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Antibacterial and irritant activities of bioactive organozinc (ll) complex with co-ligand

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Abstract
A zinc metal complex has been synthesized and characterized by physical, spectral and analytical data. The biological activity of ligand and its complex was carried out against the selected strains of two Gram positive and eight Gram negative bacteria. Agar well diffusion method was employed for screening of metal complex against Gram positive (*Xainthomonas Arconopdis, Acidovorax Tanpons, Xenorhabdus Luminescens, Kurthiagibsoni, Bordetella Pertussis, Brucella melitensis, Bacillus pumilus, Sarcina lutea, Micrococcusflavus*) *and Gram negative* (*Escherichia coli and Kurthiagibsoni*). This complex was synthesized by reacting bipyridyl and glycine with respective metal chloride and characterized by using FTIR, UV-Vis spectroscopy, conductivity measurements, elemental analysis and X-ray crystallography. Thermal behavior suggest more ordered activated state in complex formation. The biological activities data showed that activity enhances upon complexation. The complex showed different activity against different strains of bacteria.

Keywords: Ligand, metal complex, antibacterial activity, agar disc diffusion method

Introduction
Transition metal complexes of suitably substituted organic compounds are of increasing importance both academically and industrially. They are used as high quality solvent dyes, as lakes in pigments and in printing ink technology. The field of bioorganic chemistry helps to produce organometallic compounds that exhibit biological activity. The antibacterial activity was potentiated in case of zinc complex. The transition metal complexes of anilide ligands has applications in various photochemical reactions as well as in biological systems (Samy *et al*., 1988) [1]. Cu(ll), Ce(III), Bi(III) and Cd(II) complexes possessesanti bacterial activity(Fanny *et al*.,2007) [2]. Synthesis of zinc complexes have focused extensive interest in many fields such as blue or green emitting materials in LEDs (Youngmikim *et al*., 2010) [3]. Gatifloxacin complexes were synthesized with organometallic compounds. The complexes shows excellent anti-inflammatory activity (Najma *et al*., 2010) [4]. The structural diversity in borohydride lanthanide series was observed by X-ray crystal structure analysis (M. Ahmed *et al*., 2013) [5]. ”Bromoethyl sulfonium trifluoromethanesulfonate” a complex salt having three covalent bonds which result in the formation of pharmaceutically important hetrocycles in excellent yield (E. Constable *et al*., 1989) [6]. Synthesis of mixed ligand complex is characterized by elemental analysis infrared spectra thermo gravimetric analysis and x-ray crystallography (Shiva Kumar *et al*., 2012) [7]. Transition metals play an important role in various biological process, use of cations as therapeutic agents increase drug action and show greater efficiency by coordinating with metals (A.I. Seoane *et al*., 1999) [8]. The aneugenic and dastogenic abilities of cadmium sulphate and zinc chloride using the anaphase-telophase test and to validate if possible also to distinguish between these two mentioned kinds of genetic damage (Karacan *et al*., 2013) [10]. The attractive forces between metals and biological molecules leads to bind metal ions with them. Some organotin derivatives act as non-steroidalanti-inflammatory drugs (Kuwar *et al*., 2006) [13]. In the present work, we use reported ligand for the synthesis of zinc complex. The crystals of metal complex are characterized by different physiochemical methods. However, triple solvent system used for crystallization is not reported earlier thermogravimetric analysis and antimicrobial activities by using different bacterial strains is also not reported. The complex is assumed to be very effective against some strains of bacteria.

Materials and methods

Chemicals and solvents including zinc chloride, bipyridyl, glycine, ethanol, toluene, were purchased from Merck. FTIR spectra in the range 4000-250cm^{-1}KBr disk was recorded. Functional groups were inveterate by FT-IR. The UV-Vis spectra was recorded on UV-530 spectrophotometer. Conductivity was measured on Wescan-212 conductometer in Dimethyl Sulfoxide. T Thermogravimetric analysis was performed on TGA instrument SDT Q600 T.A company, temperature maintained at the rate of 20^{0}per minute. The elemental analysis of the complexes was carried on LECO Truspec micro Sr No. 4021 Model No. 630-200-200. The dimensions of the crystal were 0.30x0.25x0.10 and measurements were found by using 100(2k with the AXS SMART APX diffractometers using graphite λ (MoKα) Aº(0.71073).Unit cell parameter and orientation matrix were determined by least square calculations based on angles and 6651 reflections ranging from 1.332to 27.916 deg. The formation was solved by direct method (SHELX 86 AND SHELX 93). HPLC analysis was performed by using C:/Penexe/TcWS/ver 6.3.2.Microorganisms were obtained from Department of Agriculture University of Punjab, Lahore.

General procedure for the synthesis of zinc complex.

The zinc complex was synthesized by modification of reported method (Cheng-Juan *et al*., 2006) [11]. Zinc chloride and bipyridyl were reacted in 1:2 molar ratio. Metal chloride 0.14gm in ethanol was mixed with ligand in 250ml flask and reflux, keep on stirring for six hours. The completion of the reaction was confirmed by TLC when the reaction was completed, the solvent was evaporated by the rotary evaporator. The final product after filtration was dried under vacuum. The complex was re-dissolved in toluene and was put in the refrigerator to get the crystals. Micro crystalline product was gained. The product was re-dissolved in triple component solvent system (combination of three solvents (acetonitrile: methanol: water in the 2:3:1 ratio).Colored (orange) crystals were obtained fine crystals obtained was subjected for FTIR, TGA, UV, elemental and XRD.

Results and discussion

The physical data of ligand and zinc complex is given in table 1.The CHNS analysis shows metal to ligand ratio1: 2 stoichiometry for metal complexes (Moustafa *et al*., 2013) [14]. The zinc complex solution shows minimum conductance in DMSO which supports its nonelectrolyte nature. The U. Vis spectara analysis was performed to find out λmax of bipyridyl and zinc complex. λmax was foundat 265 nm respectively.

FTIR spectra

Table 1: Analytical data of Ligand and Metal complex.

Compound	Molecular formula	M. wt gm/mole	M.P	Elemental analysis of complexes			
Bipyridyl (L_1)	$C_{10}H_8N_2$	156.18	73 °C	Nitrogen Found (calcd) (%)	Sulphur	Hydrogen	Carbon
Glycine (L_2)	$C_2H_5NO_2$	75.06	233 °C	10.40(12.42)	Nil	5.05(4.45)	51.82(52.58)
Zn –Complex	Zn-Ligand	417	217 °C				

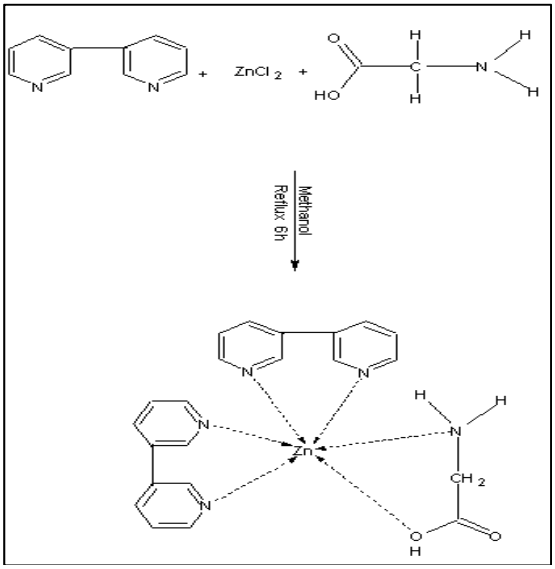
The results are presented on the basis of earlier reported work (Kuwar *et al*., 2006) [13]

Table 2: Crystallographic data for complex

Color	Orange
Shape	Irregular
Size(mm)	0.30x0.25x0.10
Chemical formula	$C_{30}H_{25}N_9O_9Zn$
Formula weight	720.96
Crystal system	Triclinic
T(K)	293
aº(A)	10.2711(11)
bº(A)	10.2718(10)
cº(A)	16.2730(17)
αº	102.390(5)
B	102.420(5)
Γ	102.196(5)
V(A^3)	1576.86(16)
μ(MoKα) Mm^{-1}	2.659
λ (MoKα) Aº	0.71073
Θmin	1.332
Θmax	27.916
hkl ranges	
H	[-12,13]
K	[-13,13]
L	[-21,20]
Refinement	[-15,21]
Space group	P-1
Cell formula unit Z	2
Collected reflections	6651
Unique reflections	4577
Parameters	367
Restrains	0
R-factor	0.3158

The FTIR. spectrum of free ligand bipyridyl when compared with spectra of Zinc complex which give medium band at 725cm^{-1}, M-N stretching band at 935cm^{-1} C-N stretching frequency at 1480cm^{-1}and C-H stretching at 2900cm^{-1} and glycine shows its characteristic absorption bands in 910-665cm^{-1} and 3400-3250cm^{-1} for N-H vibrations, 900-675cm^{-1} for aromatic C-H vibrations 1760-1690cm^{-1} for C=O vibrations similarly 1250-1020cm^{-1}for aliphatic C-N and 1335-1250cm^{-1} for aromatic C-N vibration. (Mamdouh S. Masoud *et al*., 2008) [12]. It is evident that the coordination between ligands and metal is through nitrogen and OH group of the respective ligands.

Synthetic Route for the synthesis of zinc complex with L_1



Crystallization of zinc complex in triple component solvent system

In order to get fine crystals of synthetic compound, combination of three solvents system was employed reported by (Chen et al 2006)The percentage of solvents used in this system were (toluene, ethyl acetate ethanol: 10% 40% and 50%) respectively was adopted to increase the solubility. It was observed that the solubility increased when few grams of synthetic compound was experianced which may be due to intermolecular interactions between the solute and solvent particles (Table 3).

Table 3: Crystallization of complexes in triple component solvent system.

Sr #	Compd/Complex	Solubility			
		DMSO	Chloroform	Ethyl alcohol	Mixed solvent system (Ethanol. Toluene. Ethyl acetate)
1	Bipyridyl (L_1)	+	+	+	+
2	Glycine (L_2)	+	+	+	+
3	Zn –Ligand	partially soluble	partially soluble	partially soluble	+

TGA Analysis

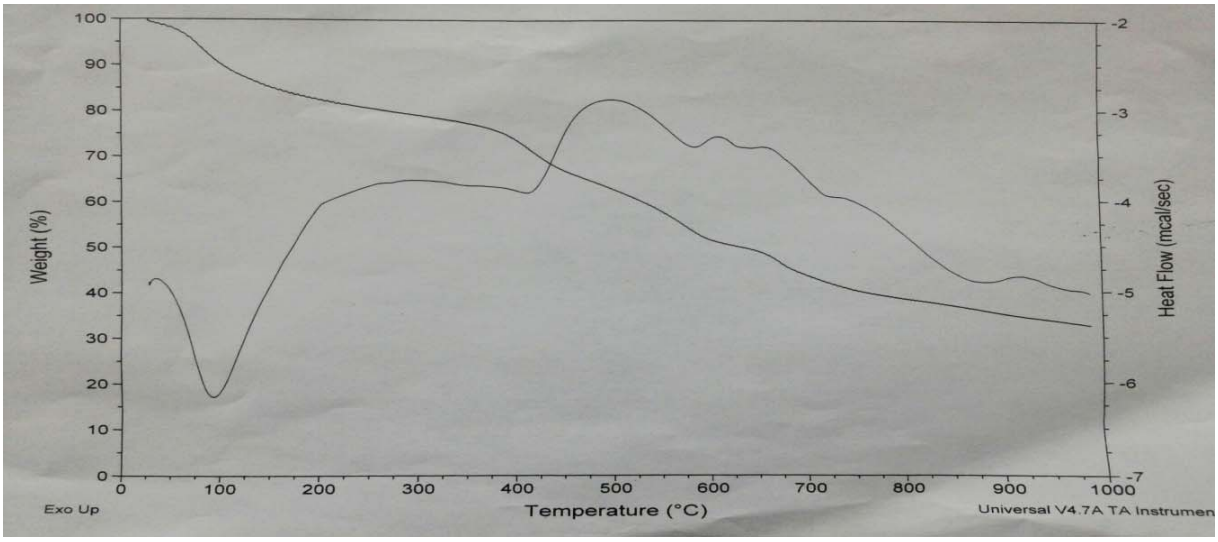


Fig: 1 TGA and DSC spectra of Zinc Complex

The Zn(II) complex was characterized by thermogravimetric analysis. The TGA of zinc complex was conceded at 1000 ^{0}C in nitrogen atmosphere using Al_2O_3 as reference (Liu. J., *et al*. 2006).In the first stage zinc complex decomposed between 180 to 220 ^{0}C with loss in mass of 48.60 percent. Similarly exothermic peaks in DSC was ascribed to decomposition of no coordinated part of ligand. Further decomposition from 480 to 600 ^{0}C corresponds to mass loss due to putrefaction of coordinated part of ligand 39.20%,afterward the weight loss remained constant corresponding to stable metal oxide ZnO 10.00% was obtained. Above graph of Zn(II) complex shows the removal of lattice and coordinated solvent molecules (H_2O or EtOH) from the complexes 200-225 ^{0}C and 650-700 ^{0}C respectively.

HPLC Analysis

The liquid RP-chromatography studies were performed in order to determine the purity of the new synthetic product in comparison to the free ligand. Aceto nitrile and water in various ratios were used as mobile phases.

Table 4

Compound	Retention time t_R	Capacity Factor K'	HPLC condition system
Bipyridyl	3.96	0.12	5
Zn-Complex	15.29	3.32	5

4. Antibacterial activity

For determining the antibacterial activity different concentrations (2%, 4%, 6%) of synthetic compound were used for testing their antibacterial response against eight gram negative and two gram positive bacteria. The selected strains were Xainthomonasarconopdis, Acidovorax Tanpons, Xenorhabdus Luminescens, Kurthiagibsoni, *Bordetella Pertussis*, Brucella melitensis Bacillus pumilus, *Sarcina lutea*, Micrococcus flavus Antibacterial activity was employed for metal complex and ligand by gram well diffusion methods. The ligand and metal complex showed antibacterial activities inhibited the growth of bacteria with the formation of clear zones, which were measured in mili meters by zone reader. Streptomycin was used as standard drug.

5. X-ray crystallography Analysis.

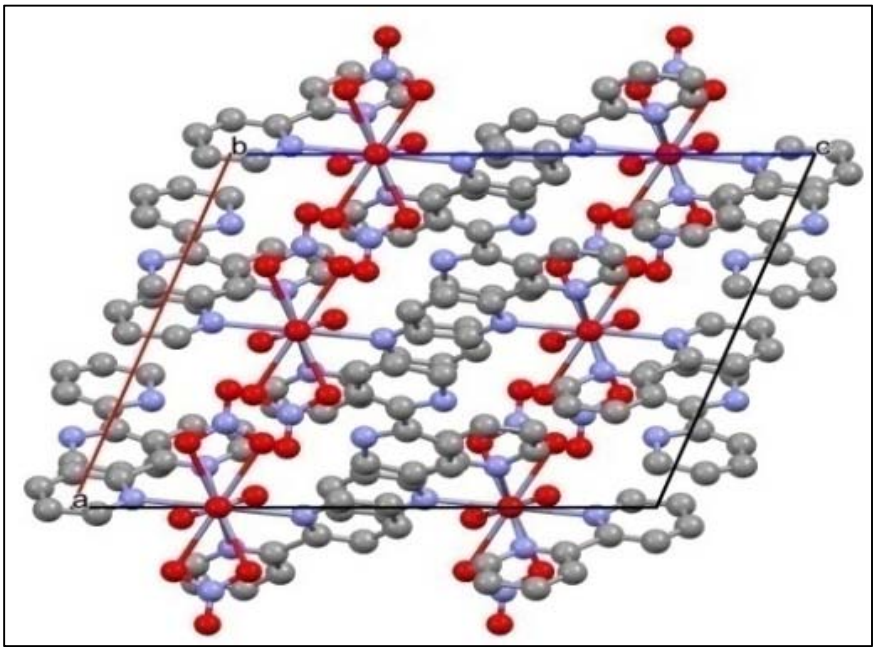


Fig 1: Arrangements of 2,2-bipyridyl and glycine Zn(II) complex in the crystal lattice.

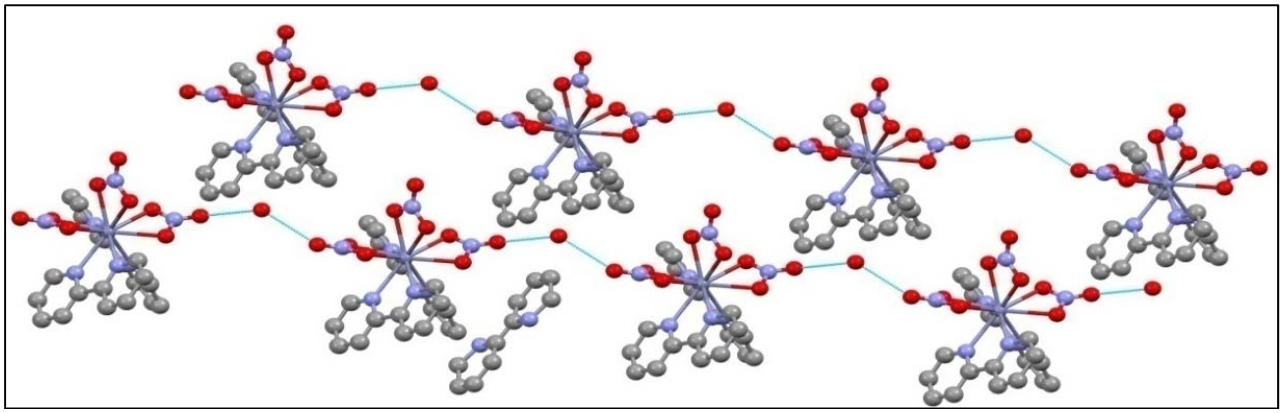


Fig 2: Crystallographic and refinement data of complex.

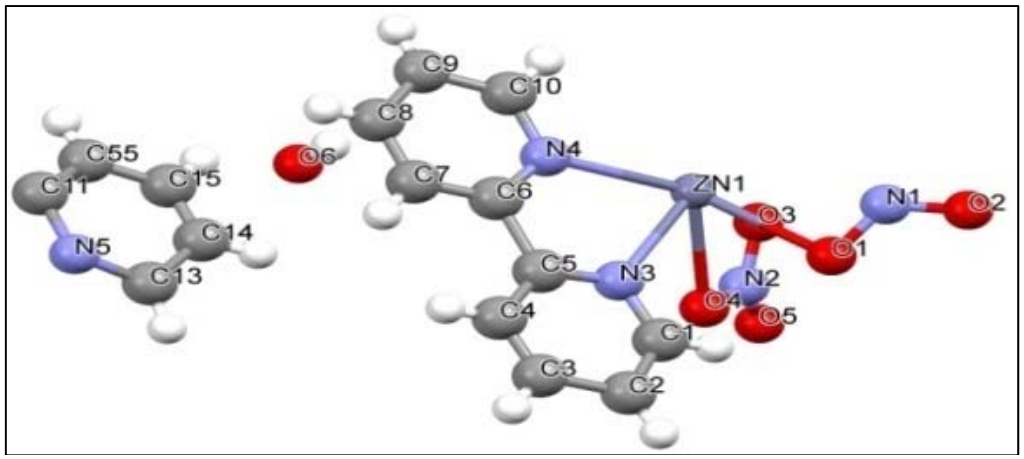


Fig 3

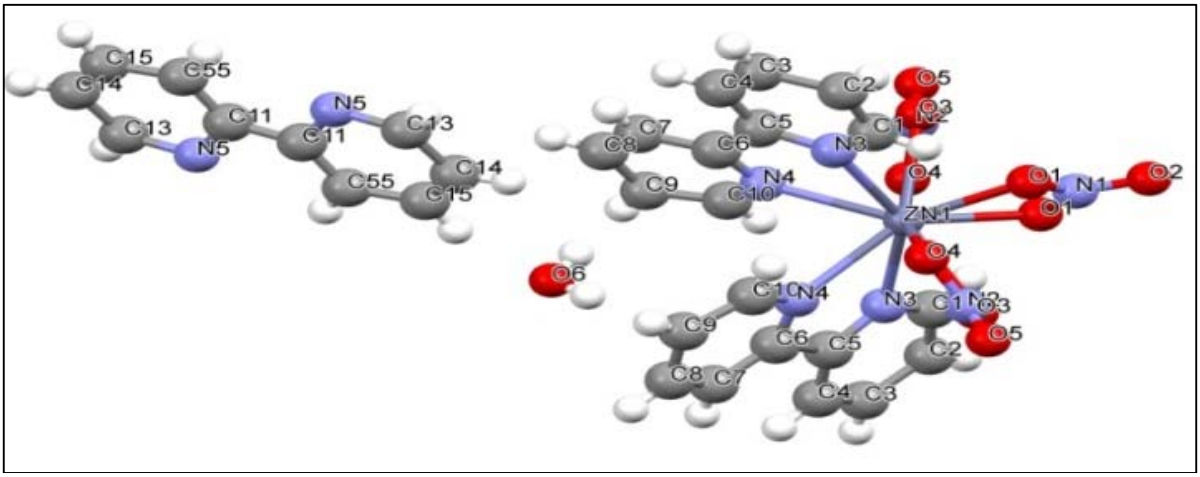


Fig 4: The H-atoms were positioned at different places.

Selected bond lengths and angles to determine the structure of Zn (bipyriclyl) glycine as determined by single X-Ray crystallography are given below

Zn_1-O_1	1.997 (2)
O_1-Zn_1-O_4	85.27 (3)
O_1-Zn-O_3	82.85 (2)
Zn_1-N_4	2.108 (2)
Zn_1-O_4	1.920 (2)
Zn_1-N_3	2.181 (2)
N_4-Zn_1-O_1	89.59 (3)
C_1-Zn_1-N_3	2.173 (3)
O_4-Zn_1-O_2	142.33
N_4-Zn_1-N_3	175.41 (4)
O_4-Zn_1-N_4C_{10}	83.17 (2)
O_1-Zn_1-N_4C_{10}	88.27 (3)
O_4-Zn_1-O_1	136.31 (2)
O_4-Zn_1-N_3	91.7 (2)

Crystallographic Data

The complex structure consists of bi layered cuboids as basic unit bonded with H –atoms. In the asymmetric unit there is one Zinc atom, two bipyridyl ligand, two nitrates one water molecule around this complex. The Zinc atom shows distorted octahedral geometry which consists of four oxygen atoms from it two symmetric glycine groups and four nitrogen atoms from two bipyridyl ligand and is interesting to note that bipyridylhavingN_4 and N_3 atoms is linked to Zn_1 atom from two ends. While the other endN_1 is linked with oxygen atoms of glycine groups (Silva *et al*., 2014) [15]. The dihedral angles show that two crystallographic bipyridyl ligands are non-coplanar. N_4-N_3(30°), N_1-N_3 (20.6°) the alternating chains are the distinctive feature of this crystallographic structure. The chains are covalently linked with bipyridyl bridges that results in two dimensional coordination sheet. Sheets located at the inversion center are connected to a two dimensional bilayer inter-layer hydrogen bonds.O_1-N_1 and O_4-$N_2$2.35° A, 82.35° A in a head to head manner, whereas nitro groups follows the orientation that aretrans to one another on other side of bilayer. In summary, novel zinc (II) coordination polymer has been hydrothermally synthesize structure is two dimensional with interlayer H bonds.

Antimicrobial Activity analysis

The antimicrobial activity against different species of bacteria was determined by using agar well diffusion method (Cuenca *et al*., 1999) [16]. The microorganisms were maintained on a slant of nutrient agar in Mc-Cartney bottles. Bacterial strains were introduced in sterile nutrient medium. The bacterial test organisms were grown in nutrient broth for 24 hours. A 100 pj nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells of 8mm diameter were prepared with the help of a sterilized stainless steel cork borer. Six wells (three are on the one half and two are on the opposite half whereas, one in the centre of petri plate) were

prepared in agar plates. The wells in each plate were loaded with 100µl of various concentrations (5,50 and 100mg/ml) of the fruit extracts. The central well in each plate was used as a control and loaded with 100µl of solvents (4.5% acacia). Two antibiotics (Ampicilline and Streptomycin 1mg/ml) were used as standard/positive controls by pouring into two cells of other half of the petri plates. The plates containing the bacteria and extracts were incubated at 37 ^{0}C for 24 hours. All the tests were repeated in six replicates. The results showed that metal complex is more active against bacterial strains as compare to ligands.

For each variable, values with different letters in a column show significant difference ($P \leq 0.05$) as determined by Turkey's HSD Test. A value of ($P \leq 0.05$) was considered to indicate a significant difference between groups. Values sharing a common superscript do not differ significantly with each other at ($P \leq 0.05$)

Table 5: Effect of different concentrations of metal complexes. Each values represents the mean + SE of diameter of zone inhibition (mm) in six replicates. All values were not significantly different at $P>0.05$, no effect

Complex concentrations	*Micrococcus flavus*	*Brucella melitensis,*	*Bacillus pumilus*	*Xenorhabdus Luminescens*	*Acidovorax Tanpons*	*Bordetella Pertussis*	*Xainthomonas Arconopdis*	*Acidovorax Tanpon*	*Xainthomonas Arconopdis,*	*Escherichia coli*	*Kurthiagibsoni*
2%	10.00±0.00	10.16±0.12	10.24±0.14	10.0±0.00	10.0±0.00	10.24±0.27	09.24±0.28	10.06±0.16	10.08±0.16	08.32±0.12	10.08±0.18
4%	10.24±0.21	10.24±0.23	10.16±0.24	10.00±0.28	------	10.28±0.24	10.16±0.24	10.16±0.24	10.00+0.00	09.18±0.34	10.00±0.00
6%	10.o8±0.23	10.16±0.23	10.25±0.42	09.91±0.18	10.00±0.00	11.16±0.24	11.00±0.28	11.23±0.22	11.16±0.23	10.08±0.00	10.25±0.24
Ampicillin 1 mg/ml	19.58±0.57	19.41±0.39	10.01±0.14	21.02±0.14	22.66±0.22	10.06±0.24	10.68±0.34	10.02±0.32	10.02±0.24	10.05±0.21	11.02±0.32
Streptomycin mg/ml	14.05±0.21	13.92±0.31	17.52±0.34	17.50±0.33	17.02±0.31	14.69±0.34	15.28±0.24	16.16±0.25	14.80±0.24	11.88±0.23	16.24±0.42

Determination of Irritant activity

Albino rabbit (*Oryctolagus cuniculus*)having weight 1kg from veterinary research institute, Lahore was purchased, it was fed on animal fodder provided with tapwater, it was used to test the irritant activity of organozinc (11) complex. For testing the irritant activity hairs present on the ears was shaved off and divided in to three parts with the help of marker about 2microliter of different concentration was applied on three portions. The other ear is taken as control. The redness of ear was observed after every 15minutes and then after 30minutes.The maximum irritancy on rabbit ear that correspond to the ++scale of Hecker (1971) after 24 hours were recorded. Neither acute nor chronic irritant activity has been exhibited by metal complex as earlier reported by (Saibabu *et al* 2007)

Results excluded from antibacterial screening is that transition metal complex show high inhibition towards bacteria Acidovorax Tanpon medium inhibition is shown by Bordetella pertussis. The diverse bioactivity help me to synthesize new bioactive compounds.

Having different mode of action against different bacteria, the pathogenic bacteria present in our environment cause serious threats to our environment and causes morbidity and mortality in living organisms Organometallic zinc complex is considered as new biologically active compound having antibacterial activity against pathogenic bacteria and has exhibited influential results Octahedral geometry for Zn(II) complex is proposed on the basis of above physiochemical and analytical data. From FTIR spectra it is presumed that metal iscoordinatedvia oxygen and nitrogen of ligands. Thermal analysis assumes that complex is thermally stable. The XRD studies suggest triclinic crystal system for Zn(II) complex. The metal complex shows strong antibacterial activity as compared to the ligand.

References

1. Samy M. abu-el-wafa, Mostafaa, RaafatmIssa, Akila A Saleh. J Chem Soc. 1988; 10:464-469.
2. Fanny bonnet, Marc visseaux, Abderrafiahafid, Estelle vigier. Inorganic Chemistry Communications. 2007; 10:690-694.
3. Youngmikim, Sujungkim, Kepyungnahm, Misookkang. J Korean Chem Soc. 2010; 31:712-749.
4. Najma Sultana, Asia Naz, M Saeed, M Ahmedmesaik. J Chem Soc. 2010; 969:17-24.
5. Maqsood Ahmed, Muhammad Yar, Claude lecomte, Christian Jelsch. J Phy Chem. 2013; 117:14267-14275.
6. Constable EC. J Adv Inorg Chem. 1989; 34:111-134.
7. Revanasiddappa HD, Shiva Kumar L, Prasad KS, Vijay B, Jayalkshmi BJ. Chem. Sc. 2012; 64,271-294.
8. Seoane AI, Dulout FN. J Gen Mic- Bio. 1999; 22:551-555.
9. RI Thompson, Al watsin. J App Chem. 2010; 43:1100-1107.
10. ALyar S. Ozbek, Ocak, Karacan. Med. Chem. Research. 2013; 22:2051-2060.
11. Cheng-Juan, Jian-Min, Da-Cheng, Da-Qi-Wang. J Mole Stru. 2006; 784:144-148.
12. Mamdouh, Masoud, MF Amira, AM Ramadan, Mole. Bio-Mole. Spectro. 2008; 69:230-238.
13. Kuwar AS, Shimpi SR, Mahulikar, Bendre RS. J Sct Ind Resc. 2006; 65:665-669.
14. Abd-elzaher M, Shakdofa Moustafa. Transc. On App. Chem. 2013; 2:2319-4847.
15. Silva Leite, Pavanand A. Cuin. Poly. 2014; 79:170-177.
16. Cuenca, Ruiz-Díez, Joaquin, Martínez-Suárez, Monzon. J Anti-Microb Chemo-Thpy. 1999; 43:149-151.
17. Saibabu KS, Timmons C, Li G. Chemical biology and Drug design. 2006; 67:101-104.
18. Liu J, WU B, Zhang B, Liu Y. Turkish journal of chemistry. 2006; 30:41-48.
19. Chen C. Indian engineering chemistry. 2006; 21:741-748.