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# Synthesis and Biological Evaluation Study of New Bis-imine Ligand and Metal Complexes

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**Abstract:** A new Schiff base bis-imine ligand was synthesized by the reaction of 1-(2-bromo, 5-methoxy benzylidene) hydrazine and 5-chlorosalicylaldehyde. This bis-imine ligand was used for the synthesis of metal complexes. The synthesized ligand and metal complexes were characterized by spectroscopic techniques. The metal complexes were formed in the ligand to metal ratio of 2:1. The synthesized metal complexes were screened for antimalarial, anti-tubercular, and antimicrobial activities.

**Keywords:** bis-imine; metal complexes; anti-malarial; anti-tubercular; anti-microbial activities.



## 1. Introduction

Schiff bases contain the C=N functional group and are an important class of compounds as they are used for the synthesis of biologically active heterocyclic compounds [1]. Schiff bases are used as chelates for complexation with metal ions [2]. Schiff base ligands containing nitrogen and oxygen donor atoms can form complexes with metal ions [3]. Imines are vital because of their stability and biological activity. Schiff base ligands and their metal complexes showed anti-depressant, anti-inflammatory, anticonvulsant, antimicrobial, anti-tubercular, antimalarial, and anti-viral activities [4-5].

Many bioactive compounds used in agricultural, material science, medicinal and pharmaceutical fields can be synthesized from bis-imines [6]. Some metal complexes of bis-imines are used as catalysts in organic reactions [7]. Cobalt (II) and iron (III) complexes of pyridine bisimine ligands showed activity in the polymerization of ethylene.

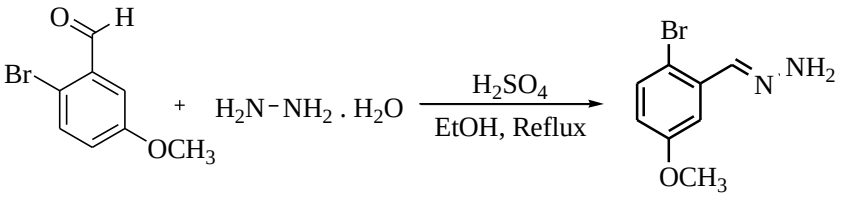
In continuation of our work in the synthesis and biological activity of Schiff base ligand and metal complexes [8-10], we report the synthesis of Schiff base (bis-imine) ligand from 1-(2-bromo, 5-methoxy benzylidene) hydrazine and 5-chlorosalicylaldehyde. The synthesized ligand was used for the synthesis of metal complexes. The synthesized compound was characterized by different spectroscopic methods, and metal complexes were evaluated for antimalarial, anti-tubercular, and antimicrobial activities.

## 2. Materials and Methods

All the chemicals used for the synthesis were of analytical grade. Distilled solvents were used [11].

*2.1. Synthesis of [1-(2-bromo, 5-methoxy benzylidene) hydrazine]-.*

2-bromo, 5-methoxy benzaldehyde (1) (1 mmol), and hydrazine hydrate (2) (7 -8mmol) were dissolved in solvent ethanol, and 1-2 drops of concentrated sulphuric acid were added as a catalyst. The resulting reaction mixture was refluxed for 4 hours at 80° C, and the progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, and the product was precipitated by the addition of ice. The product 1-(2-bromo, 5-methoxy benzylidene) hydrazine (3) was recrystallized from ethanol and confirmed by melting point and IR spectra (Scheme 1). Melting point = 161° C, color: yellow, solid. IR spectra- IR (FTIR-ATR, $\nu_{max}$ $cm^{-1}$): 1620 (C=N), 3045 (NH).

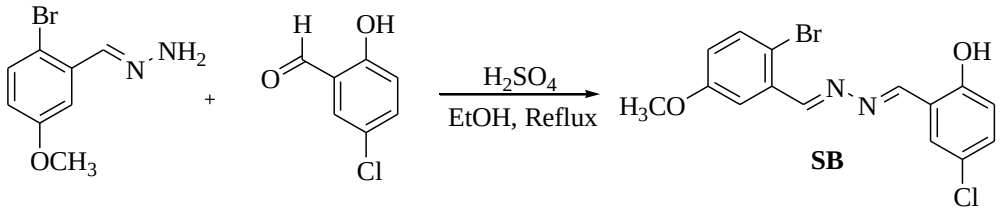



**(1)** **(2)** **(3)**

**Scheme 1.** Synthesis of 1-(2-bromo, 5-methoxy benzylidene) hydrazine.

*2.2. Synthesis of Schiff base ligand (Bis-imine) (SB).*

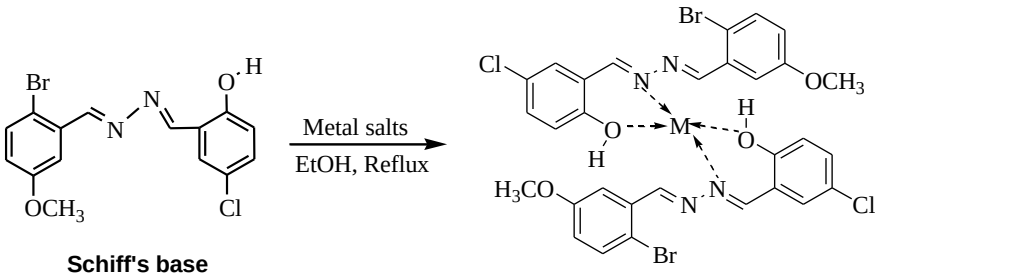
1 mmol of 5-chlorosalicyladehyde (4) was added to 1mmol of 1-(2-bromo, 5-methoxy benzylidene) hydrazine (3) in solvent ethanol, and 1-2 drops of concentrated sulphuric acid were added as a catalyst. The mixture was refluxed for 3-4 hr at 80 ° C. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, and the product was precipitated by the addition of ice. The solid product was filtered out, washed with cold water, dried, and recrystallized from ethanol. The product was confirmed by IR spectra as pure Schiff base ligand (bis-imine) (Scheme 2).




**Scheme 2.** Synthesis of Schiff base ligand (bis-imine) (SB).
IR spectra of bis-imine Ligand (SB)-: ($\nu_{max}$ $cm^{-1}$) 1616 (C=N).

*2.3. Synthesis of metal complexes (SB1-8).*

A mixture of metal salt (metal chlorides/metal nitrates) and Schiff base bis-imine ligand (SB) in solvent ethanol was prepared. The ratio of metal to the ligand in mmol was 1:2. Few drops of ammonia were added to the mixture. The mixture was refluxed for 5-6 hr. The progress of the reaction was monitored by TLC. The products were cooled, filtered out, and dried. Melting points of the products were determined (Scheme 3).




**Schiff's base**

**M=Zn(II), Fe (II),Ni(II), Sn(II), Cu(II), Cd(II), Cr(II) and Zr(II)**

**Scheme 3.** Synthesis of metal complexes (SB1-8).

The physical and analytical data of synthesized compounds are presented in Table 1.

*2.4. Infrared spectra of metal complexes.*

The IR spectra of metal complexes were recorded in the wave number region of 250 $cm^{-1}$- 4500 $cm^{-1}$ [12]. The characteristic spectral band in the region 395- 462 $cm^{-1}$ indicates the M-N band frequency i.e., the coordination of metal ion with the nitrogen atom of the ligand. The low-frequency band in the region 500-827$cm^{-1}$ was characterized for metal complexes and indicates the coordination of oxygen atom of the ligand with metal, i.e., M-O frequency bands. The frequency bands at 1610-1624 $cm^{-1}$ can be recognized for C=N group. The stretching frequency bands at 2939-3074 $cm^{-1}$ were assigned for C-H bond. IR spectra for metal complexes are reported in Table 2.

*2.5. UV -Visible spectra of metal complexes.*

UV spectra of synthesized metal complexes was recorded in DMSO solvent. The wavelength of maximum absorbance ($\lambda_{max}$ ) for metal complexes is reported in Table 3.

*2.6. Biological activity*

2.6.1. Antimalarial activity.

Newly synthesized metal complexes were screened for antimalarial activities. The *in-vitro* antimalarial analysis was performed in 96 well microtitre plates, as stated by Rieckmann and co-workers in the microassay protocol with minor changes. The *in-vitro* assay was performed to determine the susceptibility of *Plasmodium falciparum* to antimalarial metal complexes/compounds. *P. Falciparum* strain cultures were preserved in RPMI 1640 medium supplemented with 0.23% sodium bicarbonate, 10% heat-inactivated human serum, 25 mM HEPES and 1% D-glucose. The parasites of *P. Falciparum* were synchronized by the treatment with 5% D-sorbitol to have only ring stage parasitized cells. DMSO solvent was used to prepare 5 mg/mL as a stock solution of each of the test compounds, and successive dilutions were prepared with a culture medium. The diluted samples containing parasitized cells in 20 µL volume were added to the test wells so as to get concentrations in the range 0.4 µg/mL to 100 µg/mL in duplicate well. At 37°C, in a candle jar, the culture plates were incubated for 36 to 40h. After incubation, thin blood smears from each well were made and then stained with JSB (Jaswant Singh Bhattacharya) staining. The slides were studied microscopically, and the growth of ring-stage parasites into trophozoites and schizonts was noted in the presence of test compounds of different concentrations. The minimum concentration of the test compound,

which inhibits the full growth of parasite into schizonts, was reported as the Minimum Inhibitory Concentration (MIC) [13].

Standard drugs used were Chloroquine and Quinine. The antimalarial activity of synthesized metal complexes is given in Table 4.

2.6.2 Anti-tubercular activity.

The MIC (Minimal Inhibition Concentration) is one of the most well-known methods to assess anti-tubercular activity. It is non-automated *in-vitro* bacterial susceptibility tests carried out in the bottle. MIC is the lowest concentration of the test compound required to inhibit the growth of the microorganisms, while growth is defined as 20 colonies or more. This method is simple and is carried out with a test compound containing slope, whereas it is desirable to use more than one slope. Metal complexes were evaluated against the H37Rv strain (Mycobacterium tuberculosis). L.J. (Lowenstein-Jensen) medium was used as a nutrient medium to grow microbes and to dilute the test sample suspension. Inoculum size 1 mg/mL for test strain was maintained [14].

DMSO was used as diluents to prepare different concentrations of test compounds. A stock solution of 2000 microgram /mL of each synthesized metal complex was prepared.

In primary evaluation, 500 µg /mL, 250 µg /mL, and 125 µg /mL concentrations of the synthesized compounds were taken. In this primary evaluation, the synthesized compounds found active were further tested in secondary evaluation. For secondary evaluation, the synthesized compounds found active in primary evaluation were diluted to get 100 µg /mL, 50 µg /mL, 25 µg /mL, 12.5 µg /mL, 6.250 µg /mL, 3.125 µg /mL and 1.5625 µg /mL concentrations. The least concentration showing at least 99 % inhibition is reported as MIC.

Isoniazid and Rifampicin were used as standard drugs. The MIC values of synthesized compounds were compared with standard drugs, as presented in Table 5.

2.6.3. Antimicrobial activity.

For antimicrobial activity, Broth Dilution Method was applied. It is a non-automated, *in-vitro* microbial susceptibility test. It is performed in tubes. Synthesized metal complexes were analyzed against three fungi *C. Albicans* (MTCC 227), *A. Niger* ( MTCC 282) and *A. Clavatus* ( MTCC 1323), and four bacteria species *E. Coli* (MTCC 443), *P. Aeruginosa* (MTCC 1688), S. Aureus (MTCC 96) and *S. Pyogenus* (MTCC 442). The Nutrient Medium employed to grow microbes and to dilute the test compound suspension for the test microbes was Mueller Hinton Broth.

The size of the inoculum for test strain was fixed to $10^8$ Colony Forming Unit (CFU) /mL by analyzing the turbidity. DMSO solvent was used in the preparation of solutions of metal complexes. DMSO solution (without compound) was used as control.

Broth Dilution Method can be used for the determination of MIC (Minimal Inhibition Concentration). The control tube is subcultured with a medium useful for the growth of the test microorganism and incubated overnight at 37 °C. A stock solution of 2000 µg/mL concentration of each synthesized metal complex was prepared. For primary and secondary evaluation of metal complex, serial dilutions were prepared. 1000 microgram/mL, 500 microgram/mL, and 250 microgram/ml concentrations of the synthesized metal complexes were used in the primary evaluation. The synthesized metal complexes found active in the primary evaluation were taken for secondary evaluation against microorganisms. In secondary evaluation, the metal

complexes which were active in primary evaluation were diluted to get 200 microgram/mL, 100 microgram/mL, 50 microgram/mL, 25 microgram/mL, 12.5 microgram/mL, and 6.25 microgram/mL concentrations.

The Minimal Inhibition Concentration of the control microorganism is recorded to check the reliability of the concentration of the metal complex. The minimum concentration showing at least 99 % inhibition zone is recorded as MIC.

The MIC for newly synthesized metal complexes were compared with standard drugs for fungi (Nystatin and Greseofulvin) and standard drugs for bacteria (Ampicillin, Chloramphenicol, Gentamycin, Ciprofloxacin, and Norfloxacin) as reported in Table 6.

## 3. Results and Discussion

The bis-imine ligand (SB) was prepared by the reaction of 5-chlorosalicyaldehyde with synthesized 1-(2-bromo, 5-methoxy benzylidene) hydrazine. Metal complexes were synthesized from Schiff base bis-imine ligand. Synthesized ligand and metal complexes were characterized by different spectroscopic methods.

**Table 1.** Physical and analytical data of the synthesized compound.

| Sample Code | Compound | M.F. (F.W.) | M. P. (°C) | Color | Elemental analysis (%): Calculated | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | M |
| SB | Ligand(L) | $C_{15}H_{12}N_2O_2BrCl$ (367.5) | 220 | Yellow | 48.97 | 3.26 | 7.61 | - |
| SB1 | $CuL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Cu$ (798.54) | > 300 | Yellowish Brown | 45.08 | 3.00 | 7.01 | 7.95 |
| SB2 | $CdL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Cd$ (847.41) | > 300 | Lemon Yellow | 42.48 | 2.83 | 6.60 | 13.26 |
| SB3 | $ZnL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Zn$ (800.38) | 270 | Yellowish Green | 44.97 | 2.99 | 6.99 | 8.16 |
| SB4 | $FeL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Fe$ (790.84) | > 300 | Light Brown | 45.52 | 3.03 | 7.08 | 7.06 |
| SB5 | $NiL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Ni$ (793.69) | > 300 | Yellow | 45.35 | 3.02 | 7.05 | 7.39 |
| SB6 | $SnL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Sn$ (853.71) | > 300 | Off white | 42.16 | 2.81 | 6.55 | 13.90 |
| SB7 | $CrL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Cr$ (787) | > 300 | Brown | 45.74 | 3.04 | 7.11 | 6.60 |
| SB8 | $ZrL_2$ | $C_{30}H_{24}N_4O_4Br_2Cl_2Zr$ (826.22) | > 300 | Light Yellow | 43.57 | 2.90 | 6.77 | 11.04 |

Metal complexes were screened for antimalarial, anti-tubercular, and antimicrobial activities.

IR spectra of Schiff base bis-imine ligand (SB) showed a strong band at 1616 $cm^{-1}$ corresponding to the stretching frequency of the C=N group.

The IR spectra of metal complexes show stretching frequency bands in the region 395-462 $cm^{-1}$ can be recognized for coordination of metal ion and the nitrogen atom of ligand i.e., M-N bands. The IR stretching frequency bands at 500-827 $cm^{-1}$ were characterized for metal ion, and the oxygen atom of ligand was coordinated (M-O). Frequency bands at 1610-1624 $cm^{-1}$ indicate the C=N group. Frequency bands at 2939-3074 $cm^{-1}$ can be assigned to C-H bonds. The Schiff base ligand bis-imine is coordinated with metal ion through phenolic oxygen and azomethine nitrogen. Thus, the ligand behaves as bidentate.

**Table 2**. Infrared spectra data of metal complexes.

| Sample code | Compound | ν $cm^{-1}$ (M-N) | ν $cm^{-1}$ (M-O) | ν $cm^{-1}$ (C=N) | ν $cm^{-1}$ (C-H) |
|---|---|---|---|---|---|
| SB1 | $CuL_2$ | 447 | 507 | 1610 | 3068 |
| SB2 | $CdL_2$ | 395 | 655 | 1624 | 3072 |
| SB3 | $ZnL_2$ | 450 | 555 | 1618 | 2999 |
| SB4 | $FeL_2$ | 447 | 500 | 1620 | 2974 |
| SB5 | $NiL_2$ | 462 | 691 | 1618 | 3072 |
| SB6 | $SnL_2$ | 459 | 646 | 1618 | 3074 |
| SB7 | $CrL_2$ | 405 | 827 | 1612 | 2939 |
| SB8 | $ZrL_2$ | 457 | 648 | 1618 | 2968 |

In the UV spectra of metal complexes, the wavelength of maximum absorbance ($\lambda_{max}$) ranges from 206.4-230 nm. The $\lambda_{max}$ value of Fe(II) is high at higher absorption. The metal complexes were formed in a ligand to metal ratio of 2:1.

**Table 3**. $\lambda_{max}$ Value of synthesized metal complexes.

| Code no | Compound | Wavelength ($\lambda_{max}$) |
|---|---|---|
| SB1 | $CuL_2$ | 211.5 |
| SB2 | $CdL_2$ | 213.9 |
| SB3 | $ZnL_2$ | 213.9 |
| SB4 | $FeL_2$ | 230 |
| SB5 | $NiL_2$ | 225.1 |
| SB6 | $SnL_2$ | 206.4 |
| SB7 | $CrL_2$ | 225.7 |
| SB8 | $ZrL_2$ | 222.8 |

Antimalarial activity study of the synthesized compounds showed that Zn(II) metal complex was remarkably active while Cd(II), Fe(II), Cr(II), and Zr(II) were moderately active against Plasmodium falciparum as compared to standard drugs Chloroquine and Quinine.

**Table 4.** Antimalarial activity.

| Minimal Inhibition Concentration | | |
|---|---|---|
| **Code no.** | **Compound** | **MEAN IC50 VALUES** |
| SB1 | $CuL_2$ | 2.10 µg/ml |
| SB2 | $CdL_2$ | 0.57 µg/ml |
| SB3 | $ZnL_2$ | 0.39 µg/ml |
| SB4 | $FeL_2$ | 0.65 µg/ml |
| SB5 | $NiL_2$ | 1.65 µg/ml |
| SB6 | $SnL_2$ | 2.21 µg/ml |
| SB7 | $CrL_2$ | 0.64 µg/ml |
| SB8 | $ZrL_2$ | 0.62 µg/ml |
| Standard | Chloroquine | IC50-0.020 µg/ml |
| | Quinine | IC50-0.268 µg/ml |

**Table 5.** Anti-tubercular activity.

| **Method** | **L. J. Medium [Conventional method]** | |
|---|---|---|
| Bacteria | H37Rv | |
| Standard Drug | Isoniazid and Rifampicin | |
| Code no. | Compound | MIC µg/ml |
| SB1 | $CuL_2$ | 250 |
| SB2 | $CdL_2$ | 62.5 |
| SB3 | $ZnL_2$ | 50 |
| SB4 | $FeL_2$ | 100 |
| SB5 | $NiL_2$ | 125 |
| SB6 | $SnL_2$ | 250 |
| SB7 | $CrL_2$ | 50 |
| SB8 | $ZrL_2$ | 500 |
| Standard | Isoniazid | 0.20 µg/ml |
| | | 99% inhibition |
| | Rifampicin | 40 µg/ml |
| | | 99% inhibition |

Synthesized metal complexes were screened for anti-tubercular activity by using L.J. medium (conventional method). Cd(II) Zn(II) and Cr(II) metal complexes showed activity against mycobacterium tubercular as compared to standard drugs isoniazid and Rifampicin.

The antimicrobial activity studies of synthesized metal complexes reveal that Cu(II), Fe(II), Ni(II), Sn(II), Cr(II), and Zr(II) showed excellent activity against fungus *C. Albicans* (MTCC 227). Cu(II), Ni(II), and Cr(II) were moderately active against *A. Niger* ( MTCC 282). Cr(II) and Zr(II) were active against *A. Clavatus* ( MTCC 1323) fungal species as compared to standard drug Griseofulvin.

Cu(II), Zn(II), Fe(II), Sn(II), and Zr(II) metal complexes showed excellent activity against bacteria *E. Coli* (MTCC 443). Sn(II), Zn(II), and Fe(II) showed remarkable activity against *P. Aeruginosa* (MTCC 1688). Cu(II), Zn(II), Ni(II), Sn(II), Cr(II), and Zr(II) showed moderate to excellent activity against *S. Aureus* (MTCC 96). Cu(II), Ni(II), and Zr(II) showed excellent activity against *S. Pyogenus* (MTCC 442) bacteria species as compared to standard drug Ampicillin and Chloramphenicol.

**Table 6.** Antimicrobial activity of the synthesized metal complexes.

| **Sample code** | **Compound** | **Antifungal Activity** Minimal Inhibition Concentration | | | **Antibacterial Activity** Minimal Inhibition Concentration | | | |
|---|---|---|---|---|---|---|---|---|
| | | C. A. MTCC 227 | A. N. MTCC 282 | A. C. MTCC 1323 | E. C. MTCC 443 | P. A. MTCC 1688 | S. A. MTCC 96 | S. P. MTCC 442 |
| SB1 | $CuL_2$ | 250 | 500 | >1000 | 100 | 250 | 250 | 100 |
| SB2 | $CdL_2$ | 1000 | 1000 | >1000 | 250 | 500 | 500 | 250 |
| SB3 | $ZnL_2$ | 1000 | >1000 | 1000 | 50 | 62.5 | 250 | 500 |
| SB4 | $FeL_2$ | 500 | 1000 | 1000 | 100 | 62.5 | 500 | 250 |
| SB5 | $NiL_2$ | 500 | 500 | 1000 | 250 | 500 | 50 | 100 |
| SB6 | $SnL_2$ | 500 | 1000 | 1000 | 100 | 50 | 250 | 125 |
| SB7 | $CrL_2$ | 250 | 500 | 500 | 125 | 250 | 100 | 125 |
| SB8 | $ZrL_2$ | 500 | 1000 | 500 | 62.5 | 100 | 250 | 25 |
| Standard Drug | Greseofulvin | 500 | 100 | 100 | - | - | - | - |
| | Nystatin | 100 | 100 | 100 | - | - | - | - |
| Standard Drug | Ampicillin | - | - | - | 100 | - | 250 | 100 |
| | Chloramphenicol | - | - | - | 50 | 50 | 50 | 50 |
| | Gentamycin | - | - | - | 0.05 | 1 | 0.25 | 0.5 |
| | Ciprofloxacin | - | - | - | 25 | 25 | 50 | 50 |
| | Norfloxacin | - | - | - | 10 | 10 | 10 | 10 |

*C. A. = C. Albicans, A. N. = A. niger, A. C. = A. clavatus, E. C. = E. coli, P. A. = P. aeruginosa, S. A. = S. aureus, S. P. = S. Pyogenus*

## 4. Conclusions

In the present work, a new bidentate Schiff base bis-imine ligand was synthesized with 1-(2-bromo, 5-methoxy benzylidene) hydrazine and 5-chlorosalicylaldehyde. The bis-imine ligand was used for the synthesis of metal complexes with metals Cu(II), Cd(II), Zn(II), Fe(II), Ni(II), Sn(II), Cr(II), and Zr(II). The synthesized ligand and metal complexes were characterized by different spectroscopic methods. The synthesized metal complexes were analyzed for antimalarial, anti-tubercular, and antimicrobial activities. The metal complexes Zn(II), Cd(II), Fe(II), Cr(II), and Zr(II) were active against malaria as compared to standard drug chloroquine and quinine. Metal complexes Cd(II), Zn(II), and Cr(II) showed activity against M.TB as compared to standard drug isoniazid and Rifampicin. Metal complexes Cu(II), Fe(II), Sn(II), Ni(II), Cr(II), and Zr(II) showed moderate to excellent activity against fungal species as compared to standard drug Griseofulvin. Cu(II), Zn(II), Fe(II), Sn(II), Ni(II), Cr(II), and Zr(II) metal complexes exhibited activity against bacteria species as compared with standard drug Ampicillin and Chloramphenicol.

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## Conflicts of Interest

The authors declare no conflict of interest.

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