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Synthesis, Spectroscopic Elucidation, Density Functional Theory (DFT) Calculations, Toxicological Effects and Biological aspects of Novel Heterobimetallic Cu(II) complexes containing 2,6-diaminopyridine Moiety

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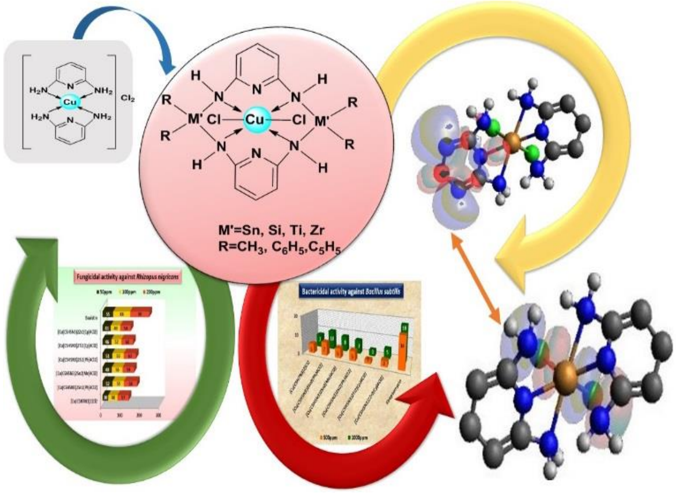
Abstract: **A novel series of heterobimetallic complexes of copper has been synthesized possessing 2,6-diaminopyridine moiety. All the synthesized complexes have been characterized in the light of various physicochemical techniques like elemental analyses, conductance measurements, molecular weight determination, electronic, infrared and electronic spin resonance X-ray, mass spectral studies and DFT analysis. The observed low conductance values of the synthesized heterobimetallic complexes suggest their non-ionic character. The studies presented in the series unveils the propensity of Cu(II) complexes with general formula $[Cu(C_5H_5N_3)_2M'_2(R)_4Cl_2]$ where M'=Sn, Si, Ti or Zr and R= CH_3,C_6H_5 or C_5H_5 against microbial pathogens *Fusarium oxysporum*, *Rhizopus nigricans*, *Escherichia coli* and *Bacillus subtilis*. Amid all the heterobimetallic complexes, the one presenting most promising antimicrobial activity was assessed on male albino rats for its toxicological effect by comparing the results with the standard fungicide Bavistin.**

Keywords: **Bimetallic complexes, Heterobimetallic complexes, DFT investigation, Antibacterial activity, Antifungal activity, Toxicological.**

Graphical Abstract:



1. INTRODUCTION

Over the past few decades studies on heterobimetallic complexes have aroused as a conspicuous field in the quest for new chemistry because of their plausible applications in fundamental, applied sciences and coordination chemistry beneficial in industrial and synthetic processes such as catalysis, photochemistry and biological systems. Heterobimetallic complexes have gained augmented research interest in recent years owing to the presence of two different metals which contributes to captivating structures and results in imperative applications.

In recent years, bimetallic complexes encompassing two different metal centers have acknowledged escalated consideration owing to the assumption that their reactivity should vary significantly from that of monometallic complexes or homobimetallic complexes [1]. The physical properties of these complexes are greatly affected by the presence of two metals in the same molecule along with an appreciable change in their reactivity. This is moreover due to the substantial modification in the individual properties of the metals or in the development of unique characteristics, which do not occur in monometallic compounds [2]. Varying applications of heterobimetallic complexes have been thoroughly explored which are found to be arising from the bifunctionality of the differing metal atoms [3].

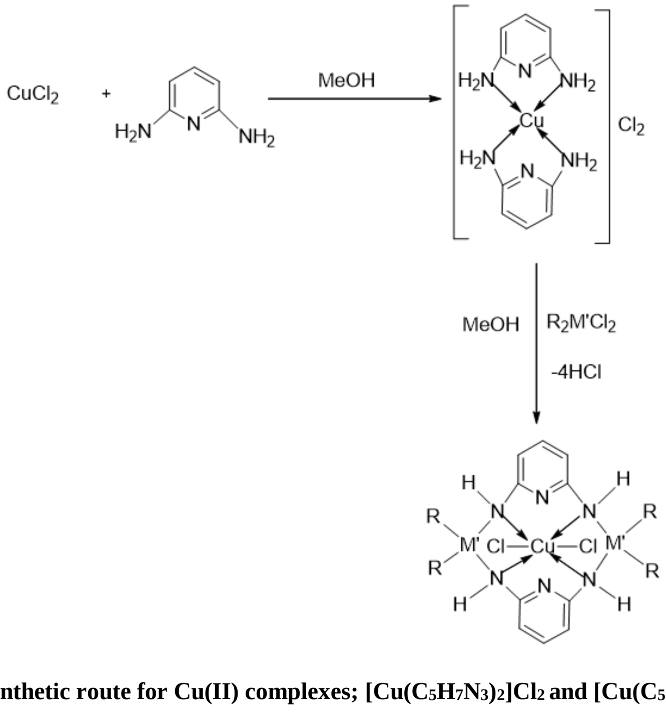
Heterobimetallic complexes with transition metal ions have been subject of interest and entangled in biological processes of life [4]. Among the essential heavy metal ions in the human body, Cu^{2+} is third in abundance after Fe^{3+} and Zn^{2+}, and it plays very important role in several biological processes. Furthermore, many bimetallic complexes embroiling transition metals have been found to have potentials as antibacterial, anticancer and anti-diabetic agents [5]**.** Keeping all these fascinating facts under consideration we have synthesized and characterized the monometallic and heterobimetallic complexes of copper along with group 4 and 14 metals which were further screened against a variety of pathogenic organisms. Apart from this these complexes were found to exhibit remarkable antifertility activity as observed from discernable reduction in sperm motility and density resulting in infertility.

2. EXPERIMENTAL

Synthesis of Monometallic Complex $[Cu(C_5H_7N_3)_2]Cl_2$

The complex was prepared by dissolving $CuCl_2$ (1.34g, 0.01mol) in hot methanol in a 100mL round bottomed flask followed by dropwise addition of 2,6-diaminopyridine in 1:2 stoichiometric proportions under constant stirring. The product thus formed was filtered, washed and recrystallized from methanol and dried in *vacuo*.

Synthesis of Heterobimetallic Complexes $[Cu(C_5H_5N_3)_2M'_2(R)_4Cl_2]$



Scheme 1. **Synthetic route for Cu(II) complexes; $[Cu(C_5H_7N_3)_2]Cl_2$ and $[Cu(C_5H_5N_3)_2M'_2(R)_4Cl_2]$.**

To the stirred solution of monometallic complex (3.52g, 0.01mol) in methanol was added a solution of 6.88g, 0.02mol of diphenyltin(IV)dichloride in 1:2 stoichiometric proportions. The reaction proceed with the liberation of hydrochloride acid leading to a solid product after keeping the reaction mixture overnight at room temperature. The product obtained was washed with cyclohexane and ether repeatedly and then dried in *vacuo*. All other heterobimetallic complexes were also obtained in a similar manner by using organometallic dichlorides such as dimethyltin (IV) dichloride, diphenyldichlorosilane, bis(cyclopentadienyl)titanium dichloride and bis(cyclopentadienyl)zirconium dichloride respectively (Scheme 1).

3. RESULTS AND DISCUSSION

Physical properties and Analytical data

The analytical data for all the synthesized complexes were in good agreement with those calculated for the suggested formulae as shown in Table 1. The compounds obtained were coloured solids and partially soluble in cold methanol but soluble in DMSO and DMF. The molar conductance values of heterobimetallic complexes in DMF solution falls in the range of 22-35 ohm^{-1} mol^{-1}cm^{2} suggesting that they are nonelectrolytes while higher conductance value of mononuclear complex of about 227 ohm^{-1}mol^{-1}cm^{2} indicates their electrolytic character [6]. These complexes were also investigated by using different spectroscopic methods.

Table 1. Physical properties and analytical data of the synthesized mononuclear and heterobimetallic complexes.

Compound	**% Yield**	**M.P.(°C) and Colour**	**Analysis % Found (Calcd.)**						**Mol. Wt. Found (Calcd.)**
			C	**H**	**N**	**Cl**	**Cu**	**M'**	
$[Cu(C_5H_7N_3)_2]Cl_2$	70	Black 112	34.08 (34.05)	4.06 (4.00)	23.85 (23.83)	20.12 (20.10)	18.04 (18.02)	-	354.65 (352.71)
$[Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2]$	65	Blackish brown 156	49.75 (45.65)	3.50 (3.38)	9.52 (9.40)	8.10 (7.93)	6.58 (7.10)	26.60 (26.54)	895.01 (894.51)
$[Cu(C_5H_5N_3)_2Sn_2(Me)_4Cl_2]$	66	Dark brown 125	25.89 (26.02)	3.12 (3.43)	12.69 (13.00)	10.56 (10.97)	9.79 (9.83)	35.12 (36.74)	638.92 (646.24)
$[Cu(C_5H_5N_3)_2Si_2(Ph)_4Cl_2]$	64	Blackish brown 165	56.52 (57.25)	3.98 (4.24)	10.99 (11.78)	9.72 (9.94)	8.54 (8.91)	6.95 (7.88)	710.03 (713.27)
$[Cu(C_5H_5N_3)_2Ti_2(Cp)_4Cl_2]$	62	Brown 230	50.34 (51.12)	4.01 (4.29)	11.51 (11.92)	9.82 (10.06)	8.76 (9.02)	13.04 (13.58)	688.94 (704.79)
$[Cu(C_5H_5N_3)_2Zr_2(Cp)_4Cl_2]$	59	Shiny brown 121	45.10 (45.52)	3.56 (3.82)	10.54 (10.62)	8.46 (8.96)	7.91 (8.03)	22.87 (23.05)	789.85 (791.50)

Infrared Spectra

The IR spectral data of the all the complexes are recorded and are presented in Table 2. The spectral data of the heterobimetallic complexes which are synthesized from mononuclear complex is very well compared with the spectra of the mononuclear complex. The primary amine exhibits a band at higher frequency than that of the corresponding secondary amine. The mononuclear complex exhibits a broad and strong band for ν(N-H) in the range of 3282-3150 cm^{-1} [7] which swings over to lower frequency region in case of bimetallic complexes evidently specifying the formation of bond between group 14 metal and nitrogen while bands due to δ(N-H) does not show any evident change after chelation. Aromatic ring (C=C) stretching appears at 1647, 1525 and 1456 cm^{-1} while (C-H) and (C-N) stretching were assigned at 3055 and 840 cm^{-1} respectively [8]. The spectra do not show any change in pyridine ring vibrations and it appears that in these complexes the nitrogen atom of pyridine does not participate in coordination [9]. Bands of weak to medium intensity are observed in the range of 415-582 cm^{-1} is attributed to ν(M-N) vibrations [10]. IR bands appear at 3000 for ν(C-H), 1435 for ν(C-C), 1028 for δ(C-H) in plane and 812 for δ(C-H) out of plane vibrations corresponding to complexes containing cyclopentadienyl ring. In addition bands due to (Ti-C_5H_5) and (Zr-C_5H_5) appears at 445 and 441 cm^{-1} respectively. A band of medium intensity was observed in the far IR region of the metal complexes (463-468) cm^{-1} was assigned to ν(Cu-N) [11]. The far IR spectra shows a distinct band at 317 cm^{-1} attributed to (Cu-Cl) vibrations [12].

Table 2. IR spectral data (in cm^{-1}) of mononuclear and heterobimetallic complexes derived using 2,6-diaminopyridine.

Compound	ν(N–H)	δ(N-H)	ν(M-N)	ν(Cu-N)	ν(Cu-Cl)
$[Cu(C_5H_7N_3)_2]Cl_2$	3282	1539	-	468	-
$[Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2]$	3185	1538	420	464	317
$[Cu(C_5H_5N_3)_2Sn_2(Me)_4Cl_2]$	3187	1536	415	464	317
$[Cu(C_5H_5N_3)_2Si_2(Ph)_4Cl_2]$	3182	1538	581	463	317
$[Cu(C_5H_5N_3)_2Ti_2(Cp)_4Cl_2]$	3165	1538	525	465	317
$[Cu(C_5H_5N_3)_2Zr_2(Cp)_4Cl_2]$	3170	1537	530	464	317

Electron Spin Resonance Spectra

The ESR spectra of monometallic and heterobimetallic complexes were recorded at the room temperature. The monometallic complex possesses $g_{\parallel}$ =2.09 and $g_{\perp}$ =2.05, suggesting that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital which indicates the square planar geometry around the Cu(II) ion [13]. The g values observed for heterobimetallic complexes has $g_{\perp} > g_{\parallel}$ suggesting a distorted octahedral geometry for these complexes with d_{z^2} as the ground state of the system [14]. On this basis it is concluded that chloride ions coordinate with Cu(II) on complexation with $R_2M'Cl_2$ achieving an octahedral environment for copper.

Electronic Spectra

The electronic spectrum of monometallic complex exhibits a strong d-d band at 590nm which is characteristic of square planar complexes. The electronic spectrum of complexes exhibits a strong band at 294nm and shoulders assignable to intraligand transitions [15]. In bimetallic octahedral complexes of copper, distortion may lead to displacement of d-d bands to lower energies and hence two bands are observed at 927nm and 875nm.

Mass spectroscopy

The mass spectra of the heterobimetallic complex $[Cu(C_5H_5N_3)_2Sn_2(C_6H_5)_4Cl_2]$ exhibited molecular ion peak $[M]^+$ at m/z = 894.06 which is in good agreement with its molecular weight 895.01 (Fig. 1). The octahedral environment around the copper is due to the two chlorine atoms which is verified by the presence of $[M+2]^+$ peak in the mass spectrum. The peaks attained at 817.89, 740.84, 621.96 and 348 has been assigned to fragments $[Cu(C_5H_5N_3)_2Sn_2(C_6H_5)_3Cl_2]^+$, $[Cu(C_5H_5N_3)_2Sn_2(C_6H_5)_2Cl_2]^+$, $[Cu(C_5H_5N_3)_2Sn(C_6H_5)_2Cl_2]^+$ and $[Cu(C_5H_5N_3)_2Cl_2]^+$ respectively. The two coordinated chlorides are removed with a mass loss of m/z= 71 with peak obtained at m/z = 277.80.

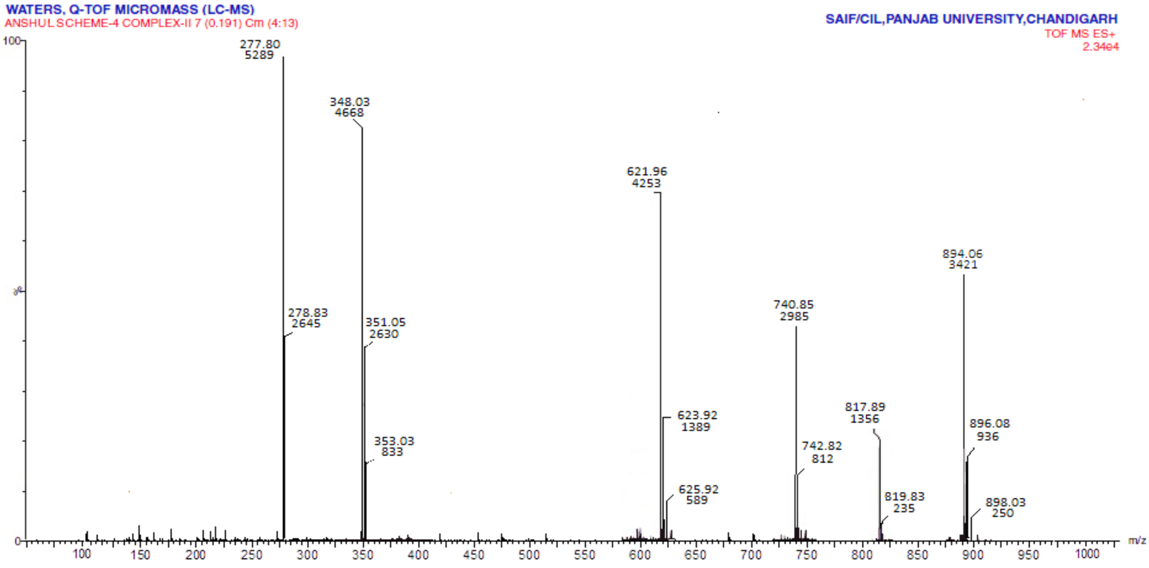


Fig. 1. Mass spectrum of the heterobimetallic complex $[Cu(C_5H_5N_3)_2Sn_2(C_6H_5)_4Cl_2]$

X-Ray Powder Diffraction Studies

The possible geometry of the finely powdered mononuclear complex $[Cu(C_5H_7N_3)_2]Cl_2$ and heterobimetallic complex $[Cu(C_5H_5N_3)_2Sn_2(CH_3)_4Cl_2]$ has been inferred on the basis of X-ray powder diffraction studies (Fig. 2 and 3). The observed interplanar spacing values ('d' in A°), have been measured from the diffractogram of this compound and the Miller indices h, k and l have been assigned to each d value. The results show that former compound belong to 'orthorhombic' crystal system having unit cell parameters as a=7.530, b=12.238, c =21.906; α=β=γ=90° while the latter belongs to 'tetragonal' crystal system having unit cell parameters a= b= 6.125 and c= 10.611; α=β=γ=90°.

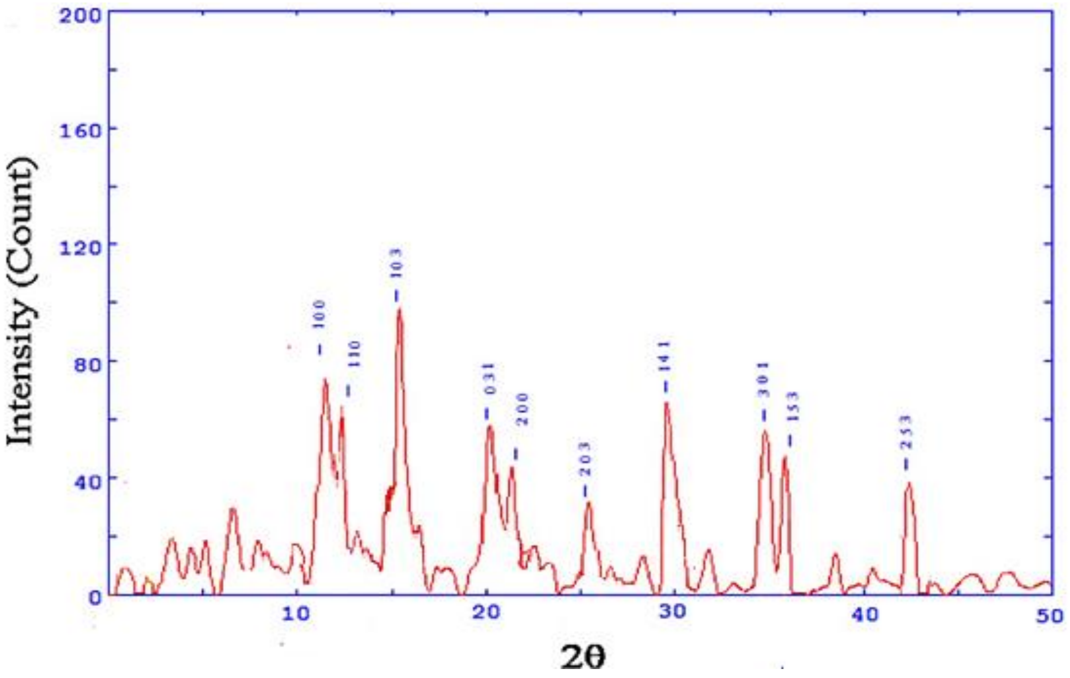


Fig. 2. X-ray powder diffraction pattern for the complex $[Cu(C_5H_7N_3)_2]Cl_2$.

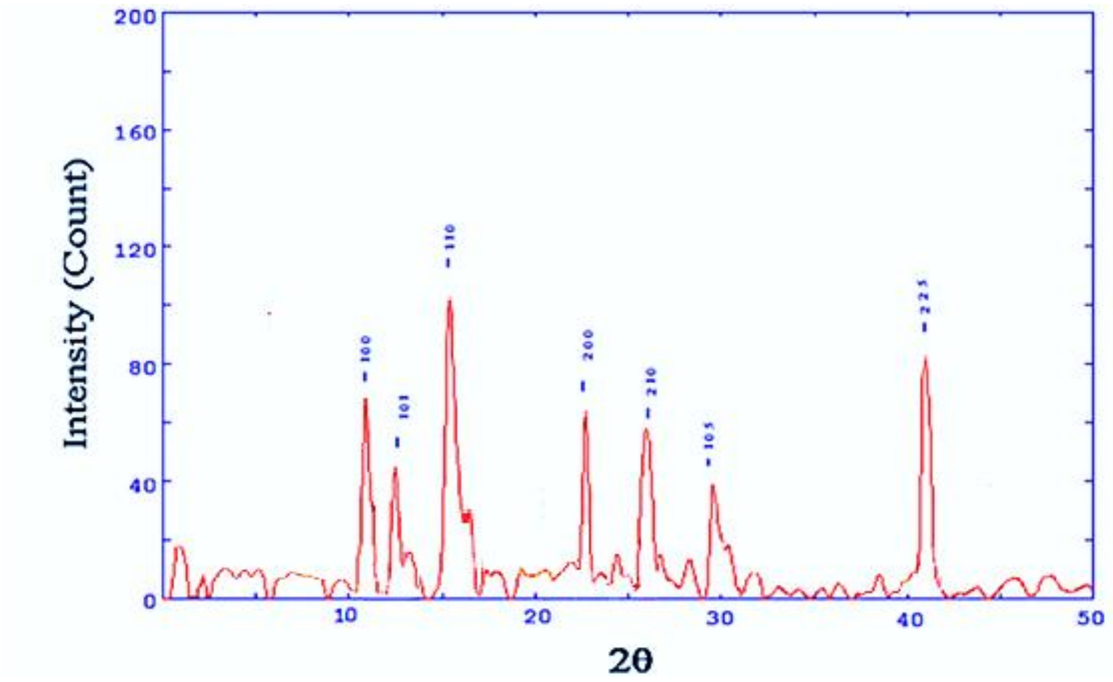


Fig. 3. X-ray powder diffraction pattern for complex $[Cu(C_5H_5N_3)_{277}Sn_2(Me)_4Cl_2]$.

Thus on the basis of the above spectral studies, the structure proposed for the mononuclear and heterobimetallic complexes seems to possess square planar and octahedral geometry, respectively (Fig. 4).

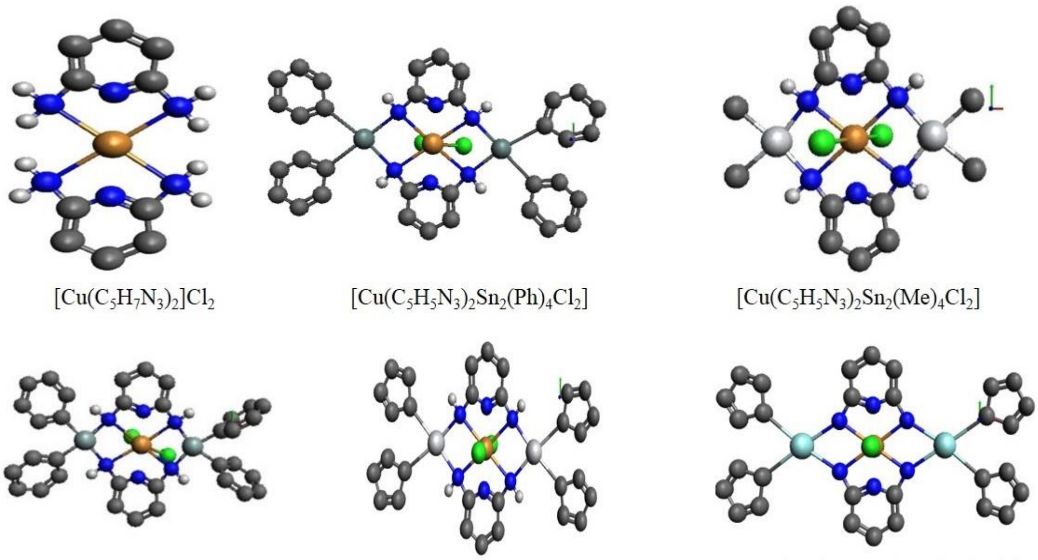
Mononuclear complex $[Cu(C_5H_7N_3)_2]Cl_2$ Heterobimetallic complex $[Cu(C_5H_5N_3)_2Sn_2(CH_3)_4Cl_2]$

Fig 4. Structures of the copper(II) complexes $[Cu(C_5H_7N_3)_2]Cl_2$ and $[Cu(C_5H_5N_3)_2Sn_2(CH_3)_4Cl_2]$.

Density Functional Theory Calculations

Density Functional Theory (DFT) calculations were performed using ORCA software and Avogadro 4.0. The Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) are known as frontier molecular orbitals and play a key role in determining the chemical reactivity of a molecule. Previous studies have shown that the energy gap (ΔE) between the HOMO and LUMO provides insight into the molecule's stability. According to Koopman's Theorem, the energies of the HOMO and LUMO can be used to estimate ionization energy (IE) and electron affinity (EA), respectively. These values further allow the calculation of various quantum chemical parameters such as electronegativity (χ), chemical hardness (η), softness (S), chemical potential (μ), and electrophilicity index (ω).

According to the frontier molecular orbital theory, the highest occupied molecular orbital (HOMO) acts as an electron donor, while the lowest unoccupied molecular orbital (LUMO) serves as an electron acceptor. The energy gap between these orbitals is an important indicator of molecular stability. Molecules with smaller HOMO–LUMO gaps generally exhibit greater chemical reactivity and lower kinetic stability. Consequently, they tend to have lower ionization potentials, making them effective as corrosion inhibitors [15,16].The energy gap is noticeably smaller in complexes, with the HOMO and LUMO energies for Cu(II) heterobimetallics complexes being 6.92 eV, 6.223eV, 5.937 eV, 5.992 eV , 4.97 eV and 4.372 eV, respectively (Fig. 5 and 6). The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are dispersed across the entire ligand's π-moiety. HOMO is dispersed over the full π-moiety in complexes, and LUMO is dispersed over the Copper metal.



$[Cu(C_5H_7N_3)_2]Cl_2$ $[Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2]$ $[Cu(C_5H_5N_3)_2Sn_2(Me)_4Cl_2]$

$[Cu(C_5H_5N_3)_2Si_2(Ph)_4Cl_2]$ $[Cu(C_5H_5N_3)_2Ti_2(Cp)_4Cl_2]$ $[Cu(C_5H_5N_3)_2Zr_2(Cp)_4Cl_2]$

Fig. 5. Optimized structure of Cu(II) heterobimetallics complexes.

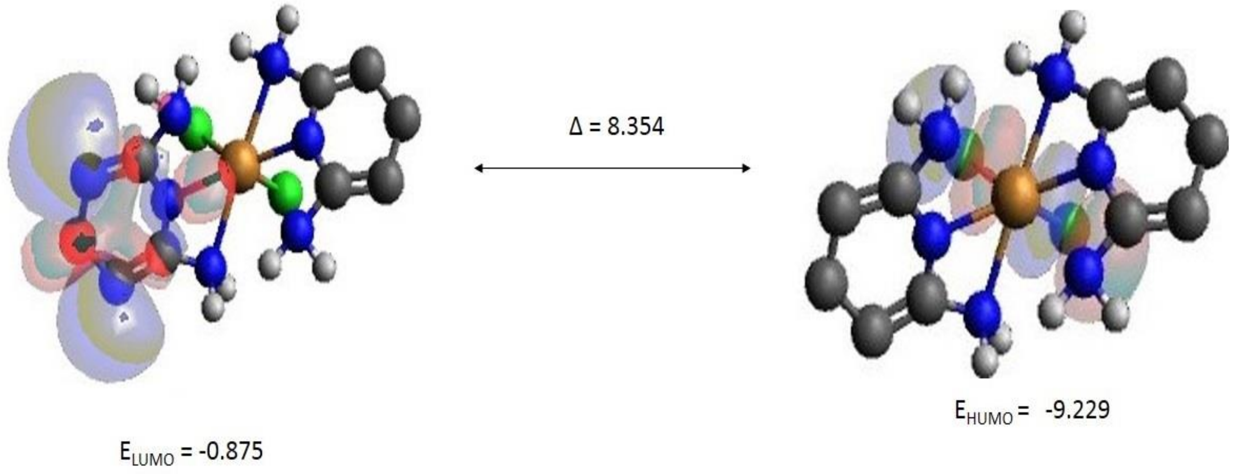


Fig. 6. Energy gap between the HOMO-LUMO of Monometallic complex Cu(II).

Biological Studies

An inspiring collection of polynuclear complexes has been reported over the last few years, preferably appropriate for chemistry involving multifarious redox processes thereby providing a varied coordination environment than a single metal ion. Cooperative interactions between the metal centers have proved to be beneficial for reactivity of these complexes [16]. In view of the above fact, in the present series we report the antimicrobial activity of the synthesized heterobimetallic complexes along with its mononuclear counterpart. MIC (Minimum inhibitory concentration) defined as the lowest concentration which inhibits the growth of microorganism was recorded for all the test compounds. The compound with most promising antimicrobial activity was assessed for its toxicological effect in male albino rats.

Antifungal activity

The antifungal activity of the freshly synthesized mononuclear and heterobimetallic complexes were tested against the fungal species *F. oxysporum* and *R. nigricans* by agar diffusion method (Fig. 7) [17]. Potato dextrose agar medium was prepared by using potato, dextrose, agar-agar and distilled water. Solutions of the synthesized compounds in methanol prepared at 50, 100 and 200ppm concentrations were mixed with potato dextrose agar medium which was then poured into Petriplates.

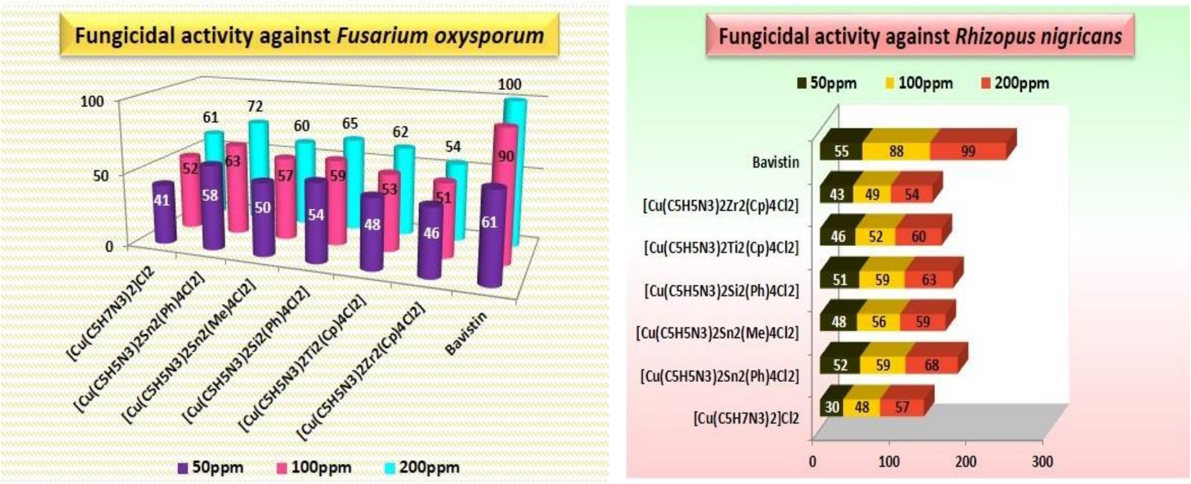

(a) **(b)**

Fig. 7. Fungicidal activity of the synthesized complexes

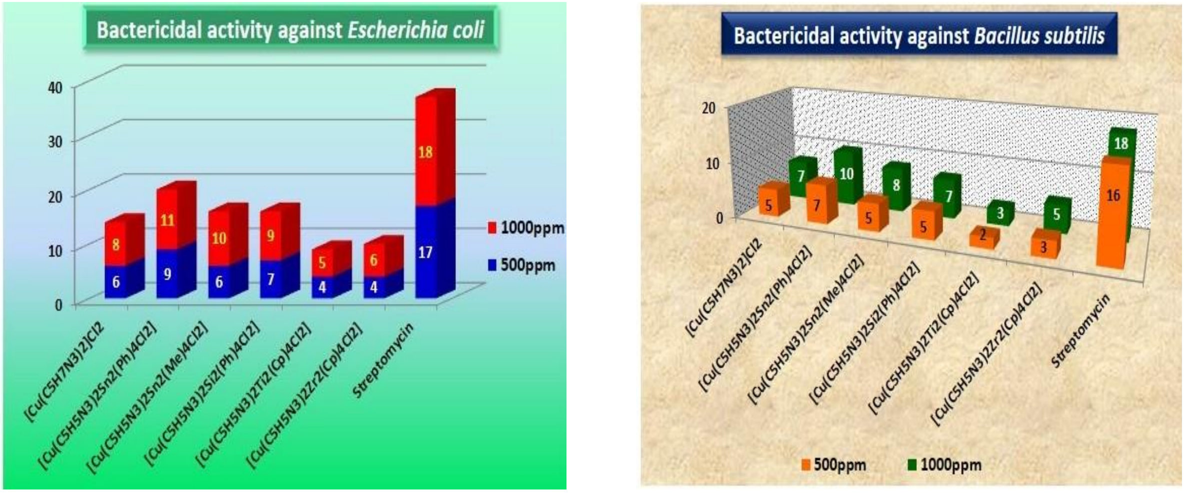
The spores of fungi were placed on this medium with the help of inoculum’s needle. These Petriplates were wrapped in the polythene bags containing a few drops of alcohol and were placed in an incubator at 25±2ºC. The activity was determined after 96hrs of incubation at room temperature (25ºC). The fungal activity of each compound was compared with Bavistin used as the standard drug. The growth of fungi was monitored and the percentage of inhibition was calculated by equation:

% inhibition = C-T/C x 100

where C and T are the diameters of the fungal colony in the control and the test plates, respectively.

Antibacterial activity

In vitro antibacterial activity is performed using paper disc plate method. The newly synthesized complexes were evaluated for their antibacterial activity against gram- negative bacteria *Escherichia coli* (MTCC 729) and gram-positive bacteria *Bacillus subtilis* (MTCC 121).



(a) **(b)**

Fig. 8. Bactericidal activity of synthesized complexes against.

Each compound was dissolved in methanol preparing solutions of 500 and 1000ppm concentrations. For the evaluation of antibacterial activity a nutrient media containing peptone, beef extract, NaCl, agar-agar and distilled water was prepared. Paper discs of Whatman filter paper (No. 1) of uniform diameter (5 mm) were soaked in different solutions of the compounds [18]. These discs were dried and placed in the petriplates containing nutrient agar media previously seeded with *E. coli* and *B. subtilis* bacteria strains separately. The Petri dishes were stored in an incubator at 28±2ºC for 24hrs. The zone of inhibition thus formed around each disc comprising the test compounds was measured accurately and were compared with standard antibiotic (Streptomycin).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration, MIC, is the lowest concentration of the test agent that inhibits visible growth of bacteria after 18hrs incubation at 37°C. The determination of the MIC involves a semi quantitative test procedure, which gives an approximation to the lowest concentration of an antimicrobial required to preclude microbial growth. The liquid dilution method was used to determine minimum inhibitory concentration [19]. Stock solutions of the ligand and the complexes with 100µg/mL concentrations were prepared with aqueous methanol. Inoculum of the overnight culture was prepared. In a series of tubes, 1 mL each of complex solutions with different concentrations was taken and 0.4 mL of the inoculum was added to each tubes. Further 3.5 mL of the sterile water was added to each of the test tubes which were further incubated for a time period of 18hrs and were observed for the occurrence of turbidity. The end result of the test was the minimum concentration of antimicrobial (test materials) which gave a clear solution with no visual growth [20] (Table 3).

The antimicrobial screening data reveals the greater inhibition effect shown by the heterobimetallic complexes as compared to monometallic complexes from which they have been derived. The reason behind the greater toxicity of metal complexes can be explained on the basis of the chelation theory [21]. The polarity of metal ion diminishes due to chelation chiefly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the whole chelation ring which upsurges the lipophilic character of the metal complex, subsequently approving its penetration through the lipid layers of the organism cell membrane, and the normal cell process being impaired along with deactivation of numerous cellular enzymes that plays imperative roles in the diverse metabolic pathways of these microorganisms. MIC values for all the synthesized complexes were recorded with best results drawn in case of compound [$Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2$], exhibiting lower MIC value at 25 µg/mL for bacterial strain *E. coli* and *B.subtilis* while in case of fungal strain *F. oxysporum* it is observed at 3.125 µg/mL respectively clearly indicating more sensitivity of metal complexes against fungal strains in comparison to bacterial strains.

Table 3. Minimum inhibitory concenteration (µg/ml) of the synthesized complexes.

Complex	***Escherichia coli***	***Bacillus subtilis***	***Fusarium oxysporum***	***Rhizopus nigricans***
[$Cu(C_5H_7N_3)_2]Cl_2$	50	50	12.5	12.5
[$Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2$]	25	25	3.125	6.25
[$Cu(C_5H_5N_3)_2Sn_2(Me)_4Cl_2$]	50	50	6.25	12.5
[$Cu(C_5H_5N_3)_2Si_2(Ph)_4Cl_2$]	25	50	3.125	6.25
[$Cu(C_5H_5N_3)_2Ti_2(Cp)_4Cl_2$]	50	50	6.25	12.5
[$Cu(C_5H_5N_3)_2Zr_2(Cp)_4Cl_2$]	50	50	6.25	6.25

Toxicological Evaluation

The mononuclear complex along with the heterobimetallic complex exhibiting superlative antimicrobial activity has been chosen for oral administration to the male albino rats for 60 days. Bavistin was used as standard for the comparison. The process was carried out by selecting twenty adult male albino rats of inbred colony (body weight 80-100gm) divided into four groups of five animals each which were maintained in an air-conditioned animal house at 24±2°C with 14hrs light availability. The animals of groups were fed with food pellet acquired from Ashirwad Industries, Chandigarh, and the tap water was supplied ad libitum. First group treated as control was served with vehicle (olive oil). The rats of the second, third and fourth groups received 30mg/kg.b.wt./day suspended in 0.2ml olive oil of Bavistin, mononuclear complex and heterobimetallic complex, respectively. At the end of the experiment i.e on 61st day the animals were weighed and autopsied under light ether anesthesia and the blood from the heart was collected in preheparinized tubes for hematological studies. Serum was obtained from blood by centrifugation at 3000rpm and stored at -20°C for biochemical estimation, done colorimetrically at a wavelength of 540 and 620nm. Fertility test (sperm dynamics) was also performed by using Neubaur's hemocytometer to check the efficacy of the compounds.

Male albino rats when exposed to the mononuclear complex **(a)** [$Cu(C_5H_7N_3)_2]Cl_2$ and the heterobimetallic complex **(b)** [$Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2$] which exhibited remarkable antimicrobial activity along with conventional fungicide, Bavistin for a period of about 60 days at a dose level of 30mg/kg.b.wt./day engendered ensuing effects:

- It is observed from Table 4 that the rats introduced to the standard fungicide Bavistin, showed highly substantial ($P \leq 0.01$ and $P \leq 0.001$) increase in alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in contrast to the control rats as well as the rats exposed to the mononuclear **(a)** [$Cu(C_5H_7N_3)_2]Cl_2$ and its heterobimetallic complex **(b)** [$Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2$]. This may possibly occur due to the necrosis of hepatocytes which causes escalation in the permeability of the cell membranes, resulting in the release of transferases into the blood stream. A noteworthy increase ($P \leq 0.05$ and $P \leq 0.01$) in the level of the cholesterol and a significant decline ($P \leq 0.05$ and $P \leq 0.01$) in the level of the serum protein and albumin was also observed in the Bavistin treated rats which may be related to cirrhosis of the liver, nephrotic syndromes or neoplastic diseases. Existing study finds support from the work of other toxicologists. Apart from this, there is momentous upsurge ($P \leq 0.05$ and $P \leq 0.001$) in the urea, creatinine and uric acid level of the Bavistin treated rats but the rats exposed to mononuclear complex showed highly significant ($P \leq 0.01$ and $P \leq 0.001$) increase in these parameters which is an indicator of the unpaired renal function. It is also observed, that the increase in the urea, uric acid and creatinine is more pronounced in the rats exposed to the mononuclear than the heterobimetallic complex.
- Significant drop in the total erythrocyte count ($P \leq 0.01$), hemoglobin ($P \leq 0.01$) and hematocrit percent ($P \leq 0.01$) values and substantial rise ($P \leq 0.001$) in leukocyte count is more pronounced in rats exposed to Bavistin than the

mononuclear **(a)** [Cu($C_5H_7N_3$)$_2$]Cl_2 and its **(b)** [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$] heterobimetallic complex (Table 5). It is also witnessed that the monometallic complex exhibited less decrease in the total erythrocyte count, hemoglobin and hematocrit percent value than its heterobimetallic complex. This may perhaps be due to the histopathological destruction of the liver and kidney so as to diminish the availability of the erythropoitin, which is produced in the juxtaglomerular apparatus in the kidney and is secreted in the plasma for the utilisation by the stem cells of the bone marrow.

- The motility of spermatozoa in cauda epididymis is diminished by 60.19% in case of rats exposed to Bavistin, 25.98% in case of monometallic **(a)** [Cu($C_5H_7N_3$)$_2$]Cl_2 and 31.83% in case of **(b)** [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$] heterobimetallic complex as compared to the control rats (Table 6). This may perhaps be due to some change in the enzymatic activities of the oxidative phosphorolytic process required for ATP production which is essential for the onward movement of spermatozoa [22]. Heterobimetallic complex was found to cause additional reduction in sperm motility and density in testes and cauda epididymis as compared to the mononuclear complex. Dropping down of the sperm counts in testes and cauda epididymis may be either due to the altered gonadotrophins (LH and FSH) essential for normal sperm production, development and maturation or altered androgen metabolism. Thus, it has been clinched that the heterobimetallic complex (b) [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$], exhibited less toxic effects on parameters related to serum biochemistry, hematology and fertility than the respective ligand i.e mononuclear complex, which in turn displayed less toxic effects than the standard fungicide Bavistin. It has also been revealed that Bavistin showed maximum antifertility effect while the heterobimetallic complex presented more antifertility effect than the ligand i.e. mononuclear complex.

Table 4. Serum analysis of male albino rats^{a} exposed to standard fungicide Bavistin

Treatment	**Urea**	**Creatinine**	**Uric acid**	**Cholesterol**	**Alanine Amino transferase**	**Aspartate amino transferase**	**Total Protein**	**Albumin**	**Alkaline phosphatase**
	(mg/dl)				**(units/ml)**		**(g/L)**		**(KA units)**
Control (vehicle treated)	32.0 ± 1.26	0.86 ±0.15	5.81 ±0.15	94.68 ±6.34	132.20 ±7.00	76.18 ±2.31	55.45 ±0.05	40.00 ±2.64	68.14 ±0.86
Bavistin	36.75* ±0.46	1.56** ±0.12	7.06* ±0.43	126.14* ±5.68	186.32*** ±4.56	119.26*** ±3.82	51.12* ±0.40	23.05* ±0.40	80.92* ±1.22
(a)	42.20* ±1.09	1.46** ±0.14	6.97** 0.33	102.34* ±1.57	147.60* ±1.52	75.13 ±1.68	53.82 ±0.17	37.78 ±1.24	69.93 ±0.52
(b)	41.15* ±0.70	1.20 ±0.08	6.66 ±0.17	100.24 ±1.32	136.14 ±1.47	72.66 ±3.78	54.16 ±0.06	36.87 ±0.57	69.13 ±0.17

Mononuclear **(a)** [Cu($C_5H_7N_3$)$_2$]Cl_2 & Heterobimetallic complex, **(b)** [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$].

a (Mean ± SEM of 5 animals); * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$

Table 5. Blood analysis of male albino rats^{a} exposed to Bavistin, mononuclear and heterobimetallic complex [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$].

Treatment	**Total Erythrocyte Count (TEC)**	**Total Leukocyte Count (TLC)**	**Hemoglobin**	**Hematocrit**
	million/mm^3	**mm^3**	**gm%**	**%**
Control (vehicle treated)	6.56 ± 0.21	5300 ± 253.34	15.25 ± 0.32	50.44 ± 1.59
Bavistin	5.04 ± 0.16**	8215.00 ± 232.73***	11.14 ± 0.18**	38.76 ± 0.52**
(a)	5.93 ± 0.31	6930.38 ± 138.46*	13.12 ± 0.34	41.59 ± 0.89*
(b)	5.98 ± 0.46	5825.65 ± 165.22	13.88 ± 1.10	45.28 ± 2.02

Mononuclear **(a)** [Cu($C_5H_7N_3$)$_2$]Cl_2 & Heterobimetallic complex **(b)** [Cu($C_5H_5N_3$)$_2$$Sn_2(Ph)_4$$Cl_2$].
a (Mean ± SEM of 5 animals); * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$

Table 6. Spermdynamics and Fertility of male albino rats^a exposed to Bavistin, mononuclear and heterobimetallic complex

Treatment	Sperm Mobility (%)	Sperm Density (million/ml)		Fertility (%)
		Testes	Cauda epididymis	
Control (vehicle treated)	69.61 ± 4.34	4.15 ± 1.86	21.70 ± 2.06	100 (+)ve
Bavistin	27.64 ± 2.35 ***	0.86 ± 0.06***	10.13 ± 1.72 *	80 (−)ve
(a)	51.13 ± 0.97	1.75 ± 0.16*	14.52 ± 1.36	55 (−)ve
(b)	46.67 ± 1.15*	1.10 ± 0.09**	10.19 ± 1.83*	60 (−)ve

Mononuclear **(a)** $[Cu(C_5H_7N_3)_2]Cl_2$ & Heterobimetallic complex **(b)** $[Cu(C_5H_5N_3)_2Sn_2(Ph)_4Cl_2]$.
a (Mean ± SEM of 5 animals); * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$

4. CONCLUSIONS

Novel Heterobimetallics complexes of Cu(II) complexes have been synthesized and characterized. Results from spectroscopic techniques such as IR, EPR, XRD and ESR support the proposed structure that the Cu (II) complex possesses an octahedral geometry. DFT calculations of the synthesised Cu(II) complexes have been calculated and found in good agreement with the experimental results. Gram positive bacteria, Gram negative bacteria, and fungi are all susceptible to the Ru(III) complexes.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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REFERENCES

[1] C.M. Thomas, *Comment on Inorganic Chemistry*, vol. 32, no. 1, pp. 14-38, 2011.

[2] M. Rakkiyasamy, K. Kalimuthu and S. Rangasamy, *Journal of Chemical Biological and Physical Sciences Sec. A*, vol. 3, no. 1, pp. 16-19, 2012.

[3] B.G. Copper, J.W. Napoline and C.M. Thomas, *Catalysis reviews*, vol. 54, no. 1, pp. 1-40, 2012.

[4] R. K. Al-Shemary, A. M. A. Al-Khazraji and A. F. S. S. M. Lateef, *International Journal of Science and Research*, vol. 5, no.5, pp. 1787-1793, 2016.

[5] A. A. Osowole, O. B. A. Agbaje and B. O. Ojo, *Asian Journal of Pharmaceutical and Cinical Research*, vol. 7, no. 3, pp. 145-149, 2014.

[6] M.S. El-Shahawi , M.S. Al-Jahdali, A.S. Bashammakh, A.A. Al-Sibaai and H.M. Nassef, *Spectrochim. Acta Pt A*, 2013, **113**, 459-465.

[7] P. Gull and A.A. Hashmi, *J. Braz. Chem. Soc.*, 2015, **26**, 1331-1337.

[8] S.M. Emam, A.S. El-Tabl, H.M. Ahmed and E.A. Emad, *Arabian J. Chem.*, 2017,**10**, S3816-S3825.

[9] D.P. Singh, V. Malik, K. Kumar, C. Sharma and K.R. Aneja, *Spectrochim. Acta Pt A*, 2010, **76**, 45-49.

[10] A. Chaudhary, E. Rawat and S. Khan, *IJCCE*, 2013, **3**, 143-148.

[11] M. Shakira, N. Banoa, M.A. Rauf and M. Owais, *J. Chem. Sci.*, 2017, **129**, 1905-1920.

[12] D.G. Thakurata, A. Kalyan, R. Sen and R. Bhattacharjee, *J. Comput. Theor. Nanosci.*, 2014, **11**, 1-5.

[13] E.E. Aranda, T.A. Matias, K. Araki, A.P. Vieira, E.A. de Mattos, P. Colepicolo, C.P. Luz, F.L.N. Marques and A. Maria da Costa Ferreira, *J.Inorg. Biochem.*,2016, **165**, 108-118.

[14] K.S. Siddiqi, H. Afaq, S.A.A. Nami and A. Umar, *Synth. React. Inorg. Met. Org. Chem.*, 2003, **33**, 1459-1468.

[15] R.P.A. Bhoopathy, M. Malathy, R. Jyalakshmi and R. Rajavel, *Int. J. Pharma.Bio. Chem. Sci.*, 2016, **5**, 11-22.

[16] A.M. Maharramov, A.V. Gurbanov, N.G. Shikhaliyev, K.T. Mahmudov and A.J.L. Pombeiro, *New Materials Comp. Appl.*, 2017, **1**, 5-9.

[17] S. Watts, R. Meena and R.V. Singh, *J. Chem. Pharm. Res.*, 2013, **5**, 260-265.

[18] N. Fahmi, S. Shrivastava, R. Meena, S.C. Joshi and R.V. Singh, *New J. Chem.*, 2013, **37**, 1445-1453.

[19] M. Balouiri, M. Sadiki and S.K. Ibnsouda, *J. Pharm. Anal.*, 2016, **6**, 71-79.

[20] M. Tyagi, S. Chandra, P. Tyagi, J. Akhtar, A. Kandan and B. Singh, *J. Taibah Univ. Sci.*, 2017, **11**, 110-120.

[21] K.M. Chauhan and S.C. Joshi, *Inorg. Lett.*, 2014, **1**, 23-35.

[22] L. Dawara, S.C. Joshi and R.V. Singh, *Int. J. Inorg. Chem.*, 2012, **2012**, 1-12.