Indian Journal of Chemistry
Vol. 65, January 2026, pp. 18-23
DOI: 10.56042/ijc.v65i1.23683

# Spectroscopic, physicochemical and antimicrobial studies of 1-(2,4-dinitrophenyl)-2-[(E)-(3,4,5-trimethoxybenzylidene)] hydrazine single crystal

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*Received 11 September 2025; accepted (revised) 7 January 2026*

Hydrazides and hydrazones are interesting because of their biological activities and metal extraction capabilities. This work focuses on the single crystal growth of a hydrazone Schiff base, 1-(2,4-dinitrophenyl)-2-[(E)-(3,4,5-trimethoxybenzylidene)]hydrazine (DPTB), using the slow evaporation solution growth technique. Single crystal X-ray diffraction, FTIR, FT-Raman and $^1$H NMR analyses have confirmed the formation of the DPTB compound. The optical and thermal properties of the title compound have been analyzed to determine the absorption range, bandgap value and melting point. To explore its biological potential, the antimicrobial activity of DPTB has been tested against several human pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and the fungus *Candida albicans*, using resazurin reduction assay. Overall, the findings suggest that DPTB shows promise as both a biologically active molecule and a material of potential interest in coordination chemistry.

**Keywords:** Organic compound, Hydrazone Schiff base, NLO studies, Antimicrobial studies

Conventional Schiff base compounds have limited use in many fields because of their low stability and solubility. However, Schiff base compounds can offer highly polarizable π-electron conjugation systems[1]. The delocalized electronic structure of π-conjugated organic compounds provides several attractive opportunities in NLO applications. Organic materials allow us to tune the chemical structure and properties as a requisite for NLO properties. Owing to structural diversity, its properties can be refined *via* molecular engineering and chemical synthesis. The phenomenon of second harmonic generation is utilized in the development of photonic devices in which materials under illumination generate light at twice the incident frequency[2].

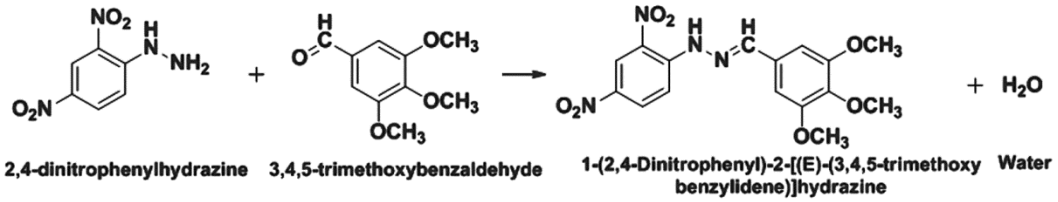
Schiff bases have been widely explored for industrial applications, and the presence of imine groups imparts biological activity[3]. Numerous substituted hydrazides are employed in the treatment of psychotic and psychoneurotic conditions[4]. The promising biological activities of hydrazones and their complexes, such as antibacterial, antifungal, antitumor, anti-inflammatory and antioxidant activities have been reported in various studies[4].

1-(2,4-Dinitrophenyl)-2-[(E)-(3,4,5-trimethoxybenzylidene)] hydrazine is an organic single crystal with the chemical formula C16H16N4O7. Within its crystal structure, the molecules are connected by weak C—H···O interactions, forming screw chains that align into sheets parallel to the bc plane. These sheets are further linked by π-π stacking interactions between the nitro and methoxy substituted aromatic rings, with a centroid-to-centroid distance of 3.9420 (13) Å. The C—H···π contacts contribute additional stability to the two-dimensional network[5]. In this study, we present the spectroscopic, physicochemical and antimicrobial characteristics of DPTB organic single crystals, providing deeper insights.

## Experimental Section

### Growth Procedure

The title compound (I) was synthesized (Scheme 1) by dissolving 2,4-dinitrophenylhydrazine (0.40 g, 2 mmol) in ethanol (10.00 mL) and $H_2SO_4$ (conc.) (98%, 0.50 mL) was added slowly with stirring. A solution of 3,4,5-trimethoxybenzaldehyde (0.40 g, 2 mmol) in ethanol (20.00 mL) was then added to the solution with continuous stirring for 1 hr, yielding an orange solid which was filtered off and washed with methanol. Orange block-shaped single crystals of the title compound (Fig. 1) suitable for X-ray structure determination were recrystallized from acetone by




Scheme 1 — Reaction scheme for DPTB

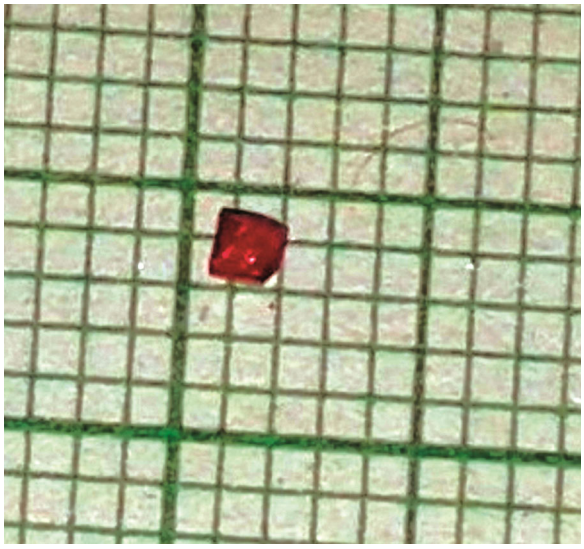


Fig. 1 — Single crystal of DPTB

slow evaporation of the solvent at room temperature over a few weeks.

## Characterization

Single crystal X-ray diffraction analysis was carried out on the grown DPTB single crystals *via* an Enraf (Bruker) Nonius CAD4 single-crystal X-ray diffractometer. A Shimadzu 2450 UV-VIS spectrophotometer was used to study the electronic absorption range of the compounds in the region of 200–800 nm. An alpha Bruker FT–IR spectrometer in the region 400–4000 $cm^{-1}$ and a Bruker 27 multiRAM stand alone FT–RAMAN spectrometer with a scanning range of 50–4000 $cm^{-1}$ were used to analyze the spectral properties of the compound. Proton NMR study of DPTB compound was performed using Bruker AVANCE III 500 MHz multinuclei solution spectrometer. Cyclic voltammetry measurements were performed for the title compound *via* a CHI 600D electrochemical analyzer (room temperature) with a 3 electrode cell in a solution of $BuNClO_4$ in dichloromethane at a scanning rate of 100 $mVs^{-1}$. A glassy carbon electrode was used as the working electrode, platinum foil was used as the counter electrode, and the reference electrode Ag/AgCl was calibrated after each measurement with ferrocene (Fc). Hot-stage optical polarized microscopy (HOPM) studies were performed on a Euromax polarizing optical microscope equipped with a Linkem HFS-91 heating stage and TP-93 temperature programmer. A small quantity of crystal was placed between two thin glass cover slips, heated and cooled at a rate of 5 μC/min, and phase changes in the crystals were observed. The photographs were taken on a Canon EOS 1000D camera. A frequency-doubled, Q-switched Nd:YAG (Spectra-Physics, INDI 40) laser, delivering 6 ns laser pulses at 1064 nm at a repetition rate of 10 Hz, passed through the powdered form of the title material, and their output intensity was measured to screen the second harmonic generation efficiency of the DPTB compound.

## Minimum Inhibitory Concentration

The minimum inhibitory concentration of the compound against human pathogens was analyzed using the resazurin reduction assay described by Sarker *et al*. (2007)[6].

## Preparation of resazurin dye solution

The resazurin dye solution was made by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure that the resazurin solution was well dissolved and formed a homogenous solution[7].

## Preparation of the activity plates

The 96-well plates were prepared under aseptic conditions. A volume of 200 μL of the compound (1 mg/mL) in 5% (v/v) di methyl sulfoxide was pipetted into the first row of the 96-well plate. To all other remaining wells, 100 μL of nutrient broth was added to the bacterial cells, and 100 μL of Sabouraud dextrose broth was added to the fungal cells. The serial dilutions were performed by micropipetting with sterile pipette tips such that each well contained 100 μL of the test material in serially descending concentrations. To all these wells, 10 μL of resazurin dye solution was added. A 10 μL bacterial suspension

($5\times10^6$ cells/mL) was added to each well to achieve a concentration of $5\times10^5$ cells/mL. The commercial antibiotics streptomycin and amphotericin B were used as positive controls in the assay plate. The plates were placed in an incubator at 37°C for 18-24 h. The color change was then observed visually. The color changes from blue to pink or colorless were recorded as the reduction of dye by the viable bacteria. The lowest concentration at which no color change occurred was taken as the MIC value.

## Results and Discussions

### Single Crystal X-ray diffraction analysis

Single crystal X-ray diffraction studies of the title compound provide the cell parameters of the crystal system. The DPTB single crystals crystallize in an orthorhombic crystal system with the $P2_12_12_1$ space group. The unit cell parameters are tabulated in Table 1, and the values are well matched with previously reported cell parameter values[5].

### FTIR and FTRAMAN Studies

FTIR and FTRaman spectroscopy studies of the title compounds revealed the presence of functional groups along with their mode of vibration. In the FTIR spectrum (Fig. 2a), the peak for azomethine is found at 1744 $cm^{-1}$, whereas in FTRaman, it is found at 1547 $cm^{-1}$ (Fig. 2b). Table 2 presents the spectral assignments[8-11].

Table 1 — Cell parameters of DPTB single crystals

| Parameters | Present values | Reported values[5] |
|---|---|---|
| Crystal system | Orthorhombic | Orthorhombic |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ |
| a (Å) | 7.479 (11) | 7.4724 (4) |
| b (Å) | 14.366 (19) | 14.3106(7) |
| c (Å) | 16.23 (2) | 16.1549(7) |
| α (°) | 90 | 90 |
| β (°) | 90 | 90 |
| γ (°) | 90 | 90 |
| V (Å$^3$) | 1743 (7) | 1727.52 (15) |

Table 2 — FTIR and FT Raman assignments of DPTB compounds

| FTIR | FTRaman | Assignments |
|---|---|---|
| 3743,3449, 3293 | 1612,1574 | N-H stretching |
| 3107, 3071 | | Aromatic C-H stretching |
| 2925 | | Methyl stretching |
| 2855 | | Asymmetric methyl stretching |
| 1744 | 1547 | C=N stretching |
| | 1518, 1453 | phenyl C-H stretching |
| 1616 | | N-H stretching |
| 1581 | | C-C stretching |
| 1500,1461 | | $CH_3$ assymetric stretching |
| 1417,1319 | | $CH_3$ symmetric stretching |
| | 1415, 1386 | C-C stretching |
| | 1347, 1323 | N=O symmetric stretching, C-N stretching |
| 1232,1152,119 | | $CH_3$ rocking |
| | 1278,1228 | $NH_2$ twisting, N-H in plane bending |
| | 1143 | C-H in plane bending |
| | 1085, 1063 | C-C-C trigonal bending |
| 998 | | C-H stretching |
| 947, 916 | | C-$OCH_3$ stretching |
| | 922 | C-C ring breathing |
| | 833 | $NO_2$ scissoring |
| | 784,731,710 | $NO_2$ wagging |
| | 568,489,456 | C-C-C ou of plane bending |
| | 342 | C-$NO_2$ out of plane bending |
| | 111 | $NO_2$ torsion |

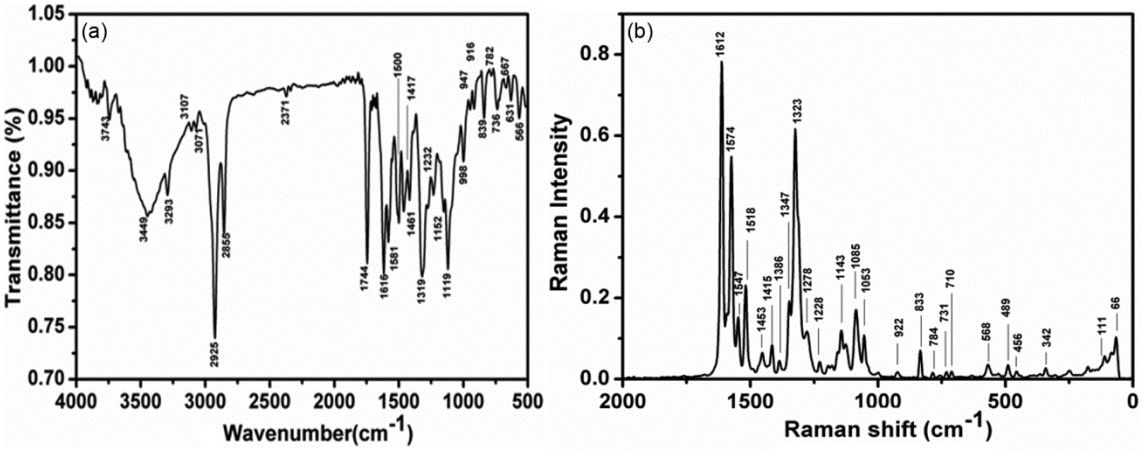



Fig. 2 — (a) FTIR spectrum of the DPTB compound and (b) FT-Raman spectrum of the DPTB compound

**FTNMR analysis**

Fig. 3 shows the proton NMR spectrum of DPTB compound. The azomethein hydrogen peak appeared at 8.38 ppm, and the NH peak appeared at 3.3 ppm. The three methoxy peaks were found in the region between 3.87 and 3.73 ppm. Two aromatic ring hydrogen peaks were observed at 8.87, 8.59, 8.14, and 7.10 ppm.

**UV–Vis spectral analysis**

UV–Vis spectroscopy (Fig. 4) was used to analyze the absorption region of the compounds. We have arrived at the maximum absorption peak at 392 nm, which arises from the n-π* transition of the C=N chromophore, and the absorption peak at 260 nm is caused by the π-π* transition of the C=N chromophore. The optical band gap of the compound was determined to be 2.64 eV using the relation, $E_g = \frac{hc}{\lambda}$.

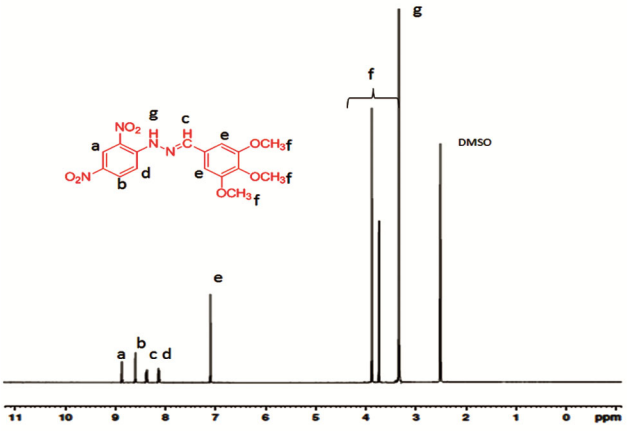



Fig. 3 — Proton NMR spectrum of the DPTB compound

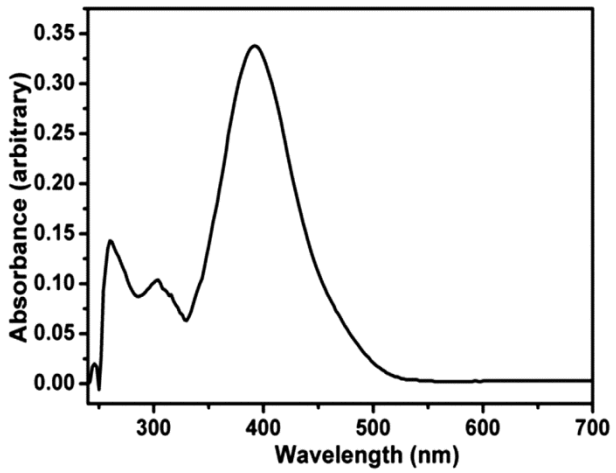



Fig. 4 — UV-Vis spectrum of the DPTB compound

**Cyclic Voltammetry**

The electrochemical behavior of the title compound (Fig. 5) was investigated using cyclic voltammetry analysis. By plotting the current against the potential, we obtained the oxidation onset potential as 0.68 and the reduction onset potential as -0.76. From these potential values, we obtained a HOMO-LUMO band gap value of 1.44 eV.

$HOMO = -[4.65\,V - E_{Ox}(onset)]$ = -0.76 V

$LUMO = -[4.65\,V - E_{Red}(onset)]$ = 0.68 V

**Thermal analysis**

The thermal properties of the title compound were studied by performing TG-DTA analysis. The TG curve (Fig. 6), revealed that the compound experienced single-step weight loss, resulting in a residual mass of 1.7132 mg. We observe a sharp endothermic peak at 240°C in the DTA curve, which corresponds to the melting point of the material, and it is also well established with the onset of weight loss in the TG curve.

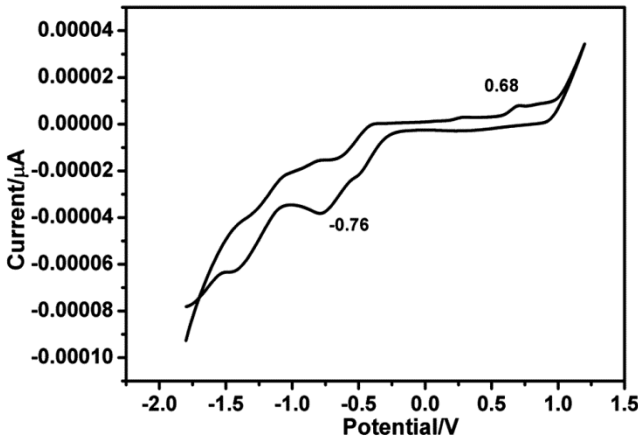



Fig. 5 — Cyclic voltammetry of the DPTB compound

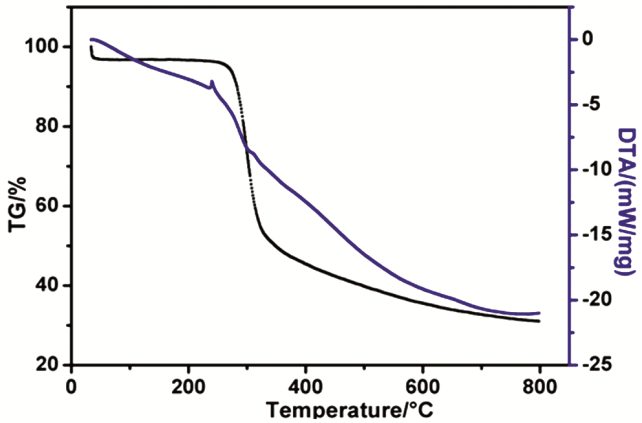



Fig. 6 — TG–DTA analysis of DPTB compound

**Hot stage optical polarized microscopy**

Hot-stage optical polarized microscopy was used to analyze the thermal properties and phase changes of the compound from crystalline to isotropic and from isotropic to crystalline phases. In Fig. 7, the isotropic phase was observed at 254°C with the melting of the compound at approximately 235°C, and upon cooling, the crystallization temperature was found to be 222°C.

**Second harmonic generation efficiency screening**

The Kurtz-Perry powder technique was used to determine the second harmonic generation efficiency of the title compound. For an input energy of approximately 0.7 J, the output signals for DPTB were 10.47 mJ and 36.5 mJ for KDP. Hence, the title compound, DPTB, was less efficient than KDP.

**Antimicrobial studies**

The MIC of the compound was determined visually using a resazurin dye reduction assay (Fig. 8). The change in dye color from blue to pink indicates that the microbial cells are viable. The enzyme oxido-reductase present inside bacterial cells converts resazurin to resorufin, which is pink in color. When the color of the dye remains blue, it indicates that there is no activity of viable cells. When the test material, was added, it killed the human pathogens during incubation. This was determined by the blue or purple color of the dye in the respective wells. The pink color of the wells, even after treatment with the test compounds or commercial drugs, indicated the presence of viable cells. Thus, the least dilution in which the color remained blue was considered the MIC value of the respective compound. All the compounds tested showed activity against bacterial and fungal pathogens. The MICs of the test compounds (Table 3) ranged from 6.25 to 100 µg/mL. Both gram-positive and gram-negative bacteria were susceptible to the test compounds. The test compound was less effective compared to the positive controls, erythromycin and fluconazole.

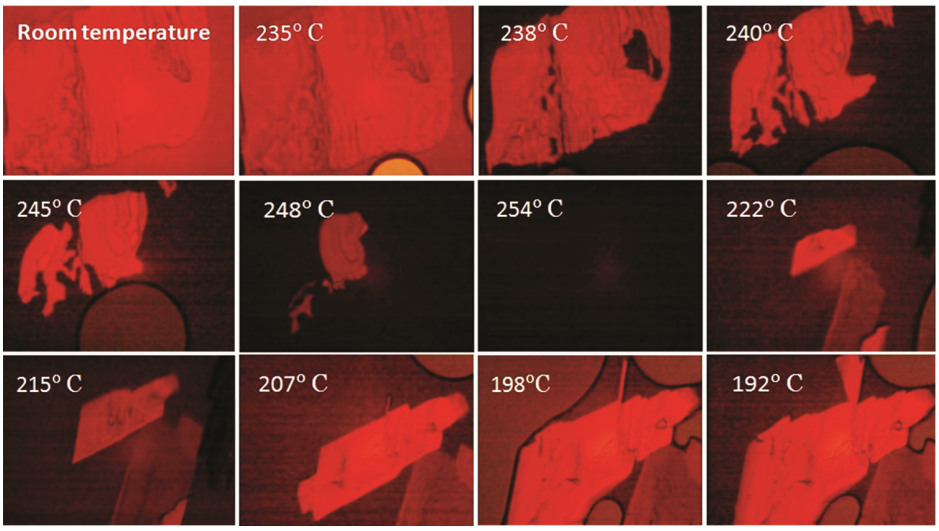



Fig. 7 — Thermomicrograph of DPTB

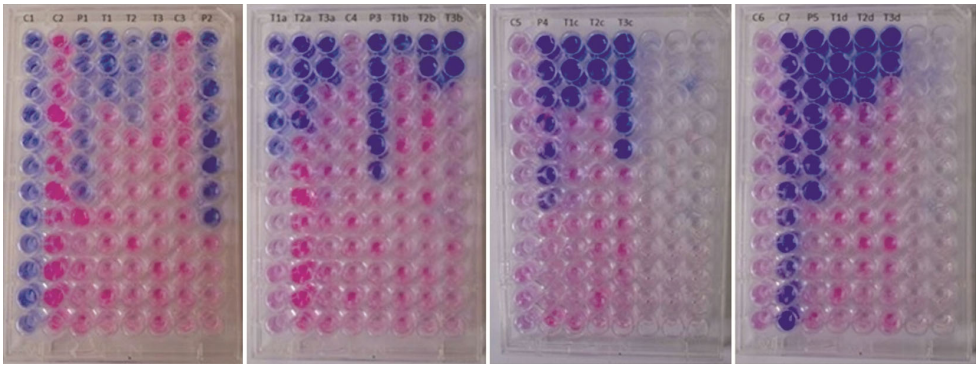


Fig. 8 — Antimicrobial screening of DPTB compounds using resazurin reduction assay

Table 3 — Minimum inhibitory concentrations of DPTB

| | MIC μg/mL | | | | |
|---|---|---|---|---|---|
| | Microbial Pathogens | | | | |
| Compd | *Staphylococcus aureus* | *Bacillus cereus* | *Escherichia coli* | *Klebsiella pneumonia* | *Candida albicans* |
| DPTB | 100 | 50 | 50 | 6.25 | 50 |
| Erythromycin | 1.56 | 0.78 | 3.12 | 1.56 | – |
| Flucanazole | – | – | – | – | 1.56 |

C1- control - Compound + dye + without bacteria
C2- control - dye + *Staphylococcus aureus* + without compound
C3- control - dye + *Bacillus cereus* + without compound
C4 -control - dye + *Escherichia coli* + without compound
C5- control- dye + *Klebsiella pneumoniae* + without compound
C6- control- dye + *Candida albicans* + without compound
C7- control - Compound + dye + without fungi
T3 – Compound + dye + *Staphylococcus aureus*
T3a – Compound + dye + *Bacillus cereus*
T3b – Compound + dye + *Escherichia coli*
T3c – Compound + dye + *Klebsiella pneumoniae*
T3d – Compound + dye + *Candida albicans*
P1 – Erythromycin + dye + *Staphylococcus aureus*
P2– Erythromycin + dye + *Bacillus cereus*
P3 – Erythromycin + dye + *Escherichia coli*
P4 – Erythromycin + dye + *Klebsiella pneumoniae*
P5 – Flucanazole + dye + *Candida albicans*

## Conclusions

This work effectively illustrates the growth and thorough description of single crystal 1-(2,4-dinitrophenyl)-2-[(E)-(3,4,5-trimethoxybenzylidene)] hydrazine (DPTB), which is synthesized using slow evaporation solution growth technique. X-ray diffraction of a single crystal confirmed that the compound has an orthorhombic crystal system belonging to the space group $P2_12_12_1$, which is consistent with existing data. Spectroscopic techniques such as FT-IR, FT-Raman, and $^1$H NMR conclusively confirmed the formation of the DPTB molecule. Optical studies revealed a significant absorption peak at 392 nm, associated with a band gap of 2.64 eV. Cyclic voltammetry results also revealed a smaller HOMO–LUMO energy gap of 1.44 eV, which suggests that the material is well suited for both optical and electronic applications. The hot-stage microscopy observations revealed that the crystal is stable, with a clear melting point at 240°C and phase changes occurring between 248 and 254°C. Antimicrobial screening indicated modest activity of the compound against several human pathogens, although it was slightly less effective than standard antibiotics and antifungal agents. These collective findings provide a solid basis for further research into DPTB, particularly in designing its structure to increase its effectiveness or in investigating its potential uses in optoelectronics and sensing technologies.

## Acknowledgement

One of the authors, G. Gomathi acknowledges Centre for Research, Anna University, Chennai 600 025, for providing Anna Centenary Research Fellowship (Proceeding no. CR/ACRF/JAN.2011/33). Authors also thank University Grants commission for having provided thermal analysis instruments to the Department of Physics, Anna University through SAP (Ref. No.: F.530/3/DRS/2012 (SAP - I)).

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