*Section A -Research paper*

# Fabrication, Characterization, DFT interpretations, and biological applications of 1-morpholin-4-ylmethyl-pyrrole-2,5-dione

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**Abstract**

A novel mannich base was synthesized from maleimide and morpholine. Its structural features have been established based on the data obtained from the FT-IR, UV-visible, Mass, and NMR spectra. The structural parameters such as the bond angle, and bond length were also been determined theoretically using the DFT calculations. The antioxidant property of the title compound was studied using DPPH free radical scavenging property. The antimicrobial activity is studied with the microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans* employing the agar-well diffusion method.

**Keywords**: Mannich condensation, ligand, maleimide, morpholine, DFT, formaldehyde, antioxidant, antimicrobial

## 1. Introduction

The need for pharmaceutically active novel compounds has increased currently in this pandemic situation. Chemistry can be a rescue and there are still numerous spots that are left unexplored. Heterocyclic compounds in particular imides or cyclic imides like the glutarimides, succinimides, phthalimides, maleimides, and other similar compounds have structural features, which enhance the high biological activities, and these compounds are found to have numerous pharmaceutical applications. All the above-said compounds have an imide ring with the general structure -CO-N(R)-CO-. They are generally found to be hydrophobic and neutral, and hence it is easy for them to diffuse into the biological cell membranes[1]. These imide structures may also possess medicinal properties by themselves. In particular, the maleimide moiety is found to be an antifungal and antibacterial agent. It can inhibit Protein Kinase C (PKC). In addition, it also has antitumor and analgesic properties[2]. When this is further linked with yet another biologically active compound, the new compound synthesized will have enhanced properties and can surely serve mankind. Condensation will be one of the methods to link the compounds. Hence we are using a mannich condensation reaction here to bridge the compounds. A lot of work has been done in this area in isolating solid complexes of various aromatic aldehydes or aliphatic aldehydes or ketones, semicarbazones together with transition metals[3, 4]. On thoroughly investigating the literature, it is found that there is no work reported on the condensation of maleimide, formaldehyde, and morpholine. Thus in

the present work, we are reporting a novel mannich base, N-(morpholinomethyl)maleimide. The structure of this ligand was predicted theoretically with the help of Schrodinger software and DFT calculations. This structure elucidated was confirmed analytically by the spectroscopic shreds of evidence from the UV-Vis, FT-IR, NMR, and Mass spectroscopic data. The novel ligand hence synthesized was tested for its antioxidant properties. Knowing these properties is of great importance as these assays can say whether the novel synthesized compounds can reduce the reactive oxygen species and hence avoid age-related diseases. Accumulation of the free radicals may increase the risk of age-related disease, and can also lead to some diseases such as diabetes[5] or Alzheimer's[6]. In our work, we have analyzed whether our novel compound can scavenge these free radicals effectively. Further, the anti-inflammatory efficiency was tested, and its result can give us an idea about the inflammation-reducing power of the compounds. The antimicrobial property of the synthesized compounds was studied using the agar well diffusion method. From these results, we can further understand the usefulness of the compounds.

## 2. Materials and Methods

### 2.1. Synthesis of the novel organic ligand (MOMM)

All the basic chemicals formaldehyde and morpholine (Merck and Aldrich) maleimide (Alfa Esser) are of AR grade. The fabrication of N-(morpholinomethyl)maleimide is by a reaction known as the Mannich condensation, which is taking place between three reactants, i.e., morpholine, formaldehyde, and maleimide in the ratio of 1:1:1. Ethanolic solution of equimolar maleimide and formaldehyde are mixed with constant stirring to which equimolar solution of morpholine (AR grade) is added at room temperature while the stirring was continued for half an hour. An oily mass was obtained. Now added petroleum ether and stirred vigorously. Allowed to settle. The unreacted solvent was decanted. The precipitate obtained was colorless. It was further filtered and dried. It was then recrystallized from hot ethanol. The percentage yield of the compound was 62%. MOMM is a colorless solid and its melting point was found to be 113 - $115^0$ C. It is insoluble in water whereas completely miscible in organic solvents like DMF, DMSO, methanol, $CHCl_3$, etc.

### 2.2. Characterization of MOMM

#### 2.2.1 Electronic (UV-Vis) Spectral Measurement

When a molecule absorbs the UV-Visible radiation a few electronic transitions may be anticipated in the molecule. The electronic spectra were measured in DMSO solutions using a double-beam UV-Visible spectrophotometer, Lambda 35, Perkin Elmer. The working range of the instrument is 190 - 1100 nm.

#### 2.2.2. Infrared (IR) Spectral Measurement

IR spectra of the ligand were compared with that of the reactants viz. maleimide, formaldehyde, and morpholine to elucidate the structures of the mannich base ligand. Infrared spectral measurements (4000-400 $cm^{-1}$) were made using KBr pellets using a Perkin Elmer spectrometer, Spectrum RX I.

#### 2.2.3. Nuclear Magnetic Resonance (NMR) Spectral Studies

The number, positions, and splitting patterns of the signals, and the area under each signal (peak integration), give the number, the positions of various magnetic nucleic numbers of neighboring magnetically active nuclei, and the total number of magnetically active nuclei of that particular category present, within the molecule. The $^1H$ NMR & $^{13}C$ spectra of the ligand were recorded with DMSO as solvent at ambient temperature.

#### 2.2.4. Mass Spectral Study

The exact molecular formula can be found with high accuracy from the mass spectrum. The mass spectral analysis of the compound was carried out using 410 Prostar Binary LC with 500 MS IT PDA

Detectors. The molecular weights together with the structure of the molecules were confirmed with the help of the electron impact mass spectra.

### 2.3. Antioxidant activity with DPPH Assay

There will be a decrease in the absorbance of methanolic DPPH solution at 517 nm if the free radical is scavenged by the sample added in a small proportion. The initial concentration of 0.1 mM solution of DPPH was prepared and to this appropriate and uniform amount of the synthesized ligand of appropriate concentration was added and after each addition, the absorbance at 517 nm was noted[7]. The same reaction was performed by varying the concentration of the ligand. The antioxidant efficiency of the prepared organic compound is expressed as % inhibition. The $IC_{50}$ values are calculated.

### 2.4. Antimicrobial Screening

Antimicrobial screening of the organic ligand MOMM has been treated against various bacteria like *Escherichia coli* (*E.Coli*) and *Staphylococcus Aureus* (*S. aureus*). The antifungal activity of MOMM was analyzed with fungi like *Aspergillus niger* (*A.niger*) and *Candida albicans* (*C.albicans*). Here Tetracyclin (antibacterial) and Fluconazole (antifungal) are used as standard drugs fro evaluation. The antimicrobial screening was carried out by earlier reported procedure[8].

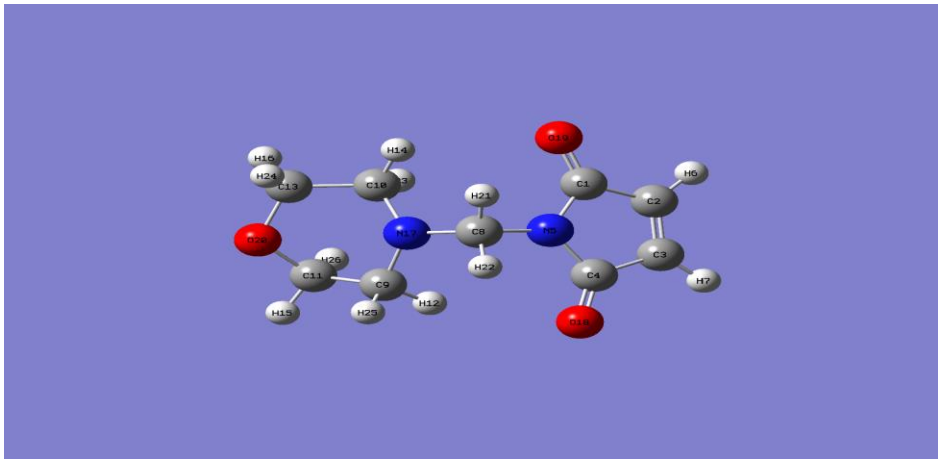
## 3. Result and Discussion

The fabricated MOMM which is a colorless powder was expected to possess several bio-medical applications. The applicability cannot be justified unless its structural aspects are clarified. For this, we have adopted many analytical and theoretical weapons, which are discussed in the sections below.

### 3.1. Computational details

The theoretical computations were carried out using B3LYP functional with 6–31G (d) basis sets for stable optimized structures in the gas phase using the Gaussian 09 Program[9] Package. The geometries were first optimized at density functional theory (DFT) employing the B3LYP which invokes Becke's three parameters, Lee-Yang Parr level of theory[10, 11]. The literature survey reveals that there is no detailed analysis so far done with molecular geometry such as bond length, bond angle, and dihedral angle by the DFT method. Fig 1 represents the optimized structure MOMM.

*Fig 1. Atom numbering scheme of MOMM*




#### 3.1.1. Molecular optimization geometry

The optimized structure parameters of molecules under study calculated by DFT– B3LYP level with the 6–31G (d) basis sets are listed in Table 1. This table shows the calculated bond length and bond angle for MOMM. The molecular bond length and bond angle are changed due to the adding nitrogen and

oxygen atom in the ring and theoretical calculations belong to isolated molecules in the gaseous phase. These theoretically calculated values of C–H bond length is 1.3961 Å, similarly, bond angles C–C–H, C–C–O are $126.372^0$ and $131.924^0$ . As a result, the oxygen atom has four electron pairs on the outermost shell out of which two pairs are in between oxygen and nitrogen atoms involved in covalent bond formation as bond pairs, and the remaining two pairs are non-bonding electron pairs as lone pairs. Moreover, each C–C–N (interior) and C–N–C (exterior) bond angle in an optimized geometry of $107.53^0$ is measured as $120^0$ , which is exactly equal to C–C–N bond angle but unequal to the C–C–O bond angle of $123.48^0$ molecule. Similarly, each torsion angle H–C–N–C, H–C–C–H, and C–C–C–H is measured as $0^0$ and $180^0$ respectively.

*Table 1*

*Optimized geometrical parameters of MOMM*

| **Optimized parameters** | **Bond length (Å)** | **Optimized parameters** | **Bond angle ($^0$ 20)** |
|---|---|---|---|
| C1-C2 | 1.4079 | C2-C1-N5 | 108.41 |
| C3-H7 | 1.0848 | C2-C1-O19 | 130.09 |
| C8-N17 | 1.5440 | N5-C8-H22 | 90.02 |
| C9-N17 | 1.3967 | C9-C11-O20 | 123.48 |
| C13-H16 | 1.0767 | C8-N17-C10 | 120.61 |

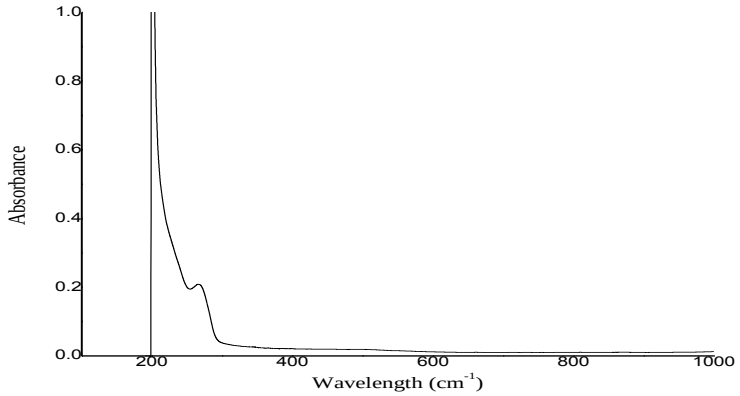
### 3.2. Determination of the structure of the organic ligand (MOMM)

The structure of the novel ligand (MOMM) was predicted with the help of the following data.

#### 3.2.1. Electronic Spectral Analysis

Fig. 2 gives the electronic spectra of the ligand in DMSO in the region 200-1100 nm, two main absorption bands are observed at 256.35 nm, and 268.75 nm, corresponding to the $\pi - \pi^*$ and $n - \pi^*$ transitions of the maleimide moiety[12] respectively.

*Fig 2. UV Spectrum of MOMM*

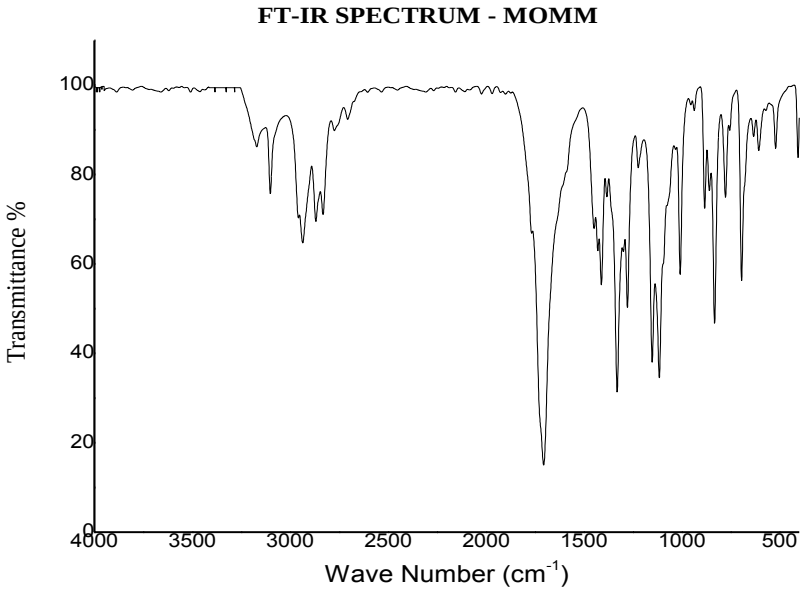


### 3.2.2. Infrared Spectral Analysis

The infrared spectrum of MOMM recorded in the range 4000- 400 $cm^{-1}$ is shown in Figure 3. It is compared with the absorption frequencies of the initial starting materials (maleimide, formaldehyde, and morpholine). On the completion of the reaction and when the Mannich base N-(morpholinomethyl) maleimide (MOMM) is formed the NH stretching vibrations of maleimide and morpholine in the range of 3442-3002 $cm^{-1}$ disappeared. Also, the out-of-plane wagging vibrations of the N-H at 713 $cm^{-1}$ for maleimide and 697 $cm^{-1}$ for morpholine are missing [13]. Both these together confirm the linkage of the maleimide and the morpholine moieties.

The aliphatic C-H stretching vibration occurring at 2949, 2931, and 2912 cm-1 in morpholine is appearing in MOMM at 2936 cm-1. In maleimide, the bands appearing at 1801, 1763, 1772, and 1711 cm-1 due to carbonyl stretching vibrations have slightly lowered their positions to 1706 cm-1. The morpholine shows its CH2 scissoring band at 1463 and 1375 cm-1 but it has been shifted to 1411 cm-1 in the case of the newly formed MOMM. The C-N-C vibrations which are observed in morpholine (1272 cm-1, 1248 cm-1) and maleimide (1302 cm-1, 1296 cm-1) are reproduced in the MOMM (1278 and 1223 cm-1) also. The C-O-C vibration was found in the morpholine at 1201 cm-1 and 1097 cm-1 and found at 1008 cm-1 in the case of the synthesized MOMM. The C-N stretching band observed at 1146 cm-1 for maleimide and at 1142 cm-1 for morpholine is lowered to 1107 cm-1 for MOMM. As the maleimide is an example of a cis-disubstituted alkene it shows a band at 681 cm-1 similarly the maleimide moiety in the MOMM shows a band at 694 cm-1. The C=C-H bending vibrations are found in the same range for maleimide (631 cm-1) and in the newly formed MOMM at 633 cm-1.

*Fig 3. FTIR Spectrum of MOMM*



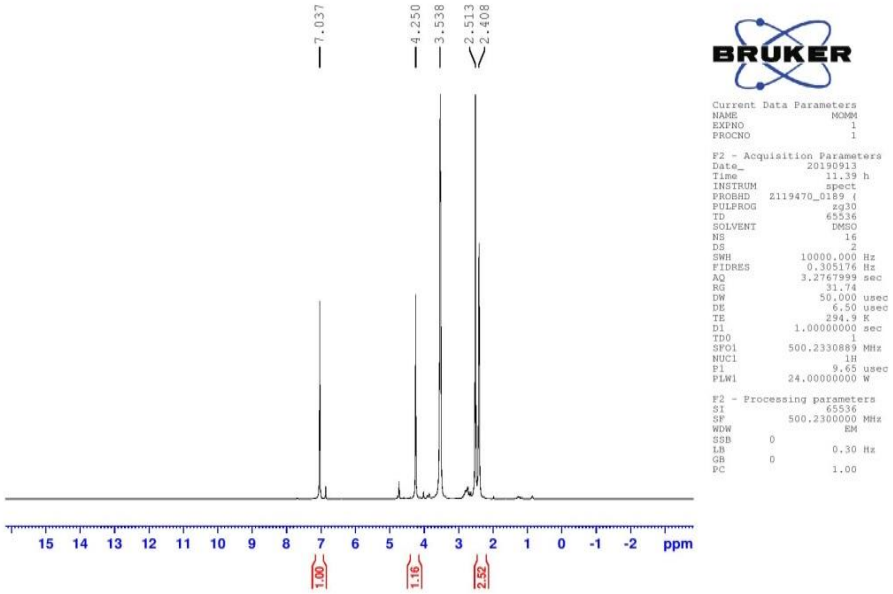

### 3.2.3. NMR Spectroscopy

#### 3.2.3.1. $^{1}$H - NMR Spectral Study of MOMM

The NMR study for the newly synthesized MOMM sample was taken with DMSO as the solvent. The information from Figure 4, conveys spectral details of MOMM, and, it is compared with that of morpholine and the maleimide.

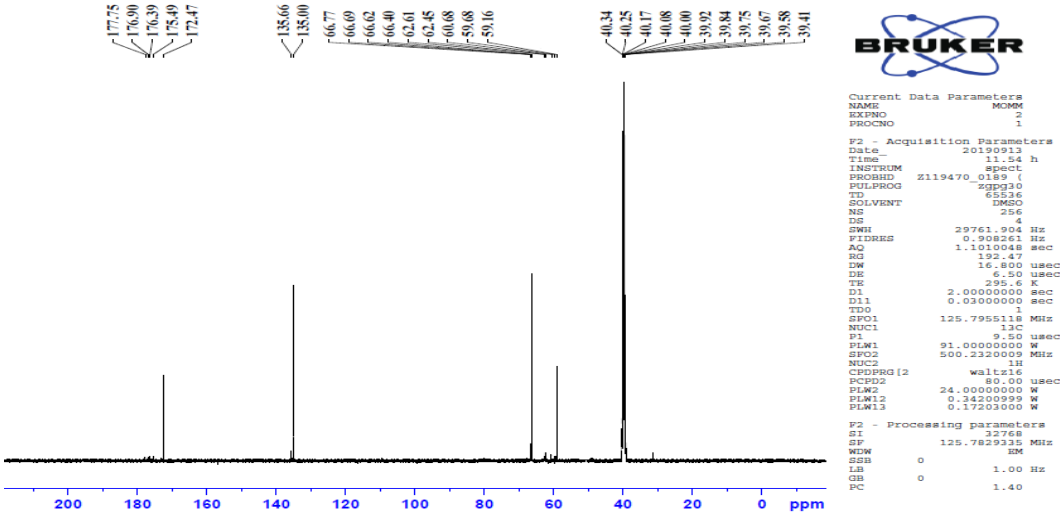
The 1H - NMR spectrum of MOMM shows 5 signals. With the help of these signals, we can predict the structure of the newly formed mannich base14. The signal at δ 2.513 ppm and δ2.408 ppm gives us information about the -N(CH2)2 protons of the morpholine moiety. The chemical shift value at δ 3.538 ppm is an intimation of the presence of -O(CH2)2 protons of the morpholine moiety in the product. The signal at δ 4.250 ppm is due to -N-CH2-N- protons. This says that the product formation is confirmed.

*Fig 4. $^{1}H$ -NMR spectrum of MOMM*




### 3.2.3.2. $^{13}C$ - NMR Spectral Study of the organic ligand (MOMM)

*Fig 5. $^{13}C$ -NMR spectrum of MOMM*

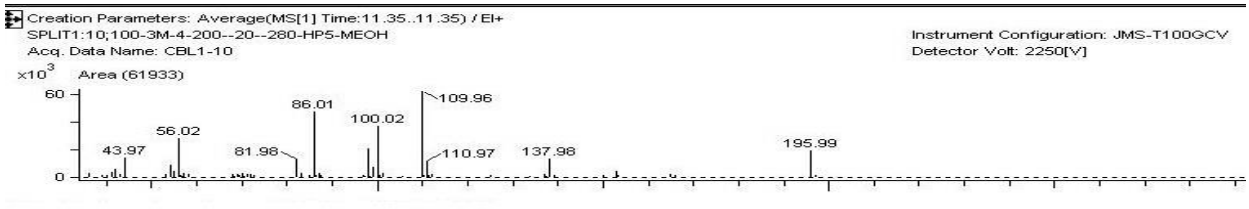



The $^{13}C$ NMR spectrum was taken for the newly prepared MOMM in DMSO. The spectrum (Figure 5) is as under. The $^{13}C$-NMR spectrum of MOMM shows signals at δ 177.75 ppm and δ172.47 ppm and this shows the presence of the carbonyl carbons in the unknown compound. These carbonyl

carbons may have come from the maleimide. The signals at δ 135.66 ppm and 135.00 ppm are due to the $sp^2$ carbon with ($C_\beta$ carbon) conjugated with a carbonyl group. The SDBS spectrum of maleimide gives a similar signal in the same range at δ 135.17 ppm. The chemical shift of δ 66.17 ppm and δ 60.68 ppm are caused due to the $–O(CH_2)_2$ carbons of the morpholine. Similarly, the signals at δ 39.41 ppm – δ40.34 ppm are due to $–N(CH_2)_2$ carbons of the morpholine moiety. The signals at δ 59.68 ppm and δ59.16 ppm are caused by the $N\text{-}CH_2\text{-}N$ carbon. This is a confirmation of the linkage between the maleimide, formaldehyde, and the morpholine molecules[15].

### 3.2.4. Mass Spectroscopy

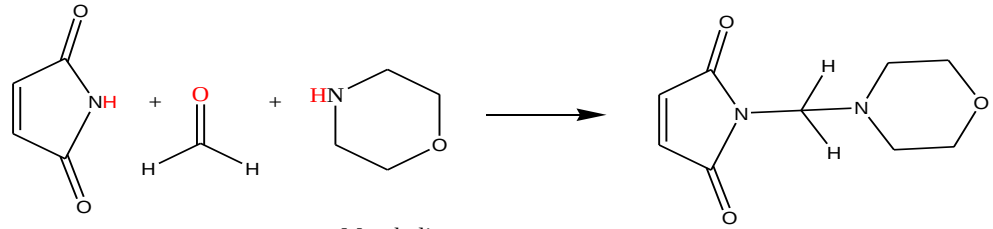
*Fig 6. Mass Spectrum of MOMM*




The mass spectrum of the ligand in Figure 6 shows the molecular ion peak at m/z 195.9, calculated being 196. Hence the structure of the organic ligand and its metal complex can be predicted to be

s

Finally the reaction is schemed as follows:-

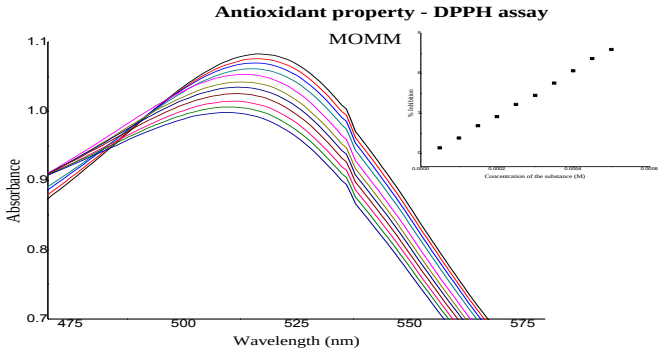



Pyrrole-2,5-dione Formaldehyde Morpholine 1-Morpholin-4-ylmethyl-pyrrole-2,5-dione

### 3.3. Antioxidant Property with DPPH Assay

The free radical scavenging property of MOMM was studied with the help of a UV 1700 spectrophotometer. There is a decrease in absorbance with the increase in concentration. This confirms that there is better antioxidant property[16]. The insight of the plot in Figure 7 gives the concentration of the substance concerning the % Inhibition. It is seen that as the concentration of the sample increases, the % inhibition increases progressively.

*Fig 7. DPPH free radical scavenging property of MOMM*



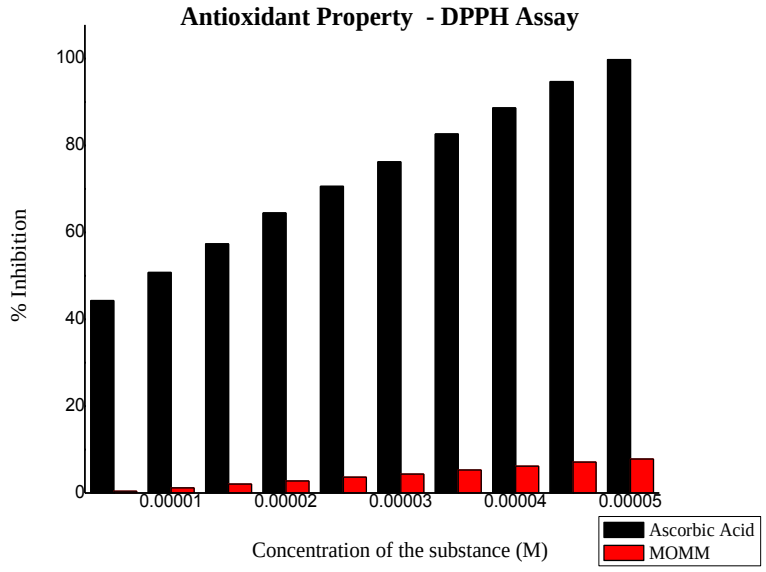
A comparison of the % inhibition of MOMM with the standard (Ascorbic acid) is plotted in Figure 8. Also, the $IC_{50}$ values are tabulated (Table 2). It gives a clear picture of the effectiveness of MOMM as a good antioxidant agent. It is already known that the lesser the $IC_{50}$ value, the greater will be the antioxidant property.

*Table 2*

*Antioxidant properties of MOMM*

| Samples | Samples | IC $_{50}$ Values |
|---|---|---|
| DPPH Free Radical Scavenging Property | Ascorbic Acid (Standard) | $9.0126 \times 10^{-6}$ |
| | MOMM | $3.0341 \times 10^{-3}$ |
| Hydrogen peroxide radical scavenging property | Ascorbic Acid (Standard) | 14.7324 |
| | MOMM | 143.9649 |
| Hydroxyl radical scavenging property | Ascorbic Acid (Standard) | 4.6835 |
| | MOMM | 112.9745 |
| NO radical scavenging activity | Ascorbic Acid (Standard) | 12.3196 |
| | MOMM | 123.1082 |
| TAC Assay | Ascorbic Acid (Standard) | 12.5370 |
| | MOMM | 139.1241 |

*Figure 8. DPPH free radical Scavenging Assay of MOMM and Standard*



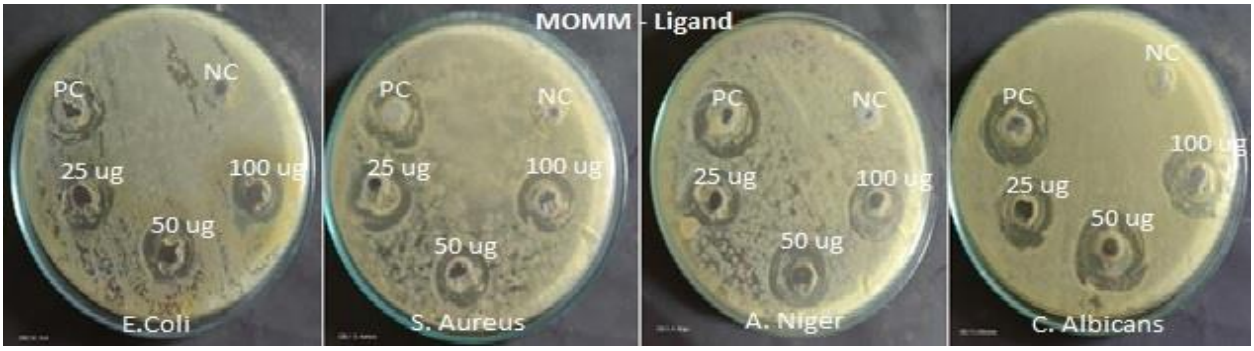

### 3.4. Antimicrobial Activity

The Mannich base ligand (MOMM) was screened for its in vitro antibacterial activity (figure 16) 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. Table 5, depicts the zones of inhibition (mm) of the test compound against the respective species.

*Table 5*
*Antimicrobial Activity of the MOMM*

| Species | Concentration (µg/ml) | Zone of Inhibition (mm) | |
|---|---|---|---|
| | | MOMM (Free ligand) | Standard (Positive control) |
| *Escherichia Coli* | 25 | 14 | 16 |
| | 50 | 17 | |
| | 100 | 18 | |
| *Staphylococcus Aureus* | 25 | 16 | 16 |
| | 50 | 18 | |
| | 100 | 19 | |
| *Aspergillus Niger* | 25 | 15 | 17 |
| | 50 | 16 | |
| | 100 | 18 | |
| *Candida Albicans* | 25 | 16 | 17 |
| | 50 | 17 | |
| | 100 | 19 | |

*Fig 16. Antimicrobial Activity of MOMM*




It is seen that the antimicrobial activity of the test samples increases with the increase of their concentrations. The MOMM exhibits activity comparable to standard drugs, which may be due to the chelation theory. It decreases the polarity of the metal ion and enhances the lipophilic or hydrophobic character which favors the permeation through a microbial cell wall. In Figure 16, the antimicrobial activity of the mannich base ligand (MOMM) towards various microbial organisms is given. It is observed that in lesser concentration (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.

## 4. Conclusion

In this, we have synthesized a maleimide derivative, MOMM, and characterized it by a theoretical approach with the DFT measurements and also with the spectral and analytical techniques. Antioxidant results show that MOMM has good radical scavenging ability. Further, this antioxidant property triggers anti-microbial activity. Antimicrobial results of synthesized compounds against selected microbial agents show that the activity is comparable to the standard drugs.

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