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## Design and Synthesis of Heterocyclic Salicyloyl Hydrazone and its Zn(II) complex: Spectro-chemical Characterization, XRD, Thermal, Antibacterial, and Antifungal study

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### Abstract

New hydrazone ligand of 2-hydroxybenzylidene-4-oxopiperidine-1-carbohydrazone (<sup>2</sup>HL) was designed and synthesized by the condensation reaction of 4-oxo-1-carboxopiperidone hydrazide with 2-hydroxy benzaldehyde. This ligand was reacts with Zn(II) chloride salts to obtained [Zn(L)(H<sub>2</sub>O)]complex. The ligand was characterized by elemental and spectral techniques such as Infrared, UV-vis, <sup>1</sup>H and <sup>13</sup>C NMR, mass) and supporting analysis molar conductance, magnetic susceptibility, powder XRD, TG analyses also performed for the confirmation of structure. The ligand and metal stoichiometric ratio found as 1:1 (M:L) in the complex. The ligand behaves as a dibasic tridentate, coordinating through the O<sup>phenolate</sup>, N<sup>azomethine</sup>, O<sup>enolate</sup> donor functionality towards Zn(II) complex. The spectral, thermo-analytical data displays tetrahedral geometry for all complexes. X-ray diffraction study confirms nanocrystalline nature of ligand and its metal complexes. The kinetic parameters were evaluated by Coats- Redfern method indicated complex shows higher degree of thermal stability. The newly synthesized (<sup>2</sup>HL) ligand and Zn(II) complex were biologically screened for its antibacterial efficiency against gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*), gram-positive bacteria (*Staphylococcus aureus* and *Bacillus substillus*) and the fungal efficiency against pathogens (*Candida albicans* and *Aspergillus niger*) and obtained data show that metal complexes are more competent than the parent ligand against bacterial species.

**KEYWORD-** Salicyloyl hydrazone, XRD, Antifungal and antimicrobial activities, Thermal properties.

### I. INTRODUCTION

Recently, the metal complexes involving active sensitive moieties have been greatly attracted by researchers. In view of their applicability in various fields, coordination compounds take take forefront position in the development of coordination chemistry and more are frequently applied for the betterment of human welfare. The metal complexes derived from hydrazone Schiff bases with triatomic (>C=N-N<) linkage reported for their extensive applicability for chelation of transitional metal ion used in the therapeutic chelation. The several other novel hydrazones and their metal complexes exhibits broad spectrum of antimicrobial activity comparable to azithromycin, ofloxacin and fluconazole as compared to positive controls [1-3].

The coordination compounds are based on the diversified structure, functional property and flexibility in the donor interaction behaviours of the ligand with the metal and vice versa. Especially, the transitional metal complexes have been extensively studied and applied due to the synthetic flexibility, multiple selectivity and sensitivity toward the central metal ions. For the many years metal complexes have gained the considerable interest due to the structural variety physiological responses [4-5].

The metal-ligand bond have been played an important role in the field of the coordination chemistry and their complexes have been shown its diversity in structures depending on the metal ions, its coordination number and the ligation. The electronegative atoms in the ligands such as nitrogen and oxygen atoms leading to forms the complexes. The metal complexation formation, reactivity, stability, physicochemical property. The complex compounds formation and reactivity is due to possessing the metal's primary and secondary valency. The architectural beauty of coordination compounds is due to the stimulating ligand systems which contains flexible donor sites such as (ONO) group which favors the strong coordination bond with metal ion which find the enormous applications. The Schiff bases and their derivatives are very important compounds in medicinal chemistry due to their reported biological activity and for the treatment of several diseases, like Alzheimer's, cancer, inflammation, and leishmaniasis. Recently, researcher significantly attracted towards the designing and synthetic strategy for the more effective bioactive, multifunctional Schiff bases for the DNA and protein binding to metal through the strong coordination bond [6-7].

The variety of technologies have been developed for assembling a metal-chelate-bimolecular complex which serve as platforms for the development of the imaging agent and metal-organic compounds, dissolved in super critical fluid (SCFs or mixtures of SCFs and organic solvents, can be deposited onto the surfaces of substrates or be infused into bulk materials to make either conventional thin metal/metal oxide films or novel hybrid (composite) coatings and materials. Now a days, the

coordination chemistry has found a great importance in many types of chemical work. It is useful to explain various phenomena and form base of various industries like leather tanning, dyeing of cloths, plant growth regulation [8-9].

In this research paper, the interest has grown up due to the attractive structural beauty, biological, thermal stability, and the good absorption phenomenon. We have synthesised and characterised of heterocyclic aroylhydrazone and their metal complexes has come from their wide applications in various fields. The easily synthetic method, good solubility in common solvents, non-toxicity, more stability at room temperature, electronic properties, thermo-kinetic and wide scope of application and justifiable steric nature has been created research interest in this area. These type of hydrazones and transition metal complexes displayed the wide applications in biology, medicine, optics, catalysis and analytical chemistry, transporting agents in organic layer photoconductors, catalysts, luminescent probes and molecular sensors etc. The hydrazones is the member of Schiff base family, biologically active drug molecules which contains triatomic linkage ( $>C=N-N=C<$ ) and lone pair on trigonally hybridized nitrogen atom of the azomethine group with flexible coordinating behaviour towards '3d' transition metals, have been stimulated research in recent years further enhance in pharmacological properties.

In spite of this, no claim have been made that the conclusion in regard with the Zn(II) complex structures and stereochemistry are unequivocal and final.

## II. EXPERIMENTAL

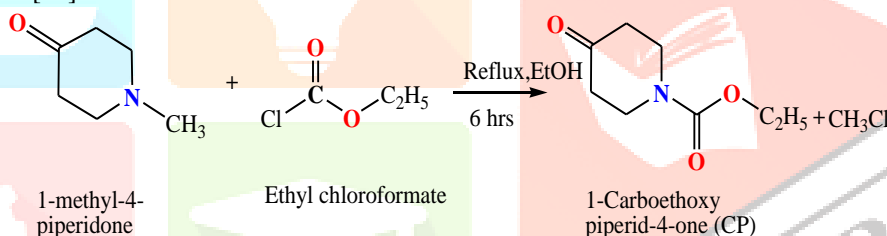
### 2.1 Material and Method

All chemicals were of AR- grade used without purification. The solvents were purified by standard methods. The completion of reaction while synthesizing compounds was routinely monitored by TLC Merck's sheets.

Infrared spectra of ligand and Zn(II) complex was recorded on adv.Brucker spectrometer. The magnetic properties were performed out by the Sherwood balance MK-1. PMR and  $^{13}C$  spectra of ligand were measured in DMSO adv.Brucker NMR spectrometer and ESI-mass spectrum of ligand was measured on Waters Micro mass Q-T. The UV-visible electronic spectra of the complexes were recorded on a Shimadzu spectrophotometer. The thermal curve ligand and its metal complexes were carried out on TGA-4000 analyzer Perkin-Elmer balance having capacity of digital resolution 0.2 $\mu$ g. XRD pattern analysis were carried on Rigaku, (600) having (10-80 $^\circ$ ). The molar conductivities of compounds was performed by using 180-Elico conductivity meter.

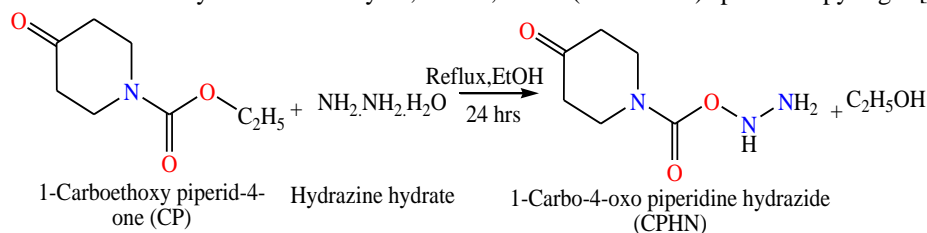
### 2.2 Synthesis of 1-carboethoxy piperid-4-one (CP):

To the round flask, 1-methyl-piperid-4-one (2.067gram, 0.02 mol) was added drop wise to the hot solution of chloroethylformate (2.485gm, 0.02mol) in 50ml dry ethanol. The reaction mixture was continuous stirred for a 1 h and then refluxed for 6 hrs. The reaction was continuously monitored by TLC (70:30 mixtures of n-hexane and ethyl acetate). The solvent ethanol was evaporated in rota vapour. The product of 1-carboethoxy piperid-4-one was collected. Yield:4.146ml (85.50%). The color of liquid was yellow with pleasant smell. B.P. 95-98 $^\circ$ c. The miscibility in common organic solvent,  $p^H$  4.6, slightly miscible in water, density 1.135 gm/ml at 25 $^\circ$ C Scheme 1 [10].



### 2.3 Synthesis of 4-oxo-1-carbo piperidone hydrazone (CPHN):

To a stirred hot ethanolic solution (25mL) 1-carboethoxypiperid-4-one (3.88gram, 0.020 mol) was added hydrazine hydrate (1.0302gm, 0.020 mol). The reaction mixture was refluxed for 22-24 hours. The reaction progress was continuous monitored by TLC in (70:30) n-hexane and ethyl acetate as a solvent. The product of 4-oxo-1-carbo piperidone hydrazone was isolated by vacuo and recrystallized by DMF and DMSO. The solubility of newly synthesised hydrazone was in ethanol, DMF and DMSO solvents. The off white solid product was dried under vacuum over  $CaCl_2$ . Yield: (4.143gram), 84.35%. M.P.:78-80  $^\circ$ C. The structure of hydrazone was confirmed by C-H-N-S analyser, FT-IR, NMR ( $^1H$  and  $^{13}C$ ) spectroscopy Fig.1 [11-12].



### Micro analytical data:

**Elemental Analysis** for ( $C_6H_{11}N_3O_2$ ) (%): Calc. C, (45.85); H,(7.05); N, (26.73); Found C (45.90); H, (7.15), N, (26.85).

**FT-IR** ( $cm^{-1}$ ): 3401 $\nu$ (-NH pri.), 2976  $\nu$ (NH sec.), 1696 $\nu$ (C=O keto), 1641  $\nu$ (C=O amide), 1228  $\nu$ (C-N aliphatic).

**UV-visible** (DMSO,  $c=2 \times 10^{-4}$  mol  $dm^{-3}$ ):  $\lambda$  max,  $\epsilon=356nm, 320nm, 243nm$  (Fig.3.1).

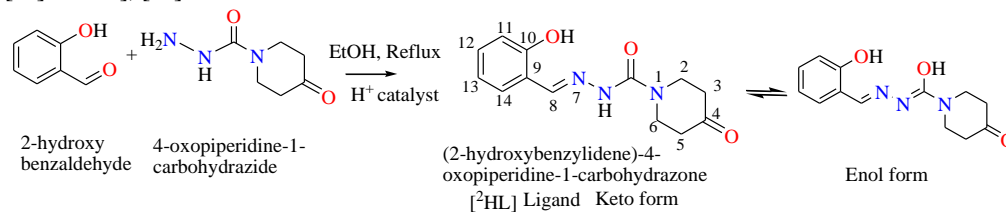
**$^1H$ -NMR** (DMSO- $d_6$ , 500MHz)ppm:  $\delta=2.5$  (s,4H, $\alpha$  position of the ring, -C(C=O)-CH $_2$ -),  $\delta 3.4$  (s,4H, $\beta$  position of the ring, -NC(=O)-CH $_2$ ),  $\delta 8$  (s,1H,NH),  $\delta 4$  (d,2H,NH $_2$ ).

**$^{13}C$ NMR** (DMSO $d_6$ ,500MHz,ppm):39.66(C2),43.57(C3),154.75(C4,C=O),C5(43.76), C6(39.75), C7(162.75,O=C-NH, Amide).

**Mass Spectrum** (ESI):  $[M^+]=158$ .

## 2.4. Synthesis of 2-hydroxybenzylidene -4-oxopiperidine-1-carbohydrazone ligand (<sup>2</sup>HL) Compounds

To a round bottom flask, 1-carbo-4-oxo- piperidone hydrazide (3.15g, 0.020 mol) and 2-hydroxy benzaldehyde (4.00g, 0.020 mol) was added in 25 ml EtOH medium and refluxed using aq. protic acid for about 3 hrs. The yellow precipitate was filtered off, washed with EtOH and finally recrystallized from DMSO. The product was dried over anhydrous CaCl<sub>2</sub> in vacuum desiccators. Product Yield 5.72 g (80%). M.P.163-165 °C. Elemental analysis, Cal. for [C<sub>13</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>]: C, 59.76; H, 5.78; N 16.08 %. Obs. C, 59.00; H, 5.50; N, 16.00 %. FT-IR: 3377 ν(OH, broad), 3260 ν(N-H), 1632 ν(C=N<sup>azomethine</sup>), 1672 ν(C=O<sup>amide</sup>), 1300 ν(C-O<sup>phenolic</sup>), 951 ν(N-N) cm<sup>-1</sup>. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 500 MHz): δ = 11.0 (s, 1H, -OH), 9.0 (s, 1H, -CH=N-azo.), 7.0 (d, 2H), 7.4 (t, 1H), 7.7 (t, 1H) (aryl), 2.5 (s, 4H, α, β, -C(C=O)-CH<sub>2</sub>-), 3.4 (s, 4H, α, β, (-NC(=O)-CH<sub>2</sub>-) ppm. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 500 MHz): 39.01 (C3), 39.38 (C2), 49.7 (C6), 39.55 (C5), 158.50 (C7 amide), 118.06 (C8), 119.46 (C9), 130.09 (C10), 133.12 (C12), 162.2 (C14) ppm. ESI-Mass: [M]<sup>+</sup>=261, [M]<sup>2+</sup>=262. Scheme 3.



Scheme 3. Synthesis of hydrazone ligand (<sup>2</sup>HL)

Table 1. Analytical data of [<sup>2</sup>HL] Ligand and its Zn(II) complex

Compounds	Molecular Formula	Mol.wt.	Elemental analysis found (calcd.) %					Molar cond. (Sm <sup>2</sup> mol <sup>-1</sup> )
			C	H	N	Cl	M	
[ <sup>2</sup> HL] Ligand	(C <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub> )	261.27	59.90 (59.76)	5.90 (5.78)	16.87 (16.08)	---	----	---
[Zn(L)(H <sub>2</sub> O)]	(ZnC <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> )	342.65	46.70 (45.56)	4.60 (4.41)	12.50 (12.26)	--	19.15 (19.08)	6.53

## 2.5. Synthesis of metal complexes – a conventional method

Zn (II) complex was dissolved in EtOH and (<sup>2</sup>HL) ligand in DMSO (0.02 mol of each) and later on each warm solution mixed in round bottom flask with magnetic stirring. The reaction mixture was refluxed for 3hrs on an oil bath and then cooled to the room temperature. The solid product of complex was filtered off, washed several times with ethanol and dried over CaCl<sub>2</sub> in a desiccator. The analytical data are shown in table 1.

## 3. RESULTS AND DISCUSSION

### 3.1. FT-IR spectral study

The free ligand shows a broad band at 3375 cm<sup>-1</sup> due to the stretching vibration of phenolic ν(O-H) bond. The FT-IR spectra of ligand are presented in fig.2 and parent hydrazide in fig.1 and selected FT-IR data in table2. The absence of this band in the spectra of complexes indicates the coordination of the ligand to the metal ion through the phenolate oxygen atom. This is further supported by the upward shifting of ν(C-O) phenolic frequency in the spectra of complexes by 25-42 cm<sup>-1</sup> from 1300 cm<sup>-1</sup> [13-14]. The IR spectrum of ligand exhibits a sharp band at 1632 cm<sup>-1</sup> due to azomethine ν(C=N) group and this band is shifted to lower frequency at 1612cm<sup>-1</sup> in complexes, indicating the donation of the lone pair of electrons of azomethine nitrogen to the metal centre. This has further been strengthened by the upward shift of ν(N-N) band from 951 to 975 cm<sup>-1</sup> in the spectra of complexes. However, the absence of bands at 3260 and 1672 cm<sup>-1</sup> due to ν(N-H) and ν(C=O) in the spectra of Zn(II) complexes and appearance of a new band at 1265 cm<sup>-1</sup> due to ν(C-O) (enolate) indicating the destruction of the (C=O) moiety as a result of the enolization and subsequent coordination of the enolate oxygen to metal after deprotonation. The presence of new band at 890 cm<sup>-1</sup> in the spectra of complex and designated to water molecule in coordination and which was further confirmed by TG analysis. The new weak intensity of non-ligand bands in the region 554 and 452 cm<sup>-1</sup> in complex are assigned to ν(M-O) and ν(M-N) bond vibrations respectively [15-16].

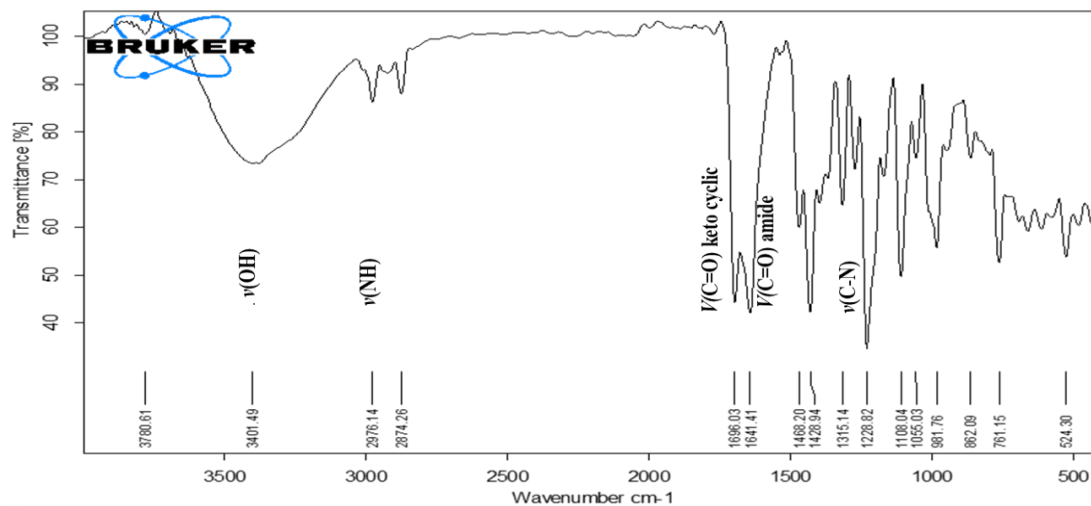


Fig.1 FT-IR spectrum of 1-carbo-4-oxo-piperidine hydrazide

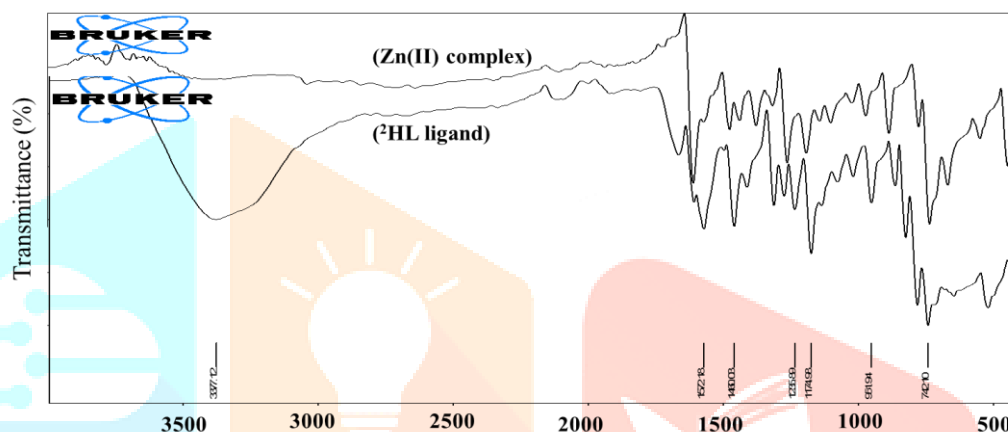


Fig.2 Comparison of FT-IR spectra of free ligand with Zn(II) complex

Table 2. FT-IR (cm<sup>-1</sup>) spectral data of free ligand (²HL) and Zn(II) complex

### 3.4. Magnetic and electronic spectral studies

The Uv-visible absorption spectra of ligand and its Zn(II) complex are shown in fig.3 The Zn(II) complex exhibits three absorption bands at 421 nm suggested LMCT and other absorption at 358, 295 nm due to intra ligand charge transfer (ILCT) at degenerate level due to forbidden in d-d ( $t_{2g} \rightarrow e_g$ ) transition. The transition appears occurred due to ligand chromophore group in ( $n \rightarrow \pi^*$ ) and ( $\pi \rightarrow \pi^*$ ) respectively. Moreover, the complex is found to be diamagnetic as expected for  $d^{10}$  configuration and suggested as a tetrahedral geometry. The complex show very low value of molar conductance of  $6.53 \text{ S m}^2 \text{ mol}^{-1}$  indicating that nonelectrolyte in nature [17-19].

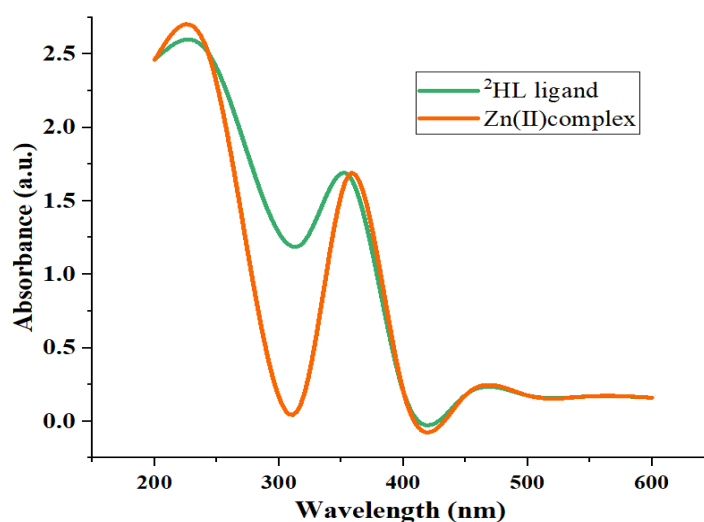


Fig.3 Uv-visible spectra of ²HL-ligand and its Zn(II) complex

### 3.5. Powder XRD study

The X-ray diffractograms of of ligand (<sup>2</sup>HL) and its Zn(II) complexes was recorded to determine the types of crystal system, lattice parameters and cell volume Figs. 4 (a-b) and data listed in Table 3. The X-ray diffraction studied using source target CuK $\alpha$  radiation = 1.540559 Å to find nature of compounds, peak intensity and relative intensity, crystallite size, interplanar spacing and unit cell dimensions. The Bragg's equation ( $n\lambda = 2d\sin\theta$ ) was used for the evaluation of angle ( $2\theta$ ) and interplanar distance (d). Miller indices were also reflected in specified directions and supported to the crystal lattice parameter of h, k, l values. Zn(II) complex crystal system is monoclinic,  $a = 8.5418$  Å,  $b = 6.300$  Å,  $c = 11.8470$  Å and  $\alpha = \gamma = 90$  deg.  $\neq \beta$  104 deg.,  $V = 608.318$  Å<sup>3</sup>, Space group=P with Z=2. The unit cell parameters for [H<sub>2</sub>L<sup>2</sup>] ligand and its complexes are as follows; <sup>2</sup>HL ligand: crystal system= monoclinic,  $a = 8.5418$  Å,  $b = 6.300$  Å,  $c = 11.8470$  Å and  $\alpha = \gamma$  (90deg.)  $\neq \beta$  (107 deg.). Interplanar distance (d) 4.1545 Å,  $V = 1899.81$  Å<sup>3</sup> The ligand [20-21].

After the calculation of the full width at half maximum intensity (FWHM), the average crystallite particle size of ligand and Zn(II) complexes was 27,21nm, respectively, which inferred their nano crystalline nature.

Crystal parameters	<sup>2</sup> HL-ligand	Zn(II) complex
Empirical formula	C <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub>	ZnC <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>
Formula weight	261.27	342.65
Temperature (K)	301k	301k
Crystal system	Monoclinic	Monoclinic
Scan speed time	10 deg/min.	10 deg/min.
X-ray	40 kV, 15 mA	40 kV, 15 mA
Refinement/ Scan range	10-80 deg.	10-80 deg.
Space group	P	P 1 21/n 1(14)
a, (Å)	8.5418	8.5418
b, (Å)	6.300	6.300
c, (Å)	11.848	11.847
$\alpha$ (°)	90.00	90.00
$\beta$ (°)	107.00	107.980
$\gamma$ (°)	90.00	90.00
Volume (Å <sup>3</sup> )	637.52	608.318
Z	2	2
Radiation ( $\lambda$ )	CuK $\alpha$ 1.54059nm	CuK $\alpha$ 1.54059 nm
2 $\theta$ range for data collection (°)	11.182-30.307	11.28 - 63.05
I/c (RIR)	0.98	0.98
FWHM ( $\beta$ )	0.193-0.59	0.30-1.6
Interplanar distance from peak position	4.1545	3.8713
Average particle size	27 nm	21nm

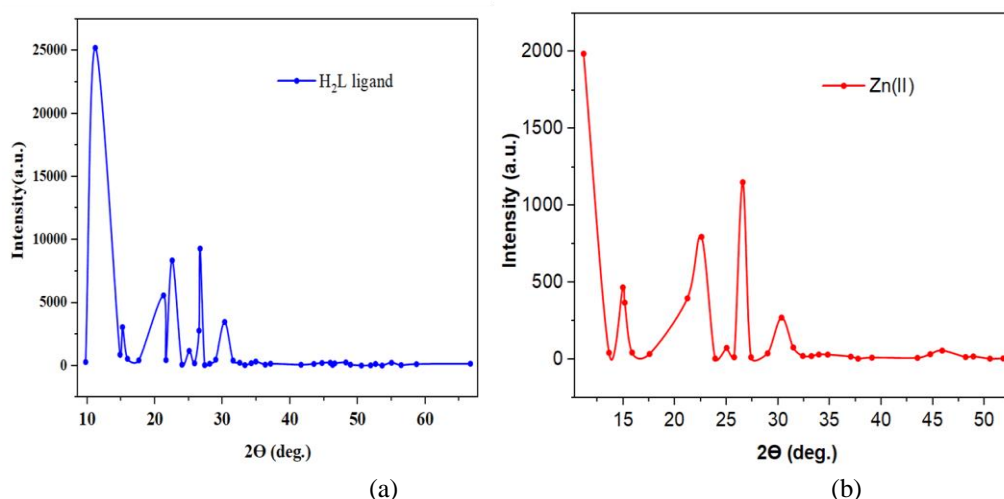


Fig.4 Powder XRD diffraction pattern of (a) ligand and (b) Zn(II) complex

**Table.3 XRD diffraction crystal data and lattice parameter for the title compound**

### 3.6. Thermal analysis

In order to investigate the thermal stability of the complexes, the simultaneous TG-DTG analysis of the  $[\text{Zn}(\text{L})(\text{H}_2\text{O})_2]$  complex undergoes two stage of decomposition in which one small and another broad decomposition steps represented in fig.5. and data summarised in 4. At  $120^\circ\text{C}$  for loss of one water coordinated water thereor.9.17% (calc. 9.85%) and the organic moieties losses  $\text{DTG}_{\text{max}}$  at  $450^\circ\text{C}$  found 80.20% (calc.79.86%) in temperature range of  $400\text{-}650^\circ\text{C}$ [22-23].The residue of (ZnO) was found 10.63% nearly equal to theoretical values (9.65%). It was also confirmed from the appearance of IR bands in the range  $850\text{-}890\text{ cm}^{-1}$  indicated the presence of coordinated water molecules. The absence of chloride ion was confirmed by the Volhard estimation in Zn(II) complex [24-25].

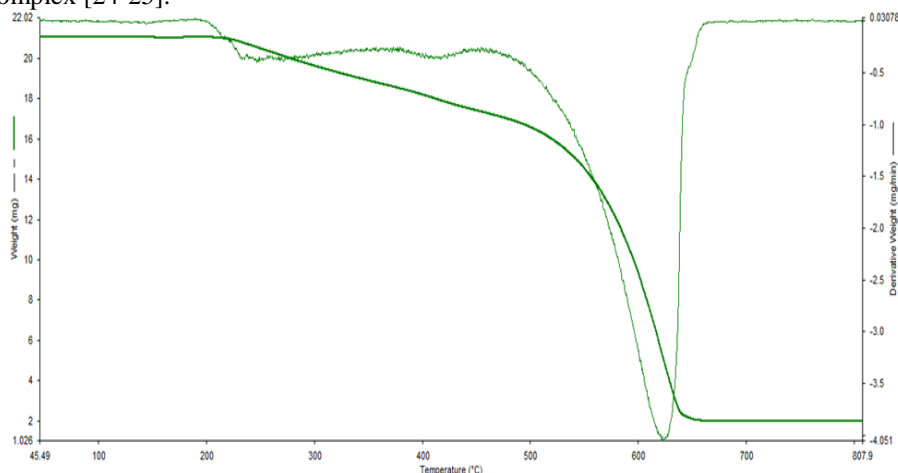
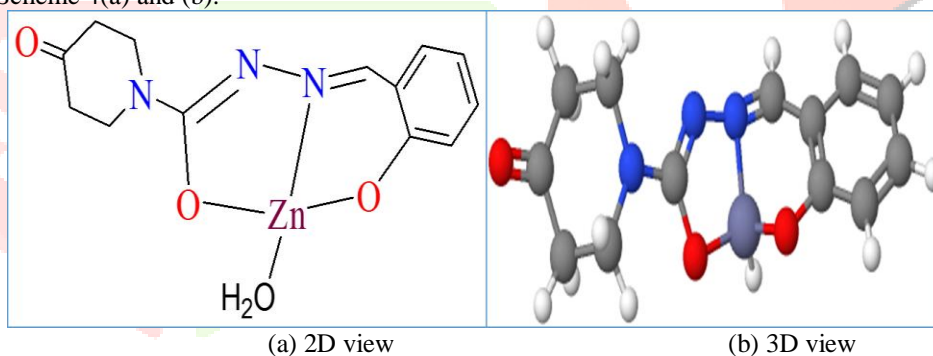


Fig.5 TG-DTG curve of Zn(II) complex

Table 4 Thermogravimetric data of Zn(II) complex

Compound	Stages	Temp.range ( $^\circ\text{C}$ )	$\text{DTG}_{\text{max}}$ ( $^\circ\text{C}$ )	% Mass loss		Decomposition Assignments
				Exptl.	Calcd.	
$[\text{Zn}(\text{L})(\text{H}_2\text{O})]$	I	45-120	120	9.17	9.85	Loss of one coord. $\text{H}_2\text{O}$ molecule
	[II]	240-650	450	80.20	79.86	Loss of organic moieties ( $\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_2$ )
	III	650-709	--	10.63	9.65	Metallic oxide residue

All thermal and spectral parameter of newly synthesised Zn(II) complexes proposed the following geometrical structure in two forms given Scheme 4(a) and (b).



Scheme 4 (a) 2D and (b) 3D view of Tetrahedral structure of Zn(II) complex

### 3.10. Biological study

The antibacterial activity of the ligand 2-hydroxybenzylidene-4-oxopiperidine-1-carbohydrazone [ $^2\text{HL}$ ] and its Zinc (II) complex was performed against gram -ve bacterial strains of *Escherichia coli*, *Salmonella typhi*; and gram +ve bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and fungal strains such as *Candida albican*, *Aspergillus niger* at  $1.0\text{ mg/mL}$  using disc diffusion method. DMSO used as negative control has negligible activity and standard compounds Ofloxacin, Azithromycin and Fluconazole were used as positive control and zone of inhibition was measured after 24 h of incubation. All the experiments were repeated thrice and the average values are presented in table 4 and shown in fig.7. The antibacterial activity increased as the concentration of the complex increased. According to the chelation theory, reducing the polarity of the metal ions due to the partial sharing of its positive charge with the ligand resulting in increase in the lipid solubility which favors its permission into normal cell of the all pathogenic microorganisms. The lipophilic solubility factor enhances the Zn(II) complex retards the microbial growth and shows poetical activity against microbial growth than the free ligand [26-30].

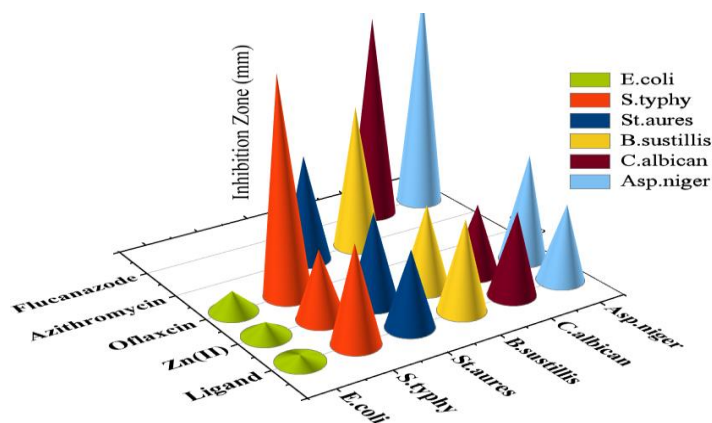


Fig.7 Graphical result of antimicrobial activities of ligand and Zn(II) complex

Table.4 Inhibition zone of antimicrobial assay of ligand and Zn(II) complex.

Compounds <sup>a</sup> and std.drugs*	Gram -ve bacteria		Gram +ve bacteria		Fungus	
	<i>E.coli</i>	<i>S.typhi</i>	<i>S. aureus</i>	<i>B.substillis</i>	<i>C.albican</i>	<i>A.niger</i>
[ <sup>2</sup> HL] Ligand	13	14	11	13	12	11
[Zn(L)(H <sub>2</sub> O)]	12	10	13	12	10	15
Ofloxacin*	30	30	-	-	-	-
Azithromycin*	-	-	15	20	-	-
Fluconazole*	-	-	-	-	28	30

## CONCLUSION

The synthesis of hydrazone and hydrazone schiff base ligand and its Zn(II) complex based on one step reaction with better yield. The mononuclear complex of the type [Zn(L)(H<sub>2</sub>O)] formed by 2-hydroxybenzylidene-4-oxopiperidine-1-carbohydrazone (<sup>2</sup>HL) have been reported in this paper. All these compounds have been characterized by spectral and analytical tools. Molar conductance values indicate that the complexes are non-electrolytic in nature. The nano crystalline nature of ligand and Zn(II) complex was concluded from powder XRD diffraction. The thermal data revealed the presence of one coordinated water and thermally stable at over high temperature. The coordination of ligand leads to the improved activity against selected bacterial and fungal species. Antibacterial activity against *Staphylococcus aureus*, *Bacillus substillis* *Escherichia coli*, *S.typhi* and fungal species *Candida albicans* and *Aspergillus niger* have been screened and results suggest that metal complexes are more potentially active than the parent hydrazone ligand.

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## Disclosure statement

Declaration of Competing Interest- The author declares that this our original work and we don't have any conflict of interest towards its publication.

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