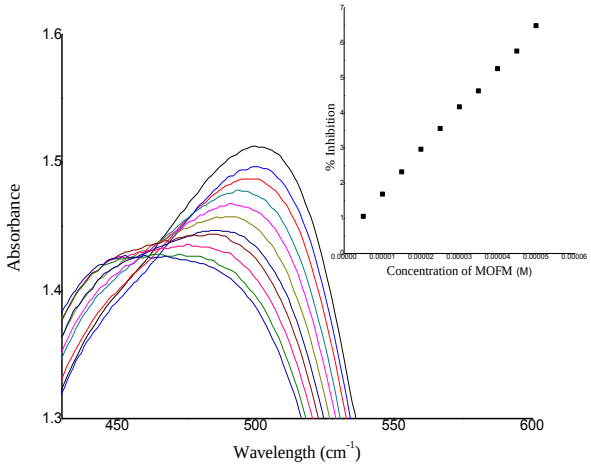


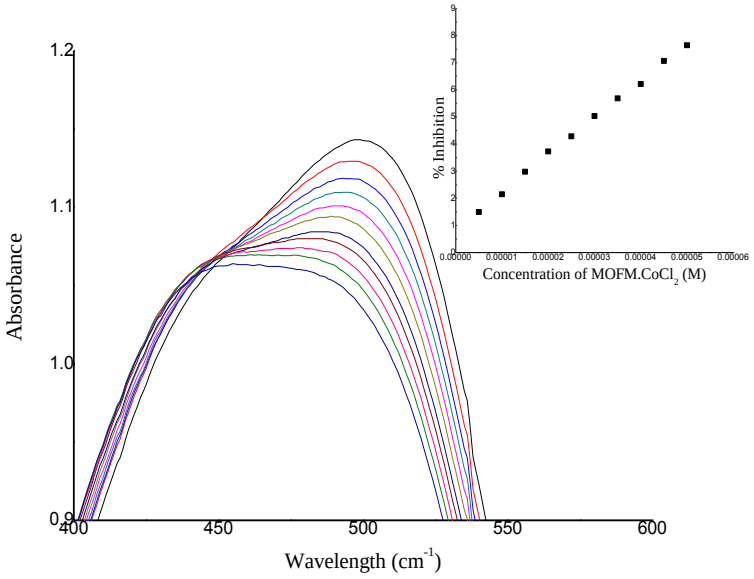
4.5. Antioxidant Property

4.5.1. DPPH Assay

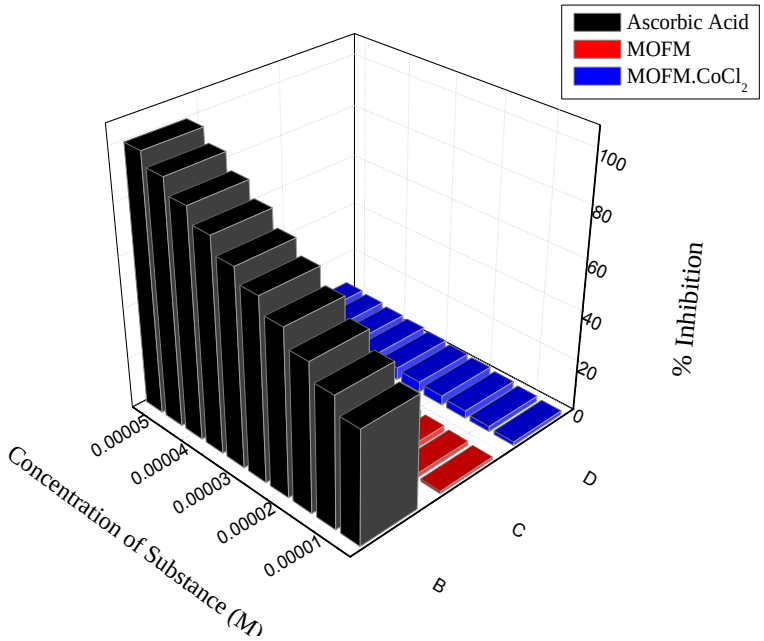
The free radical scavenging property of MOFM and $MOFM.CoCl_2$ complex was studied with the help of a UV-Vis 1700 spectrophotometer. There is a decrease in the absorbance with

the increase in the concentration of the substance added to the constant molarity of DPPH. This confirms that there is better antioxidant property.





The % Inhibition of the novel organic ligand and that of its cobalt (II) complex with respect to various concentration are shown as under.



The IC $_{50}$ values obtained are tabulated as follows.

Samples	**IC $_{50}$ Values**
STANDARD (ASCORBIC ACID)	9.0126×10^{-6}
MOFM	$4.176164666 \times 10^{-4}$
$MOFM.CoCl_2$	$3.600790137 \times 10^{-4}$

It is already known that the lesser the IC_{50} value the greater is the antioxidant property. Here the ligand (MOFM) shows greater IC_{50} values in comparison with the cobalt complexes. The increasing order of the IC_{50} values is Ascorbic Acid < $MOFM.CoCl_2$ < MOFM. Thus and increasing order of the antioxidant property of the samples can be shown as MOFM < $MOFM.CoCl_2$ < Ascorbic Acid.

4.6. Antimicrobial Activity

The Mannich base ligand (MOFM) and $MOFM.CoCl_2$ were screened for their in vitro antibacterial activity against Staphylococcus aureus (gram +ve) and Escherichia coli (gram -ve) and antifungal activity against Candida albicans and Aspergillus niger at various concentrations in DMSO by agar-well diffusion method. The zones of inhibition produced by the test compounds are presented in the table.

Antimicrobial Activity of the ligand MOFM and its Cobalt (II) complexes

Species	Concentration (µg/ml)	Zone of Inhibition (mm)		
		MOFM (Free ligand)	MOFM.$CoCl_2$	Standard (Positive control)
Escherichia Coli	25	15	16	16
	50	17	17	
	75	17	18	
	100	18	19	
Staphylococcus Aureus	25	16	17	16
	50	18	18	
	75	19	19	
	100	19	20	
Aspergillus Niger	25	16	18	17
	50	16	19	
	75	17	19	
	100	18	19	
Candida Albicans	25	17	17	17
	50	17	18	
	75	18	19	
	100	18	20	

It is seen that the antimicrobial activity of the test samples increases with an increase in their concentrations. It is observed that the test compounds exhibit activity comparable to standard drugs and the cobalt complex shows more activity than the free organic ligand. The increased activity of the metal complex may be explained based on chelation theory. Chelation reduces the polarity of the metal ion and enhances the lipophilic or hydrophobic character of the metal chelate which favors the permeation through the microbial cell wall. In the figure, the antimicrobial activity of the Mannich base ligand (MOFM) towards various microbial organisms is given. It is seen that when the concentration is less i.e 25 µL, the activity was less than the standard, whereas the activity increases as the concentration increases and it exceeds the standard used at 100 µL.

As mentioned earlier the antimicrobial activity of the MOFM.$CoCl_2$ was higher in comparison with the standard (ascorbic acid) or with MOFM itself.

5. Conclusion

In this research work, we have synthesized a morpholine derivative (mannich base ligand) (MOFM) and its cobalt (II) complex. Characterization was performed by spectral and analytical techniques. The synthesized complex has square planar geometry which has been

identified from the comparison of the spectral results. Antioxidant results show that the synthesized ligand and the complex have good radical scavenging ability. Antimicrobial results of synthesized compounds against selected microbial agents show that MOFM.$CoCl_2$ has better properties than the ligand MOFM.

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