

## Eco-friendly synthesis of azo Schiff base ligand and its metal complexes: Anticancer, antidiabetic and antimicrobial activities

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### ABSTRACT

Now a days Cancer, Diabetics and other diseases are become major issue of the society. Due to this synthesis of potential medicines against it is a major challenge to the researcher and hence Schiff base ligand became an attracting class of researcher. The azo Schiff base ligands are most widely used in various fields such as medicinal, pharmacological, biological etc. due to its broad spectrum of biological activity. In this research paper we have synthesized the Azo-Schiff base ligand and its transition metal complexes by simple grinding method. Synthesis via green approach and biological evolution of azo-Schiff base ligand and its Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and VO(II) metal complexes. These compounds were characterized by Mass, <sup>1</sup>H-NMR, FT-IR, Elemental analysis, Molar conductance, magnetic susceptibility, UV-Vis., P-XRD, TGA etc. and were screened for biological activities. Synthesized azo-Schiff base ligand and its metal complexes were evaluated for their antimicrobial, antidiabetic as well as anticancer activities against various bacteria and fungi, acarbose and MCF-7 breast cancer cell line respectively. From the findings of various results we can conclude that the synthesized metal complexes exhibit higher biological activities than that of azo-Schiff base ligand.

**Keywords:** Grinding, coordination, spectroscopy, stability, standard drug

### INTRODUCTION

The Schiff base ligand have been subject of substantial research in the last two decades since it has boundless utilizations in various fields. There are plenty of uses of Schiff base ligand including antibacterial, antifungal, antiviral, antimalarial, anti-tumor, anti-tuberculosis, anthelmintic, anti-HIV, antidiuretic, anti-inflammatory, antiviral, antiprotozoal, anticonvulsant, analgesic, antioxidant, anti-Alzheimer, anti-hypertension, anti-ulcer, herbicidal properties have made them more and more important [1-13]. Schiff base ligands and metal complexes have multiple applications including polymers, dyes, pharmaceuticals, corrosion inhibitors and catalysts in biological systems inorganic, biological as well as analytical chemistry [14-28]. The green synthesis is an important alternate method for preparation of Schiff base ligand as well as metal complexes. It eliminates the uses of heat energy as well as the hazardous solvents also it increases the practical yields. The Schiff base ligand derived from condensation of aldehyde and amine gives broad spectrum of antimicrobial, anti-inflammatory [29] anticancer, antiviral, anti-fungal, antibacterial, anti-Alzheimer, antidiabetic activity. The Schiff base ligands

based on the Azo dye have attracted to the researcher as well as scientist in the last two decades because of its extraordinary application in various fields like medicinal, biological, industrial, chemical etc. Azo Schiff base ligands are the compounds that contains two main functional groups like azo (-N=N-) and Schiff base (-C=N). These ligands are formed by the procedure same as that of Schiff base ligand. The term co-ordination chemistry makes stable metal complexes by using Schiff base ligand, transition metals are known to exhibit the wild spectrum of biological activities such as: antiproliferative, chemotherapeutic, antineoplastic, antiviral, antifungal, antibacterial, anticancer etc. [30-31]. If we coordinate the Schiff base ligand to the transition metal ions then the spectrum of these activities will be enhanced. The coordination of the azo Schiff base ligand to the transition metal salts can be achieved by various modes including monodentate, bidentate, tridentate or chelating coordination. This is depending on the structure of ligand and metal ion. Over the past two decades, the green approach to the synthesis of Schiff base ligands and their metal complexes has gained significant attention due to its sustainability and environmental benefits. This method

enables eco-friendly synthesis by eliminating the use of hazardous solvents and harmful reaction conditions, thereby aligning with the principles of green chemistry. In this research paper we have represented alternate method of synthesis of azo-Schiff base ligand and its met-al complexes via green approach. We have used simple grinding method to prepare the compounds. The synthesized compounds were confirmed by various spectroscopic technique. Further these compounds were characterized by different biological techniques like: anticancer, antifungal, antibacterial, antidiabetic etc. against standard drugs. The metal complexes also studied by powder XRD to confirm crystal systems.

## EXPERIMENTAL

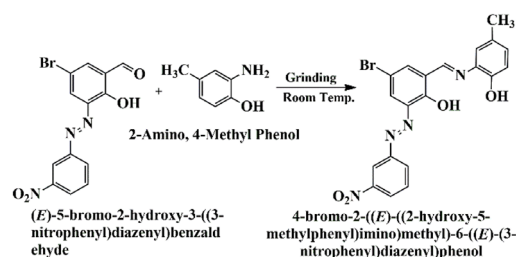
**Materials and methods:** All chemicals; 5-bromosalicylaldehyde, 3-nitroaniline, 2-amino-4-methylphenol were used as received from Sigma Aldrich Private Limited. In addition to the metal salts, the manga-nese(II), cobalt(II), copper(II), nickel(II) and zinc(II) acetates and vanadium(II) oxysulphate used are also commercially available. The various solvents such as ethanol, n-hexane, petroleum ether and ethyl acetate were purchased from local suppliers and purified by standard techniques. The progress of reaction completion was monitored using thin layer chromatography (TLC) on aluminium foil plates precoated with silica gel using n-hexane: ethyl acetate system. <sup>1</sup>H-NMR spectra were recorded on a BRUKER 400 MHz <sup>1</sup>H-NMR instrument using CDCl<sub>3</sub> as solvent. The melting points of the synthesized Schiff base ligands and met-al complexes were observed on an OPTIC TECHNOLOGY digital melting point instrument. The EI-MS spectra were observed on a MALDI-TOF-Mass spectrum. FTIR spectra were performed using a potassium.

### Synthesis

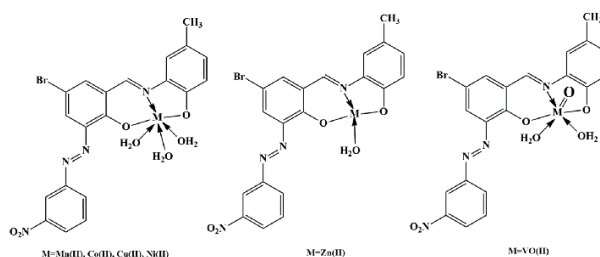
**General procedure of synthesis of azo-Schiff base ligand:** The synthesis of azo-Schiff base ligands was carried out as described in previous studies [32]. 3.50 g (0.01 mol) of azo-aldehyde was taken in mortar and 1.23 g (0.01 mol) of 4-Methyl, 2-aminophenol was added to it and few drops of ethyl alcohol was added in this mixture. The mixed component grinded for 50 minutes. The reaction progress observed by TLC. The orange-colored solid product was obtained in the mortar. The orange-colored solid product has been taken out, washed by using warm ethanol. This is shown in Scheme 1.

**General procedure of synthesis of Metal complexes:** The synthesis of azo-Schiff base ligands was carried out as described in previous studies [32]. The metal complexes has been synthesized by the addition of equimolar metal salts (acetate) of Mn(II), Ni(II), Co(II), Zn(II), Cu(II), and sulphate of VO(II) (0.001 mol) to the azo-Schiff base ligand (0.001 mol) in mortar with few drops of ethyl alcohol at ambient temperature. The mixture has been grinded for 20-35 minutes and

different coloured solid metal complexes were obtained in the reaction mixture. These metal complexes have been then collected, washed with warm ethyl alcohol and dried under vacuum. This is given in Scheme 2.



Scheme 1. Synthesis of Schiff base ligand



Scheme 2. Synthesis of metal complexes

### Biological Activity

**Anticancer activity:** For the anticancer activity of synthesized azo-Schiff base ligand and its metal complexes MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay on the human breast cancer cell line MCF-7 (Michigan Cancer Foundation-7) has been utilised. The human breast cancer cell line MCF-7 was procured from National centre for cell sciences (NCCS), Pune maintained in DMEM medium supplemented with 10% fetal bovine serum. The preparation of sample concentration at 25 µg/mL, 50 µg/mL, 100 µg/mL in DMSO. The method is previously described [32]. The percent of inhibition is calculated by formula given below:

$$\text{Inhibition Percentage} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

**Antidiabetic activity:** For the antidiabetic activity we have used the  $\alpha$ -amylase inhibition assay. For this study firstly made 500 µL of 0.1 M phosphate buffer solution having pH of 6.9 containing 0.5% enzyme. The test sample 500 µL taken and added in the above solution allowed to react and incubated for 10 minutes at 25 °C. After this 500 µL of 1% starch solution, extra pure 0.1 M phosphate buffer was mixed and placed in incubator for 10 min at 25 °C. The comparable procedure was conducted for the controls where 500 µL of the enzyme were substituted with buffer. After incubation, 1000 µL of DNS was transferred to both test and control samples. The drug acarbose used as standard drug. At 540 nm, the absorbance was measured with a spectrophotometer and the percentage inhibition of

$\alpha$ -amylase enzyme was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540 (control)}} \times 100$$

**Antimicrobial activity:** The antimicrobial activity was evaluated using the disk diffusion method to test its effectiveness against a selected microorganisms including two gram-positive bacteria, two gram-negative bacteria and three fungi. This assessment adhered to the procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The process involved the use of standard agar, implemented in accordance with established protocols described in relevant literature [32]. DMSO was used as a negative control which showed no inhibition activities against any of the organisms and it was utilized for dissolving all compounds concentration at 100  $\mu\text{g/mL}$ . The zone of inhibition around each disk was measured using sliding callipers in millimetres and comparison with standard reference drug Tetracycline and fluconazole as positive control.

## RESULTS AND DISCUSSION

The synthesized azo-Schiff base ligand and its metal complexes display distinct colors. Generally, the metal complexes are insoluble in water but readily soluble in common organic solvents such as chloroform, dimethyl sulfoxide and dimethyl formamide. The observed solubility behavior indicates that these complexes are likely more compatible with organic solvents than with aqueous media. The differences in color may reflect variations in the coordination environments or oxidation states of the metal ions, which could significantly influence their chemical properties and reactivity. All prepared compounds are stable in air. Physicochemical data of azo-Schiff base ligand and metal complexes shown in Table 1.

suggested structure molecular weight, as depicted in Fig. S1.

**<sup>1</sup>H-NMR spectra of azo-aldehyde and azo-Schiff base ligand:** Azo-Schiff base ligand: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  12.44 (s, 1H), 8.64 (s, 1H), 7.43 (d, *J* = 2.2 Hz, 1H), 7.41 – 7.34 (m, 1H), 7.29 (s, 1H), 7.07 (d, *J* = 6.8 Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 1H), 6.98 (s, 1H), 6.94 (d, *J* = 7.9 Hz, 1H), 5.60 (s, 1H), 2.36 (s, 3H) [32] illustrated in Fig. S2.

**FTIR spectra of azo-Schiff base ligand and metal complexes:** The IR spectra of azo-Schiff base ligand characteristic band appeared at 1623 cm<sup>-1</sup> which shifted to lower frequencies within the range of 1593-1604 cm<sup>-1</sup> in all the metal complexes. This change implies that the metal atom and azo-imine nitrogen is in coordination resulting in the formation of azo-imine complexes [33]. The phenolic -OH group was identified by a large band in ligand located at 3664 cm<sup>-1</sup> and 3477 cm<sup>-1</sup>. Interestingly, this band vanished in every metal complex indicating that the phenolic oxygen atoms had coordinated by deprotonation. This observation is further supported by the IR spectra of ligand where a >C-O band at 1227 cm<sup>-1</sup> shifted to higher frequencies ranging from 1246-1296 cm<sup>-1</sup> in all the metal complexes [34-35]. The azo group's band of -N=N- was originally observed at 1483 cm<sup>-1</sup>. New bands of the metal complexes, in the range of 441-449 cm<sup>-1</sup> and 522-526 cm<sup>-1</sup> respectively appeared in the metal complexes and were attributed to M-N and M-O vibrations. These findings suggest the formation of coordination bonds among the metal ions and ligand leading to changes in IR spectra which provide insights into the chelation process. The band of >C-Br exhibited at 569-616 cm<sup>-1</sup> in ligand and metal complexes. A band of -OH rocking at 813-824 cm<sup>-1</sup> suggests the presence of coordinated water in all metal complexes [36]. According to overall IR data, the two phenolic oxygen atoms and one nitrogen atom of imine group coordinated with metal ion by tridentate manner.

Table 1. Physical and chemical data of azo-Schiff base ligand and metal complexes

| Compounds              | Physical Appearance | Physical Constant | Time min | Yield in % | Elemental analysis in % found (Calculated) |            |              |
|------------------------|---------------------|-------------------|----------|------------|--|------------|--------------|
|                        |                     |                   |          |            | C  | H          | N            |
| Azo-Schiff base ligand | Orange              | 150-160 °C        | 55-60    | 87         | 52.31(52.76)                               | 3.10(3.32) | 12.58(12.31) |
| Mn(II) complex         | Coffee              | >300 °C           | 25-30    | 85         | 42.29(42.73)                               | 3.47(3.41) | 9.79(9.97)   |
| Co(II) complex         | Brown               | >300 °C           | 30-35    | 83         | 42.12(42.42)                               | 3.48(3.38) | 9.59(9.89)   |
| Ni(II) complex         | Dark brown          | >300 °C           | 20-25    | 78         | 42.26(42.44)                               | 3.56(3.38) | 9.81(9.90)   |
| Cu(II) complex         | Green               | >300 °C           | 30-35    | 81         | 41.87(42.05)                               | 3.56(3.35) | 9.45(9.81)   |
| Zn(II) complex         | Yellow              | >300 °C           | 20-25    | 78         | 44.89(44.76)                               | 3.18(2.82) | 10.87(10.44) |
| VO(II) complex         | Faint Green         | >300 °C           | 30-35    | 79         | 42.72(42.19)                               | 3.00(3.08) | 9.78(10.08)  |

### Spectroscopic Analysis

**Mass spectra of azo-aldehyde and azo-Schiff base ligand:** The azo-Schiff base ligand molecular ion peak had been found at *m/z* 455(M), 457(M+2) in the spectrum of mass. This peak is consistent with the

These findings are shown in Fig. S3-S6 and Table 2.

**Electronic spectrum and magnetic susceptibility of azo-Schiff base ligand and metal complexes:** The electronic spectrum of azo-Schiff base ligand and its metal complexes were recorded in dimethylsulfoxide

Table 2. FTIR stretching frequency of azo-Schiff base ligand and metal complexes in  $\text{cm}^{-1}$ 

| Sr. No. | Sample         | -OH        | >C=N- | -N=N- | M-O | M-N | >C-O | >C-Br |
|---------|----------------|------------|-------|-------|-----|-----|------|-------|
| 1       | Ligand         | 3664, 3477 | 1623  | 1483  | --  | --  | 1227 | 580   |
| 2       | Mn(II) complex | --         | 1600  | 1490  | 526 | 441 | 1296 | 569   |
| 3       | Co(II) complex | --         | 1598  | 1487  | 526 | 449 | 1257 | 578   |
| 4       | Ni(II) complex | --         | 1602  | 1490  | 522 | 445 | 1246 | 576   |
| 5       | Cu(II) complex | --         | 1593  | 1491  | 526 | 449 | 1258 | 580   |
| 6       | Zn(II) complex | --         | 1600  | 1488  | 526 | 449 | 1291 | 580   |
| 7       | VO(II) complex | --         | 1604  | 1488  | 522 | 441 | 1283 | 616   |

at concentration at ( $\approx 5 \times 10^{-4}$ ), molar range of 50000 to  $16666 \text{ cm}^{-1}$  and 200 to 600 nm. The electronic spectra have been used to deduce the nature of the ligand field surrounding the central metal ion and magnetic susceptibility confirms that whether the metal complex is paramagnetic or diamagnetic [37]. Paramagnetic complexes exhibit a positive affinity toward external magnetic fields whereas diamagnetic complexes are repelled by such fields. In paramagnetic materials the magnetic flux density within the substance exceeds that present in a vacuum. Consequently, paramagnetic complexes demonstrate a positive magnetic susceptibility. The magnetic analysis reveals that all metal complexes exhibit paramagnetic behavior with the exception of the Zn(II) complex [32]. The electronic spectrum of the azo-Schiff base ligand displays absorption bands at 334 nm and 240 nm corresponding to  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions respectively. The electronic spectra of Mn(II) complexes exhibit a band at 277 nm which can be attributed to a metal-to-ligand charge transfer (MLCT) transition suggesting an octahedral geometry. The electronic spectra of Co(II) complexes exhibit broad absorption peaks at 397 nm, 330 nm and 301 nm which can be tentatively attributed to the transitions  ${}^4T_{1g} \rightarrow {}^4T_{2g}$ ,  ${}^4T_{1g} \rightarrow {}^4T_{1g(P)}$  and  ${}^4T_{1g} \rightarrow {}^4A_{2g}$  respectively. The magnetic moment value of  $4.10 \mu\text{B}$  suggests the presence of an octahedral configuration around the Co(II) ion. The electronic spectra of Ni(II) complexes display absorption bands at 420 nm, 347 nm and 311 nm which can be assigned to the transitions  ${}^3A_{2g} \rightarrow {}^3T_{2g}$ ,  ${}^3A_{2g} \rightarrow {}^3T_{1g(f)}$  and  ${}^3A_{2g} \rightarrow {}^3T_{1g(P)}$  respectively. The magnetic moment value of  $3.1 \mu\text{B}$  indicates the presence of an octahedral geometry around the Ni(II) ion. The electronic spectra of Cu(II) complexes exhibit absorption bands at 430 nm, 400 nm and 340 nm which can be attributed to the transitions  ${}^2E_g \rightarrow {}^2T_{2g}$ ,  ${}^2E_g \rightarrow {}^2T_{2g}$  and ligand-to-metal charge transfer (LMCT) transitions respectively. The magnetic moment value of  $2.0 \mu\text{B}$  indicates a distorted octahedral geometry around the Cu(II) ion attributed to Jahn-Teller distortion [32]. The electronic spectra of Zn(II) complexes exhibit an absorption band at 336 nm, which can be attributed to a metal-to-ligand charge transfer (MLCT) transition. The diamagnetic nature of the complex suggests a tetrahedral geometry. The VO(II) complex displays a

band at 346 nm corresponding to the  ${}^2B_2 \rightarrow {}^2E$  transition. The magnetic moment value of  $1.80 \mu\text{B}$  is indicative of a square-pyramidal geometry. The electronic spectra of Zn(II) complexes 336 nm which may metal to ligand charge transition and shows diamagnetic moment suggesting tetrahedral geometry [32]. The electronic spectral data and magnetic moment shown in **Fig. S7-S9** and Table 3.

**Thermal Analysis of metal complexes:** The decomposition stages of the metal complexes highlight their thermal behavior and provide insights into their structural stability. The release of coordinated water molecules at lower temperatures indicates relatively weak bonding interactions with the metal center. The subsequent breakdown of ligand molecules at higher temperatures suggests stronger metal-ligand interactions, which require greater thermal energy for dissociation. Finally, the formation of stable metal oxides at elevated temperatures reflects the complete thermal decomposition of the complexes. The thermal stability of the synthesized metal complexes was investigated across a temperature range from ambient conditions to  $1000^\circ\text{C}$ . Analysis of the results indicates that the decomposition of the metal complexes occurs in two distinct stages. In the first stage, between ambient temperature and  $400^\circ\text{C}$ , the coordinated water molecules are released. The second stage involves the removal of the ligand molecules, occurring within the temperature range of  $400$  to  $800^\circ\text{C}$ . Beyond  $800^\circ\text{C}$ , the formation of stable metal oxides is observed [33] shown in **Fig. S10-S11**.

**Powder X-Ray Diffraction (XRD) of metal complexes:** The powder X-ray diffraction analysis of the metal complexes has been carried out to confirm whether the nature of the sample is amorphous or crystalline. The P-XRD of metal complexes were scanned in range from  $2\theta = 20-80^\circ$  at wavelength of  $1.540 \text{ \AA}$  for prediction of cell parameter, crystal system, lattice parameter etc. the metal complexes of Mn(II), Co(II) and Cu(II) shows monoclinic crystal system. Ni(II) and VO(II) complexes shows orthorhombic crystal system. The Zn(II) complex shows triclinic crystal system [34] shown in **Fig. S12-S14** and Table 4.

Table 3. Magnetic moment and molar conductance of azo-Schiff base ligand and metal complexes

| Sr. No. | Compounds      | $\lambda_{\max}$ (nm) | Absorption band (cm <sup>-1</sup> ) | Tentative transition                   | Molar conductance s.mol <sup>-1</sup> cm <sup>2</sup> | Magnetic moments in $\mu_B$ | Proposed geometry |
|---------|----------------|-----------------------|-------------------------------------|--|---|-----------------------------|-------------------|
| 1       | Ligand         | 334                   | 29940                               | $n \rightarrow \pi^*$                  | 7.1   | --                          | --                |
|         |                | 240                   | 41666                               | $\pi \rightarrow \pi^*$                |   |                             |                   |
| 2       | Mn(II) complex | 277                   | 36101                               | MLCT                                   | 10.1  | 6.01                        | Octahedral        |
| 3       | Co(II) complex | 397                   | 25188                               | ${}^4T_{1g} \rightarrow {}^4T_{2g}$    | 9.2   | 4.10                        | Octahedral        |
|         |                | 330                   | 30303                               | ${}^4T_{1g} \rightarrow {}^4T_{1g(P)}$ |   |                             |                   |
|         |                | 301                   | 33222                               | ${}^4T_{1g} \rightarrow {}^4A_{2g}$    |   |                             |                   |
| 4       | Ni(II) complex | 420                   | 23809                               | ${}^3A_{2g} \rightarrow {}^3T_{2g}$    | 12.3  | 2.99                        | Octahedral        |
|         |                | 347                   | 28818                               | ${}^3A_{2g} \rightarrow {}^3T_{1g(f)}$ |   |                             |                   |
|         |                | 311                   | 32154                               | ${}^3A_{2g} \rightarrow {}^3T_{1g(P)}$ |   |                             |                   |
| 5       | Cu(II) complex | 430                   | 29441                               | ${}^2E_g \rightarrow {}^2T_{2g}$       | 11.2  | 2.00                        | Octahedral        |
|         |                | 400                   | 25000                               | ${}^2E_g \rightarrow {}^2T_{2g}$       |   |                             |                   |
|         |                | 340                   | 23255                               | LMCT                                   |   |                             |                   |
| 6       | Zn(II) complex | 336                   | 29761                               | MLCT                                   | 9.4   | Dia.                        | Tetrahedral       |

Table 4. P-XRD of metal complexes

| Compounds      | No. of reflections | Maxima (2 $\theta$ ) | d-Value | Lattice constant (Å)                      | Unit cell volume | Axis and axis angle  | Z-Value | Crystal system |
|----------------|--------------------|----------------------|---------|---|------------------|--|---------|----------------|
| Mn(II) complex | 11                 | 54.62                | 13.768  | a = 7.8800<br>b = 7.03407<br>c = 13.8900  | 763.131          | a $\neq$ b $\neq$ c and<br>$\alpha = \gamma = 90^\circ \neq \beta$       | 2       | Monoclinic     |
| Co(II) complex | 12                 | 49.82                | 10.887  | a = 11.2800<br>b = 7.9200<br>c = 13.4500  | 1159.774         | a $\neq$ b $\neq$ c and<br>$\alpha = \gamma = 90^\circ \neq \beta$       | 4       | Monoclinic     |
| Ni(II) complex | 12                 | 48.42                | 11.665  | a = 12.1332<br>b = 16.7980<br>c = 46.6580 | 9509.530         | a $\neq$ b $\neq$ c and<br>$\alpha = \beta = \gamma = 90^\circ$          | 4       | Orthorhombic   |
| Cu(II) complex | 14                 | 78.49                | 5.584   | a = 5.1352<br>b = 9.8020<br>c = 6.8500    | 342.017          | a $\neq$ b $\neq$ c and<br>$\alpha = \gamma = 90^\circ \neq \beta$       | 4       | Monoclinic     |
| Zn(II) complex | 13                 | 48.78                | 11.129  | a = 5.3350<br>b = 10.6250<br>c = 11.8860  | 630.806          | a $\neq$ b $\neq$ c and<br>$\alpha \neq \beta \neq \gamma \neq 90^\circ$ | 2       | Triclinic      |
| VO(II) complex | 12                 | 26.57                | 3.352   | a = 3.9800<br>b = 13.7590<br>c = 15.4610  | 846.530          | a $\neq$ b $\neq$ c and<br>$\alpha = \beta = \gamma = 90^\circ$          | 4       | Orthorhombic   |

### Biological activity of azo-Schiff base ligand and metal complexes

**Antimicrobial activity:** The disc diffusion method was used to screen the antimicrobial activity of the synthesized ligand and its metal complexes against gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) as well as gram-negative bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*) and three fungi (*Penicillium chrysogenum*, *Trichoderma viride*, and *Aspergillus niger*). From the findings, in the antibacterial activity the all metal complexes shows better inhibition but Mn(II), Co(II), and VO(II) show higher inhibition than that of ligand and standard reference drug tetracycline against *S. aureus*. Only Mn(II) complex shows better inhibition against *K. pneumonia*. The Mn(II) and VO(II) shows better inhibition against *P. aeruginosa*.

In antifungal activity no metal complex shows better or higher inhibition than that of ligand and standard reference drug fluconazole [39]. Mn(II) and Co(II) complexes exist in various oxidation state allowing them to participate in redox reactions important for many biochemical processes. Based on Tweedy's chelation theory, chelation plays a role in enhancing the biological activity of metal complexes. It has been observed that while the azo-Schiff base ligand demonstrates limited biological activity, its corresponding metal complexes show significantly improved activity against both bacteria and fungi. This indicates that the biological activity of azo-Schiff base ligands improves through chelation with various metals that are biologically active [32]. The findings are given in Table 5.

Table 5. Antimicrobial activity of azo-Schiff base ligand and metal complexes

| Compounds      | Antibacterial activity |                    |                      |                      | Antifungal activity   |                  |                 |
|----------------|------------------------|--------------------|----------------------|----------------------|-----------------------|------------------|-----------------|
|                | <i>S. aureus</i>       | <i>B. subtilis</i> | <i>K. pneumoniae</i> | <i>P. aeruginosa</i> | <i>P. chrysogenum</i> | <i>T. viride</i> | <i>A. niger</i> |
| Ligand         | 18                     | 13.5               | 8                    | 15                   | 8                     | 8                | 8               |
| Mn(II) Complex | 32.5                   | 8                  | 18                   | 18                   | 8                     | 8                | 8               |
| Co(II) Complex | 33.5                   | 8                  | 8                    | 8                    | 8                     | 8                | 8               |
| Ni(II) Complex | 20                     | 8                  | 8                    | 8                    | 8                     | 8                | 8               |
| Cu(II) Complex | 23                     | 8                  | 8                    | 8                    | 8                     | 8                | 8               |
| Zn(II) Complex | 23.5                   | 12.5               | 8                    | 15                   | 8                     | 8                | 8               |
| VO(II) Complex | 25                     | 8                  | 8                    | 30                   | 8                     | 8                | 8               |
| Tetracycline   | 25                     | 19                 | 32                   | 30                   | --                    | --               | --              |
| Fluconazole    | --                     | --                 | --                   | --                   | 10                    | 20               | 22              |

**Anticancer activity:** The activity of the anticancer activity of azo-Schiff base ligand as well as the metal complexes has been performed by utilizing the MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) test on MCF-7 human breast cancer cell line. The metal complexes involving Cu(II), Zn(II), and Co(II) demonstrated significantly superior anticancer activity compared to ligand against standard drug 5-FU[40]. The cytotoxic effectiveness can be attributed to the metals positive charge, which increases the acidity of the coordinated ligand. This, in turn, releases protons that form stronger hydrogen bonds thereby enhancing biological activity [40]. The findings are given in Table 6 and photos are enclosed in Fig. S15.

Table 6. Anticancer activity of azo-Schiff base ligand and metal complexes

| Compounds      | Concentration of compounds ( $\mu\text{g/mL}$ ) | Cell Inhibition in % |
|----------------|---|----------------------|
| Standard 5-FU  | 10  | 79.81                |
|                | 40  | 83.69                |
|                | 100   | 89.02                |
| Ligand         | 10  | 46.19                |
|                | 40  | 52.78                |
|                | 100   | 64.73                |
| Co(II) Complex | 10  | 40.47                |
|                | 40  | 59.06                |
|                | 100   | 68.98                |
| Cu(II) Complex | 10  | 30.95                |
|                | 40  | 62.27                |
|                | 100   | 69.08                |
| Zn(II) Complex | 10  | 40.90                |
|                | 40  | 57.55                |
|                | 100   | 66.99                |

**Antidiabetic activity:** The anti-diabetic activity of synthesized azo-Schiff base ligand and metal complexes was done by utilizing  $\alpha$ -amylase inhibition assay. The Co(II) complex shows higher inhibition than azo-Schiff base ligand. Other metal complexes such as Cu(II) and Zn(II) also exhibit better inhibition than azo-Schiff base ligand compared with standard drug Acarbose [41] shown in Table 7 and photos are provided Fig. S16.

Table 7. Antidiabetic activity of azo-schiff base ligand and metal complexes.

| Compounds           | Concentration of sample ( $\mu\text{g/mL}$ ) | Inhibition (%) at 540 nm |
|---------------------|--|--------------------------|
| Acarbose (standard) | 250  | 54.54                    |
|                     | 500  | 61.93                    |
|                     | 1000   | 74.43                    |
| Ligand              | 250  | 20.45                    |
|                     | 500  | 31.81                    |
|                     | 1000   | 39.77                    |
| Co(II) Complex      | 250  | 25.00                    |
|                     | 500  | 36.36                    |
|                     | 1000   | 55.68                    |
| Cu(II) Complex      | 250  | 22.72                    |
|                     | 500  | 35.22                    |
|                     | 1000   | 54.54                    |
| Zn(II) Complex      | 250  | 31.25                    |
|                     | 500  | 38.63                    |
|                     | 1000   | 52.27                    |

## CONCLUSION

In this study, we successfully synthesized an azo-Schiff base ligand and its transition metal complexes using a green approach. Spectroscopic analyses confirm the tridentate nature of the ligand, facilitating stable coordination with metal centers. Powder X-ray diffraction (XRD) studies reveal distinct crystallographic structures among the complexes: Mn(II), Co(II),

and Cu(II) complexes exhibit monoclinic systems, whereas Ni(II) and VO(II) complexes crystallize in the orthorhombic system, and the Zn(II) complex adopts a triclinic arrangement.

Biological evaluations indicate that the metal complexes exhibit enhanced pharmacological activities compared to the free azo-Schiff base ligand, demonstrating better antimicrobial and antioxidant properties relative to standard drugs. Furthermore, cytotoxicity assays highlight the promising anticancer potential of these metal complexes, reinforcing their applicability in medicinal chemistry. The findings emphasize the role of green synthesis in developing biologically active coordination compounds with significant therapeutic relevance.

### AUTHOR CONTRIBUTIONS

Study conception and design: Kuldeep B. Sakhare, Mahadeo A. Sakhare data collection: Kuldeep B. Sakhare, Kirti N. Sarwade, Analysis and interpretation of results: Mahadeo Abarao Sakhare, Kirti N. Sarwade, Yogesh N. Bharate, Writing manuscript: Yogesh N. Bharate, Kuldeep B. Sakhare. All authors reviewed the results and approved the final version of the manuscript.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### DATA AVAILABILITY

The data and supportive information are available within the article.

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