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**RESEARCH ARTICLE**

# ANTIMICROBIAL ACTIVITY OF HYDROCHLOROTHIAZIDE SCHIFF BASE AND THEIR METAL ION COMPLEXES.

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## Abstract

The synthesis of four metal complexes of hydrochlorothiazide Schiff base is reported. The compounds were obtained by the condensation of hydrochlorothiazide and salicylaldehyde, followed by the synthesis of their Cd(II), Ni(II), Zn(II) and Co(II) metal complexes. Structural characterization was done using $^{1}H$ and $^{13}C$ NMR, MASS, IR, UV-Vis spectroscopy and elemental analysis. Prepared compounds were screened for their antimicrobial activity. Obtained results were compared with those of parent drug hydrochlorothiazide. The increased antimicrobial potency of all compounds were recorded; excellent activity was reported for Co(II) and Cd(II) complexes.

## Introduction:-

Diuretic activity is a function by which excess water and toxic elements are excreted from the cell and circulatory system, the drugs which induces this activity is called as diuretic drugs [1-4]. Many diuretic drugs are available in the market with strong activity. However, serious side effects like hyperuricemia, uridosis, gastric irritation and high level of blood sugar is associated with them [5-7]. Hydrochlorothiazide is diuretic thiazide drugs have been well studied for their bioactivity as antibiotics and tumor metastasis inhibitors [8]-[9]. This drug is used to treat hypertension, originally marketed as Hygroton in the USA. Compared with other medications of the thiazide class, hydrochlorothiazide has the longest duration of action, but a similar diuretic effect at maximal therapeutic doses [10].

It is often used in the management of hypertension and edema. Metal ions also play an essential role in biological system. Transition metal complexes have attracted attention in exploring their role in such antimicrobial activities [11,12]. Various sulfonamide and thiazide Schiff bases have also been extensively investigated because of their bioactivity. There is enormous interest presently in the field of coordination chemistry of 3dtransition metal ion with Schiff bases. Metal complexes of Schiff bases have occupied a major role in the development of coordination chemistry [13].

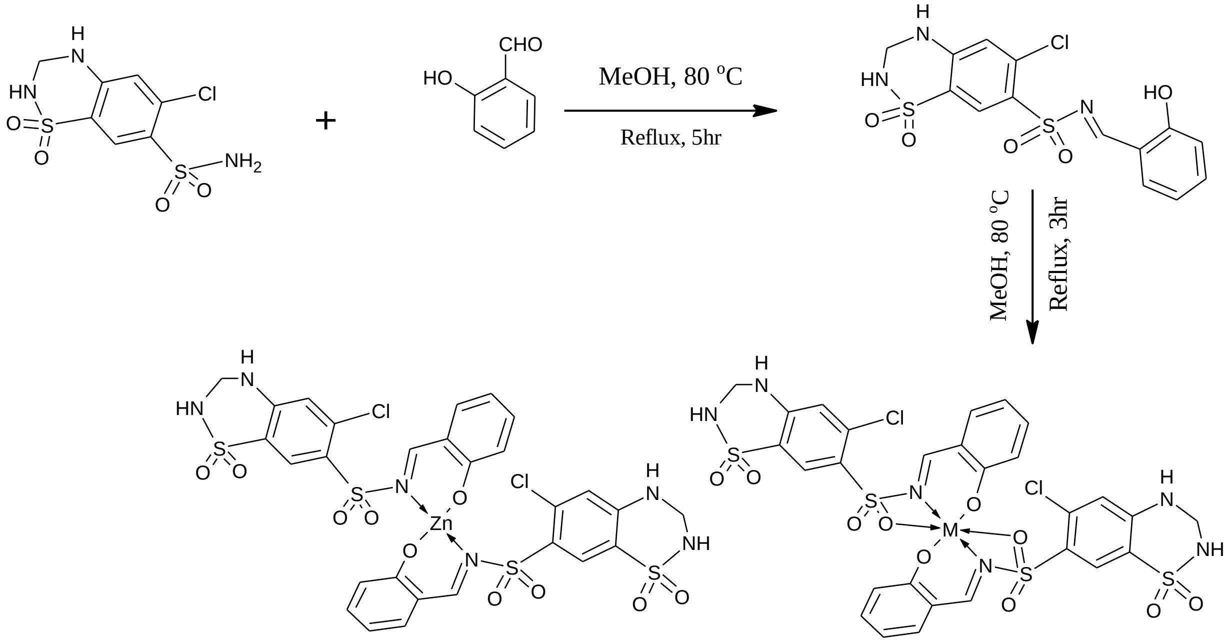
Study of metal complexes has been of great importance, metal ions play a vital role in the biological activity and certain metal complexes of the drug are more potent than their parent drug. Schiff base metal chelates are widely applicable because of their industrial and biological importance and hence have well been studied in the past era. A detailed survey of literature revealed that very little work has been done on metal complexes of diuretic drugs. We herein continue our previous work report the synthesis, characterization and antimicrobial activity of Cd(II), Ni(II), Zn(II) and Co(II) metal complexes of hydrochlorothiazide Schiff base.

## Materials and methods:-

All chemicals used were of analytical grade. Diuretic drug Hydrochlorothiazide was purchased from IPCA laboratories, Ratlam (M.P.), which was recrystallized and analyzed for percentage purity using HPLC. Metal salts used were purchased from Merck chemicals and recrystallized using methanol. Methanol was purchased from Merck chemicals and redistilled using magnesium turnings; anhydrous methanol was collected in a dark colored glass bottle.

### General procedure for the synthesis of hydrochlorothiazide Schiff base using salicylaldehydeand their transition metal complexes:-

Schiff base ligand was prepared according to **Scheme 1**. An equimolar methanolic solution of hydrochlorothiazide and salicylaldehyde were mixed in a 250 ml conical flask and transferred to a round bottom flask with constant stirring. The solution was refluxed for 5hr using water bath. As the reaction completed solution was cooled at room temperature.



where, M= Co(II), Cd(II) and Ni(II)

**Scheme1.**Synthesis of Schiff base ligand from 2.1.1. 6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, (ligand) and their metal complexes.

Solvent was removed under reduced pressure, yellowish brown crystals were separated. Obtained crystals were washed thoroughly with ethanol, afforded TLC pure products in good yield [14,15]. The transition metal complexes have been synthesized by refluxing methanolic solution of ligand with metal salts (M:L, 2:1)for 3 hour, purification was followed by the same procedure reported for the ligand (**Scheme 1**).

6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCSligand **(1)**

Yellow crystals, Yield: 81% (3.5 g); mp: 125 $^{o}$C; $\lambda_{max}$: 380 nm (2.07); IR (KBr, cm$^{-1}$): 3093 (NH, pyrollidine), 1604 (HC=N imine), 1716 (C-Cl), 1427 (C-N, pyrollidine), 1556, 1300, 3361 (C-OH), 1604 (C=O pyrollidine), 860 (S-N), 1024 (S=O); $^{1}$H NMR (DMSO-d$_6$, 400 MHz, ppm): 11.10 (aldehydic -OH), 7.96, 8.01 (NH benzothiadiazine), 8.59 (CH=N imine), 6.92-7.33 (C-H benzene ring); $^{13}$C NMR (DMSO-d$_6$, ppm):142.4 (C25), 155.61 (C23 imine), 167.33 (C-OH aldehydic), 121.09 (C-Cl), 71.22 (N-C-N), 111.37 (C-S); Anal. Calcd. For: C14H12ClN3O5S2

(401.84): C (41.84), H (3.01), Cl (8.82), N (10.46), O (19.91), S (15.96).

6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCSZn(II) complex**(2)**

Yellow crystal; Yield: 78% (2.00 g); mp: 95$^{o}$C; UV-Vis: $\lambda_{max}$ 410 nm (1.78); IR (KBr,cm$^{-1}$): 2833 (NH, pyrollidine), 1566 (HC=N, imine), 1717 (C-Cl), 1444 (C-N, pyrollidine), 1454-1556 (C=O pyrollidine), 908 (S-N), 1300 (M-O), 584 (M-N) ; $^{1}$H NMR (DMSO-d$_6$, 400 MHz, ppm): 7.96, 8.01 (NH, benzothiadiazine), 8.10 (CH=N, imine), 6.92-7.65 (C-H, benzene ring); $^{13}$C NMR (DMSO-d$_6$, ppm):147.6 (C25), 154.53 (C-imine), 131.60 (C-Cl), 55.89 (N-C-N), 124.83 (C-S); Anal. Calcd. : C28H22Cl2N6ZnO10S4 (867.08): C (38.79%), H (2.56%), Cl (8.18%), N (9.69%), O (18.45%), S (14.79%), Zn (7.54%).

6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCSNi(II) complex**(3)**

Yellowish green powder; Yield: 68% (1.85 g); mp : 101$^{o}$C; $\lambda_{max}$ 460 nm (0.92); IR (KBr, cm$^{-1}$): 2845 (NH, pyrollidine), 1531 (HC=N, imine), 1715 (C-Cl), 1440 (C-N, pyrollidine), 1454-1556 (C=O pyrollidine), 907 (S-N), 1304 (M-O), 575 (M-N) ; $^{1}$H NMR (DMSO-d$_6$, 400 MHz, ppm): 7.87, 8.00 (NH, benzothiadiazine), 8.20 (CH=N, imine), 6.77-7.50 (C-H, benzene ring); $^{13}$C NMR (DMSO-d$_6$, ppm):145.22 (C25), 149.14 (C-imine), 134.47 (C-Cl), 58.53 (N-C-N), 124.11 (C-S); Anal. Calcd. : C28H22Cl2N6NiO10S4 (860.36): C (39.09%), H (2.58%), Cl (8.24%), N (9.77%), O (18.60%), S (14.91%), Ni (6.82%).

6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCSCd(II) complex**(4)**

Light green powder; Yield: 95% (3.01 g); mp: 140 $^{o}$C; $\lambda_{max}$: 610 nm(1.96); IR (KBr, cm-1): 2830 (NH, pyrollidine), 1565 (HC=N, imine), 1716 (C-Cl), 1441 (C-N, pyrollidine), 1454-1556 (C=O pyrollidine), 905 (S-N), 1307 (M-O), 543 (M-N) ; $^{1}$H NMR (DMSO-d$_6$, 400 MHz, ppm): 7.90, 8.08 (NH, benzothiadiazine), 8.25 (CH=N, imine), 6.80-7.65 (C-H, benzene ring); $^{13}$C NMR (DMSO-d$_6$, ppm):146.6 (C25), 154.5 (C-imine), 125.9 (C-Cl), 115.3 (N-C-N), 70.4 (C-S); Anal. Calctd. : C28H22Cl2N6CdO10S4 (914.08): C (36.79%), H (2.43%), Cl (7.76%), N (9.19%), O (17.50%), S (14.03%), Cd (12.30%).

6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, HCSCo(II) complex**(5)**

Light green powder; Yield: 70% (2.07 g); mp: 196 $^{o}$C; $\lambda_{max}$ 390 nm(2.94); IR (KBr, cm-1): 2830 (NH, pyrollidine), 1557 (HC=N, imine), 1700 (C-Cl), 1438 (C-N, pyrollidine), 1440-1551 (C=O pyrollidine), 900 (S-N), 1310 (M-O), 580 (M-N) ; $^{1}$H NMR (DMSO-d$_6$, 400 MHz, ppm): 8.01, 8.10 (NH, benzothiadiazine), 8.25 (CH=N, imine), 6.90-7.69 (C-H, benzene ring); $^{13}$C NMR (DMSO-d$_6$, ppm):141.6 (C25), 154.53 (C-imine), 134.40 (C-Cl), 55.77 (N-C-N), 123.63 (C-S); Anal. Calcd. : C28H22Cl2N6CoO10S4 (860.60): C (39.08%), H (2.58%), Cl (8.24%), N (9.77%), O (18.59%), S (14.90%), Co (6.85%).

**In-vitro biological activity:-**

Antibacterial activity

In this research work the antibacterial activity of Schiff base ligand, and their metal(II) complexes were studied against four E.coli, S.flexenari, S.pyrugenes, B.subtilisusing agar-well diffusion method, according to the literature protocol [16]. Obtained results were compared with those of standard drug trimethoprim. Bacterial culture was incubated for 24 hr into nutrient broth. By using a sterilized cork borer (7 mm diameter), wells were then dug in the culture plates. Test compounds dissolved in DMSO were added (0.2 µl) to these wells and left for 2 hr at 4 $^{o}$C. Culture plates were incubated at 30 $^{o}$C for 18–24 hr. Inhibition zones formed on the medium were measured as millimeters (mm) diameter [17].

Antifungal activity

Schiff base and their metal(II) complexes were studied for their activity against T. longifusus, C. albican, A. flavus and C.glabrata fungal strains according to procedure reported elsewhere [18,19]. Obtained results were compared with those of standard drug miconazole and recorded in Table 3 and 4. All the compounds were dissolved in DMSO, fungi were cultivated in sabouraud dextrose agar (Merck). Tested samples were applied to the culture plates and incubated at 36 oC for 48 hr. At the end of the incubation period, minimum inhibition concentration (MIC) was recorded as the lowest concentrations of the substances that gave no visible turbidity.

## Results and discussion:-

The Schiff base ligand and its Cd(II), Ni(II),Co(II) and Zn(II), complexes were synthesized and characterized by spectroscopic and elemental analysis techniques. The complexes were found to be air stable. The ligand and metal complexes were soluble only in $CH_3OH$ and DMSO at room temperature. The composition of ligands was consistent with their mass spectral, nuclear resonance and IR data.

### Spectroscopic characterization of ligand and their metal complexes:-

### $^{1}$H NMR spectra:-

The $^{1}$H NMR spectrum of Schiff base ligand and their metal(II) complexes in duterated DMSO exhibited signals consistent with the proposed structure. A singlet of hydroxyl proton H-25of ligand was appeared at 11.10 ppm, whereas doublet of H-21 and H-24 proton was observed at 6.92 and 7.24 ppm respectively, triplet was observed between 7.14-7.15 ppm for H-23 proton. The H-2 and H-4 proton from NH moiety was observed at 7.96 and 8.01ppm respectively. The H-7 and H-10 proton appeared at 7.04 and 8.17ppm respectively. The H-3 proton was shifted lower downfield at 4.68ppm (fig.1 and 2), similarly a doublet of aromatic H-28 proton was appeared at 6.92 ppm. Singlet of H-45, H-43. H-7, H-10 and H-19 were observed at 7.96, 8.01, 8.17, 7.09 and 8.10 ppm.

Other aromatic proton can be seen in Cd(II) and Co(II) complexes between 7.03-7.65 ppm. The aromatic proton was observed between 7.14-7.65 ppm in Zn(II) and Ni(II) complexes, a singlet was observed at 7.03 ppm for H-27 proton. The N=CH proton was shifted to 8.10 ppm, H-19 can be seen at 7.64 ppm as doublet.

On complex formation, imine proton (–CH=N) shifted to less downfield at 8.10, 8.20 8.25 and 8.25 ppm correspond to Zn(II), Ni(II), Co(II) and Cd(II) complexes (fig.2). Aromatic proton (–CH) in metal complexes was.

### $^{13}$C NMR spectra:-

Schiff base ligand and their complexes were analyzed for $^{13}$C NMR in Bruker’s 400 MHz NMR using DMSO-d6 as solvent. The $^{13}$C NMR spectral information is reported along with their possible assignments in the experimental section and all the carbons were found in the expected region. The spectra of Cd(II) and Co(II) complexes displayed the imine (CH=N) carbon at 154.5 ppm, benzothiadiazine C41 and C38 at 55.89 and 118.31 ppm as singlet, while C45 as doublet at 124.14ppm. The aldehydic carbon (C-OH) shift at 112.5 and 129.8 ppm due to the presence of electronegative (-OH) group. The carbon in C-Cl, C-S and C-N-C bond were assigned to the shift observed at 125.9, 70.4 and 115.3 ppm respectively.

The spectra of Cd(II) and Co(II) complexes displayed the imine (CH=N) carbon at 154.5 ppm, benzothiadiazine C41 and C38 at 55.89 and 118.31 ppm as singlet, while C45 as doublet at 124.14ppm. The spectra of Cd(II), Zn(II), Ni(II) and Co(II) complexes exhibited downfield shifting indicating the coordination of azomethine nitrogen to the metal ion. The C-Cl bond was recognized by the shift observed at 134.75 ppm for Cd(II) and Co(II) complexes while in Ni(II) complex it was shifted to more downfield at 134.47 ppm. Similar spectra was observed in Zn(II) complex at 131.60 ppm. The N-C-N, carbon was confirmed between 53.12-55.89 ppm in metal complexes. All other shifts of the metal (II) complexes underwent downfield shifting by 0.24–0.8 ppm due to the increased conjugation and coordination with the metal.

### IR spectral studies:-

The characteristic IR spectra of ligand and their metal(II) complexes possessed potential donor sites azomethine linkage, benzothiadiazine secondary amine (NH-C-NH), sulphonamide (C-S=O)

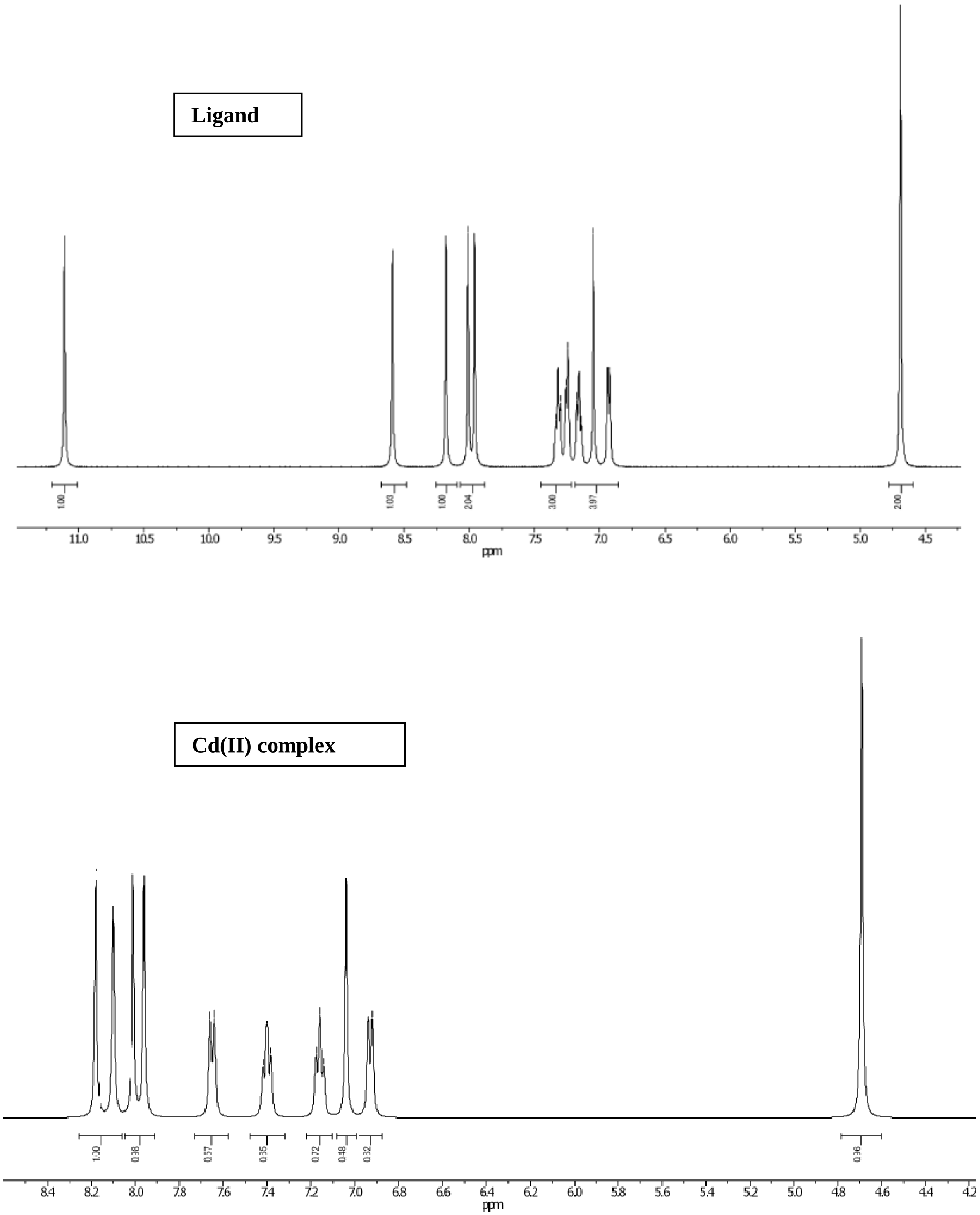


**Fig. 2.**$^1$H NMR chemical shift ofligand and Cd(II) complex.

andaldehydic hydroxyl group (Ar-OH) which have tendency to coordinate with the metal ions. The IR spectra of ligand was observed in the range2833-2936 and 2568 cm$^{-1}$ correspond to sulphonamide amino (-$NH_2$) and N-H (benzothiadiazine) vibration respectively. Anew sharp band appeared at 1607 cm$^{-1}$ assigned to the azomethine linkage (C=N), another strong peak was observed

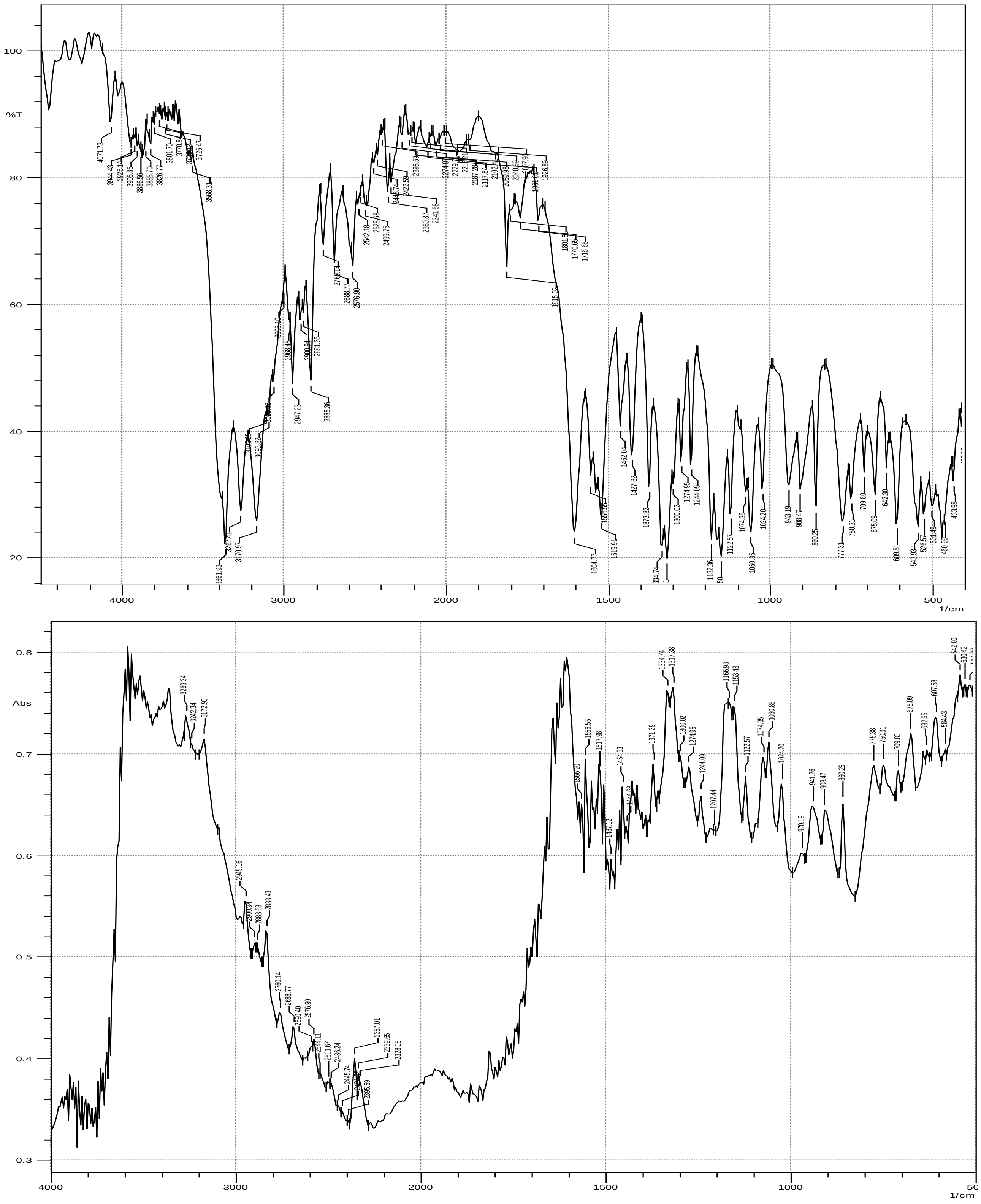


Fig 4. IR spectra of ligand and their Zn(II) metal ion complex

at 1837 cm$^{-1}$ due to vibration of C-Cl bond. The hydroxyl (O-H) bond of benzene moiety appeared as broad peak at 3040-3373 cm$^{-1}$ (Table 1, fig. 4).

A new band appeared between 420-590 cm$^{-1}$ in all metal complexes, due to M–N vibration indicating the coordination of imine nitrogen atom with the metal ions. The disappearance of one O-H proton and appearance of new band at 2850 and 2940 cm$^{-1}$ confirms deprotonation and coordination of hydroxyl group (O-H) to the metal atom. It also indicates coordination of sulphonamide moiety to the central metal ion. In case of Zn(II) complex the S=O bond was observed at higher region shows participation of sulphonamide moiety in complex formation. All other bands remain unchanged in the spectra of all ligands and their corresponding metal complexes

**Table 1:-** IR vibrational spectra of ligand and complexes.

| **Compounds** | **IR frequencies v (cm$^{-1}$)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **C=N** | **$NH_2$** | **M-N** | **M-O** | **Ar-OH** | **Ar-H** | **C-NH-C** | **C=O** |
| **Ligand** | 1604 | 3120 | - | - | 3361 | 1273 | 3120 | 1693 |
| **2** | 1566 | - | 584 | 1300 | 2358 | 1140 | 2831 | 1556 |
| **3** | 1531 | - | 575 | 1304 | 2400 | 938 | 2850 | 1560 |
| **4** | 1565 | - | 543 | 1307 | 2521 | 996 | 3045 | 1570 |
| **5** | 1557 | - | 580 | 1310 | 2386 | 1250 | 3029 | 1551 |

**Mass spectra:-**

The mass spectral data and fragmentation pattern of Schiff base ligand and their metal complexes justifies the formation of the proposed structures and their bonding pattern. The spectra of ligand showed molecular ion peak m/z 401 (Calcd.401.84) of [C14H12ClN3O5S2]. Its base peak [C14H11ClN3O5S2]$^+$, was observed at m/z 400.74. The molecular ion peak of Cd(II), Co(II), Zn(II) and Ni(II) complex was observed at m/z; 914, 860, 867 and 860 respectively. The first fragmentation pattern followed the cleavage of O-H, C-Cl bonds confirming the proposed structure of ligand and metal complexes.

**Biological activity:-**

**In vitro antibacterial study:-**

The hydrochlorothiazide Schiff base and its complexes were studied for their antibacterial activity against four bacterial strainsE.coli, S.flexenari, S.pyrogenesand B.subtilisusing disk diffusion method. All the compounds exhibited varied degree of inhibitory effects on the growth of selected bacterial strains (Fig. 5). Data showed that ligand is inactive against B.subtiliswhereas excellent activity was observed against S.pyrugenes and S.flexenari. Amongst all synthesized compounds Zn(II) complex show good activity against E.coli while poor to moderate activity was recorded for other strains. The excellent activity of Ni(II) complex was recorded against B.subtilis and S.flexenari, whereas no activity was recorded against E.coli, poor activity was recorded against S.pyrugenesto those of standard drug trimethoprim. The Cd(II) complex showed less activity against S.flexenari whereas excellent activity was recorded against B.subtilis and S.pyrogenes. The Co(II) complex showed no activity against B.subtilisand E.coli, whereas moderate activity was observed against S.flexenari and S.pyrogenes. The results can be compared with the obtain MIC values recorded in Table 2 and Table 3.magnetic flux density B or magnetic field strength symbolized as $\mu_0H$. Use the center dot to separate compound units, e.g., “A$\cdot$m$^2$.”

**Table 2.** Zone of inhibition of ligand and its complexes against selected bacterial strains.

| Compounds | Zone of inhibition (in 10 mm) | | | |
|---|---|---|---|---|
| | Ecoli | S.flexenari | S.pyrogenes | B.subtilis |
| **Ligand** | 5 | 7 | 10 | - |
| **2** | 10 | 5 | 6 | 4 |
| **3** | - | 8 | 1 | 9 |
| **4** | 5 | 3 | 10 | 9 |
| **5** | - | 7 | 5 | - |
| Trimethoprim | 9 | 8 | 10 | 7 |

**Table 3:-** Minimum inhibitory concentration (MIC) of Schiff base and their metal complexes against selected bacterial culture.

| Compounds | Minimum inhibitory concentration (μg/ml) | | | |
|---|---|---|---|---|
| | E.coli | S.flexenari | S.pyrogenes | B.subtilis |
| **Ligand** | 500 | 290 | 310 | - |
| **2** | 150 | 600 | 810 | 650 |
| **3** | - | 375 | 780 | 535 |
| **4** | 425 | 540 | 110 | 200 |
| **5** | - | 510 | 370 | - |
| Trimethoprim | 150 | 200 | 300 | 600 |

**In vitro antifungal study:-**

In this research work Co(II), Ni(II), Cd(II) and Zn(II) metal complexes of ligand have been prepared and their antifungal activities were evaluated against two fungal species F.solony and C.glabrata. The minimum inhibitory concentration (MIC) of tested compounds recorded are shown in Tables 4 and 5. It is found that ligand possess moderate activity against F.solony and C.glabrata; whereas, Zn(II) and Co(II) complexes have no activity against C.glabrata. The result showed that Cd(II) complex possess maximum activity against both C.glabrata and F.solony. The Co(II) and Cd(II) complexes showed excellent activity against F.solony. The Ni(II) complex was found inactive against F.solony, whereas poor activity was observed against C.glabrata. The results were supported by the minimum inhibitory concentration (MIC) obtained by the experiments (Table 4 and 5).

**Table4:-** Zone of inhibition against selected fungal culture.

| Compounds | Zone of inhibition (in 30 mm) | | | |
|---|---|---|---|---|
| | F.solony | C.glabrata | | |
| **Ligand** | 10 | 15 | | |
| **2** | - | - | | |
| **3** | - | 10 | | |
| **4** | 30 | 30 | | |
| **5** | 22 | - | | |
| **Miconazole** | 28 | 26 | | |

**Table 5.** Minimum inhibitory concentration (MIC) against fungal culture

| Compounds | Minimum inhibitory concentration (μg/ml ) | | | |
|---|---|---|---|---|
| | F.solony | C.glabrata | | |
| **Ligand** | 670 | 540 | | |
| **2** | - | - | | |
| **3** | - | 750 | | |
| **4** | 80 | 100 | | |
| **5** | 220 | - | | |
| **Miconazole** | 300 | 350 | | |

## Conclusion:-

The hydrochlorothiazide Schiff base and their metal complexes were synthesized; structural characterization confirms their geometry and composition. The Zn(II) complex has tetrahedral geometry, whereas Ni(II), Cd(II) and Co(II) complexes have octahedral geometry. The antimicrobial activity of all compounds were found encouraging; All the synthesized compounds show moderate to good activity against selected fungal and bacterial strain. Results are encouraging compared to our previous research with the use of Schiff base as ligand. More study on these compounds may lead as good antimicrobial agents.

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## Abbreviations:-

**HCS-** Schiff base of hydrochlorothiazide with salicylaldehyde6-chloro-N-[(E)-(2-hydroxyphenyl)methylidene]-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; **HCSZn**, **HCSNi**, **HCSCd**, **HCS**Co-Schiff base Zn(II), Ni(II), Cd(II) and Co(II) complex; **TMP-** Trimethoprim.

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