Tropical Journal of Pharmaceutical Research May 2017; 16 (5): 1137-1146
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i5.23

Original Research Article

Synthesis, characterization and pharmacological evaluation of mixed ligand-metal complexes containing omeprazole and 8-hydroxyquinoline

Waqar Ahmad[1], Shakeel Ahmad Khan[2]*, Khurram Shahzad Munawar[1], Asma Khalid[3] and Sadia Kawanl[4]

[1]Department of Chemistry, University of Management and Technology, Lahore-54000, Pakistan, [2]Department of chemistry, Hallym University, Chuncheon, Gangwon-do, 200-702, Republic of Korea, [3]Institute of Chemistry, University of the Punjab, Lahore-54590, [4]Department of Biochemistry, University of Agriculture, Faisalabad-38000, Pakistan

For correspondence: **Email: Shakilahmad56@gmail.com; **Tel:** 01032692977*

Sent for review: 24 January 2017 *Revised accepted: 15 April 2017*

Abstract

Purpose: *To synthesize a series of mixed ligand-metal complexes and to evaluate their alkaline phosphatase inhibitory capacities, antioxidant potential and antimicrobial activities.*
Method: *Mixed ligand-metal complexes of Zn (II), Ni (II), Co (II), Cu (II), omeprazole and 8-hydroxyquinoline were synthesized. The ligand-metal complexes were characterized by various physicochemical techniques, including elemental analysis, magnetic susceptibility, scanning electron microscope (SEM), mass spectrometry (EI-MS), ultraviolet-visible (UV-Vis) spectrophotometry, Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR) and conductance studies. The new compounds were also investigated for their alkaline phosphatase (ALPs) inhibition, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and antimicrobial activities.*
Results: *Spectroscopic studies revealed the chemical composition of synthesized compounds as well as the bidentate bonding behavior of the coordinating ligands with metal ions. Conductance measurement suggested that the metal complexes were non-electrolytes. Ni(II) complex exhibited antioxidant activity (30.48 ± 0.32 μM) higher than those of BHT (standard) and other complexes. Stronger inhibition of ALPs by Ni (II) mixed ligand complex compared to the other complexes was evident. The synthesized compounds showed moderate to very good antimicrobial activity against bacterial strains, i.e., Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Actinomyces viscosus, Staphylococcus aureus, Bacillus subtilis; as well as against the fungal strains, Candida albicans, Aspergillus flavus and Aspergillus niger.*
Conclusion: *All the mixed ligand complexes demonstrate higher antioxidant, ALPs-inhibitory and antimicrobial activities than their corresponding ligands. This indicates their therapeutic potential as future drug candidates for the concerned diseases.*

Keywords: *Omeprazole, 8-Hydroxyquinoline, Mixed-ligand complexes, Alkaline phosphatase, Bidentate bonding behaviors*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Omeprazole belongs to a class of substituted benzimidazole and has some clinical applications in treatment of peptic-acid diseases like gastric and esophageal ulceration and prevention of their relapse [1]. Acid inhibition strategies such as histamine H_2-receptor antagonist and use of anti-cholinergic agents have been employed to treat peptic ulcer disease. Omeprazole is a proton pump inhibitor which promotes reduction of parietal cells by a series of prototropic reactions. The long duration of action of parietal cell H^+-K^+-ATPase is due to prolonged binding of the active form of the drug [2-4]. Omeprazole has a privileged structure in pharmacophore and medicine chemistry. The importance of this compound is due to its bicyclic structure which is a fusion of benzene and imidazole rings. The plasma elimination half-life ($t_{1/2\beta}$) of omeprazole is usually shorter than one hour, although a few individuals exhibit slower elimination ($t_{1/2\beta} \approx 2h$). These individuals have recently been shown to be poor hydroxylators of S-mephenytoin, which suggests that a major part of omeprazole metabolism is mediated by S-mephenytoin hydroxylase, an enzyme within the cytochrome P450 system [5,6].

Omeprazole is completely metabolized and its average plasma clearance is 30 L/h. The drug also shows good antimicrobial activity against *Helicobacter pylori* [7-9]. 8-hydroxyquinoline is well-known for its antimicrobial activity. It has been comprehensively used for analytical determination of metal ions [10]. Cheatum *et al* [11] studied the dynamic excited state of HQL dimmers.

In the present study, an attempt has been made to synthesize Zn (II), Co(II), Ni(II) and Cu(II) mixed ligand complexes of omeprazole and 8-hydroxyquinoline. All the complexes were characterized by spectroscopic techniques and their biological activities were studied.

EXPERIMENTAL

All chemicals and solvents of analytical grade were purchased from Merck and Sigma-Aldrich. UV-visible spectra were recorded from 200 - 800 nm on Jasco UV visible spectrophotometer model V-770 UV-VIS. FT-IR spectra were recorded on Nicolet IS50, FT-IR spectrophotometer from 400-4000 cm^{-1}. Elemental analyses were performed on a CHNS/O-2400 Series II elemental analyzer. Magnetic susceptibility was determined using Holmarc's Magnetic Susceptibility (model HO-ED-EM-07). Mass spectral measurement was performed on Finnigan MAT LCQ^{TM} mass spectrometer. The molar conductance of the synthesized complexes in DMSO was measured using a Conductivity Meter, Model WD-35604-00 at 25 °C temperature. SEM images were recorded in a Hitachi SEM analyzer. ^1H-NMR spectra were recorded in DMSO-d_6 at room temperature using TMS as internal standard on a Bruker 400 MHz spectrophotometer.

Synthesis of mixed ligand metal complexes

Mixed ligand complexes were synthesized by mixing water-ethanol solutions (1:1, v/v) of metal salts (1 mmol), ethanolic solution of primary ligand (L) omeprazole (0.345 g, 1mmol) and secondary ligands 8-hydroxyquinoline (L2, 0.145 g, 1 mmol) in molar ratio of 1:1:1 (M:L:L2).The resultant mixture was refluxed for 3-4 h and colored precipitates were obtained in each case. The solids precipitated were filtered and washed with diethyl ether-ethanol (1:1 v/v). Stability constants were also calculated. Figure 1 shows the structural formula of the mixed ligand metal complexes.

Assay of inhibitory effects of metal complexes on ALPs

The assay of inhibition of ALPs was done spectrophotometrically as described earlier [12]. Working substrate was prepared by mixing four parts of diethanolamine (pH = 9.8, 2 mol/dm^3) and $MgCl_2$ (0.5 mmol/dm^3), and one part of *p*-NPP (50 mmol/dm^3). This substrate was incubated at 37°C for 5 min. The ALP assay of OMZ, HQL and metals complexes were determined by addition of 2 mL of working substrate to 40 µL of human serum.

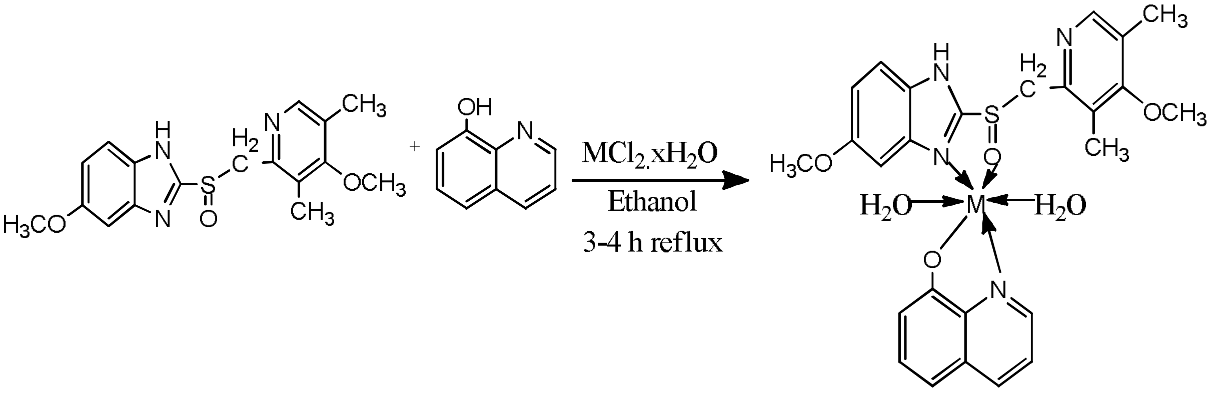

Figure 1: Reaction mechanism of mixed ligand metal complexes. (M = Zn(II), Ni(II), Co(II) and Cu(II))

ALP activity was assayed with 25 mM stock solutions of OMZ, HQL and the synthesized complexes. After the incubation period, absorbance was recorded at 405 nm [13]. All measurements were done in triplicate and the mean of these values was used to calculate the percentage inhibition of ALPs activity. The principle of this assay is that colorless *p*-Nitrophenyl phosphate (substrate) in alkaline medium changes to a yellow-colored due to liberation of *p*-Nitrophenol by ALPs.

Determination of DPPH radical scavenging activity

DPPH free radical scavenging activity is a rapid technique for assay of antioxidant activities of compounds. Using the method as described by Khan *et al* [14] the DPPH free radical scavenging potential of the mixed ligand complexes were determined relative to standard i.e. butylated hydroxytoluene (BHT). In the assays, OMZ, HQL and synthesized mixed ligand metals complexes at various concentrations (500, 250, 125 and 60 µg/mL) were incubated with 3 ml of 0.1 mM DPPH in methanol for 60 min at room temperature. Absorbance was measured at 517 nm against methanol. The absorbance was decreased by conversion of DPPH to the more stable $DPPH_2$ molecules, which serves as an indication of the capacity of antioxidant compounds to donate hydrogen ion [15]. All measurements were taken in triplicates. DPPH radical scavenging activity was calculated using the formula:

DPPH scavenging (%) = {(Ac - As)/Ac}100… (1)

where Ac is the absorbance of control and As is the absorbance of tested compounds.

Antimicrobial assay

The antimicrobial properties of OMZ, HQL and synthesized complexes were determined using the agar-well diffusion method [16]. Six bacterial strains including three Gram-negative strains, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and three Gram-positive strains, *Actinomyces viscosus, Staphylococcus aureus, and Bacillus subtilis* were used. Antifungal activity was determined against *Candida albicans*, *Aspergillus nigar* and *Aspergillus flavus.* The culture media was prepared by dissolving 28 g of nutrient agar in 1L of distilled water (for bacterial strains), and 38 g of potato dextrose agar in 1L of distilled water for fungi. The media were autoclaved at 121°C and 15 LD/sq inch for 15 min. The stock solution (100 µL in each dish) was put into Petri dishes and then allowed to attain room temperature. The microorganisms were cultured and incubated for 24 h at 37 °C (for bacterial cultures) and for 48 h at 28 °C (for fungal cultures) [17,18]. Zones of inhibition in specimen Petri dishes were measured in mm with venire caliper in mm. The anti-fungal drug fluconazole and antibacterial drug ampicillin (100 µg/mL) were used as standard controls for comparison with the tested specimens under similar conditions. These assays were performed in triplicate, and the mean was taken as the final reading.

Statistical analysis

Analysis of data was carried out using Microsoft Excel-2016 software. All computations were executed in triplicate and the results were expressed as mean ± SEM (n = 3). Antimicrobial, DPPH and ALP assays were computed with suitable dilutions for each sample and different statistical techniques such as Duncan multiple range method, one-way analysis of variance were used for analysis of data obtained from different samples. $P < 0.05$ was taken as indicative of statistical significance.

RESULTS

The synthesized mixture of ligand complexes showed yields that ranged from good to excellent yield (up to 89 %). The purity of the complexes was checked by melting point determination. Sharp melting points were obtained with fairly pure complexes. Low values of conductance measurements suggested the non-electrolyte nature of synthesized mixed ligand metal complexes. The chemical structures of all the synthesized compounds were deduced with the aid of physical and spectroscopic methods. Physical data and elemental (CHNS/O) analysis of these complexes were in good agreement with the proposed formulae. The general reaction equation (2) for the synthesis of the complexes is shown in Eq 2.

$$\text{OMZ} + \text{HQL} + \text{MCl}_2.n\text{H}_2\text{O} \longrightarrow \text{M(LL2)}.n\text{H}_2\text{O} + 2\text{HCl} + \text{H}_2 \text{ .... } (2)$$

where M = Zn(II) (n=0); M = Ni(II), Co(II) and Cu(II) (n = 2)

Zn (II) Complex

Yield: 89 %; colour: White solid; m.p: 266-268 °C; Molar conductance, Λ_m (in 10^{-3} M DMSO): 5.6 $ohm^{-1}cm^2mol^{-1}$; μ_{eff}: 1.71; Stability constant: 11.647 log K (L/mole); Selected IR (KBr, cm^{-1}): (S=O): 1023; $(C=N)_{bnz}$: 1555; $(C=N)_{HQL}$: 1632; (CO): 1723; (M-O): 479; (M-N): 435. Electronic spectra (λmax in nm):289, 229. ^{1}H NMR (400 MHz, δ, DMSO-d_6): 11.23 (s, 1H); 8.02, 8.41 (s, 2H); 7.00-7.34 (m, 7H); 6.97 (d, 1H); 4.69 (s, 2H, *CH$_2$-pyridine*); 3.51 (s, 6 H); 2.16 (d, 6H). Anal. Calc. for $C_{26}H_{25}N_4O_4SZn$ (MW: 554.95 g/mol): C, 56.27; H, 4.54; N, 10.10; S, 5.78; O, 11.53; Zn, 11.78% and Found: C, 56.36; H, 4.91; N, 10.19; S, 5.61; O, 11.59; Zn, 11.68%; MS (*m/*z, (relative abundance, %)): 554 [M$^+$, 9.5].

Co (II) Complex

Yield: 77 %; colour: Light green; m.p: 249-251 °C; Molar conductance, Λ_m (in 10^{-3} M DMSO): 5.9 ohm^{-1}cm^{2}mol^{-1}; μ_{eff}: 3.21; Stability constant: 11.421 log K (L/mole); Selected IR (KBr, *v*, cm^{-1}): (S=O): 1027; (C=N) $_{bnz}$: 1549; (C=N)$_{8HQ}$: 1635; (CO): 1731; (H_2O): 3600-2700; (M-O): 486; (M-N): 439. Electronic spectra (λmax in nm): 290, 226. ^{1}H NMR (400 MHz, δ, DMSO-d_6): 12.09 (s, 1H); 8.03, 8.36 (s, 2H); 7.03-7.43 (m, 7H); 6.81 (d, 1H); 4.63 (s, 2H, *CH$_2$-pyridine*); 3.53 (s, 4H; $2H_2O$); 2.11 (d, 6H). Anal. Calc. for $C_{26}H_{29}N_4O_6SCo$ (MW: 584.529 g/mol): C, 53.42; H, 5.00; N, 9.58; S, 5.49; O, 16.42; Co, 10.08% and found: C, 53.56; H, 4.91; N, 9.42; S, 5.69; O, 16.61; Co, 9.89%. MS (*m/*z, (relative abundance, %)):584 [M$^+$, 14.9].

Ni (II) Complex

Yield: 83 %; colour: Light Brown; m.p, 241-244 °C; Molar conductance, Λ_m (in 10^{-3} M DMSO): 5.7 ohm^{-1}cm^{2}mol^{-1}; μ_{eff}: 3.42; Stability constant: 10.567 logK(L/mole); Selected IR (KBr, *v*, cm^{-1}): (S=O): 1032; (C=N)$_{bnz}$: 1534; (C=N)$_{8HQ}$: 1637; (CO): 1733; (H_2O): 3600-2700; (M-O): 488; (M-N): 443. Electronic spectra (λmax in nm): 294, 223. ^{1}H NMR (400 MHz, δ, DMSO-d_6): 11.23 (s, 1H); 8.03, 8.41 (s, 2H); 7.02-7.31 (m, 7H); 6.99 (d, 1H); 4.64 (s, 2H, *CH$_2$-pyridine*); 3.53 (s, 6 H); 3.48 (s, 4H, $2H_2O$); 2.16 (d, 6H). Anal. Calc. for $C_{26}H_{29}N_4O_6SNi$ (MW: 584.29 g/mol): C, 53.45; H, 5.00; N, 9.59; S, 5.49; O, 16.43; Ni, 10.05% and Found: C, 53.78; H, 4.93; N, 9.64; S, 5.44; O, 16.49; Ni, 10.16%. MS (*m/*z, (relative abundance, %)):584 [M$^+$, 12.3].

Cu (II) Complex

Yield: 88 %; colour: Yellowish white: m.p., 223-227 °C; Molar conductance, Λ_m (in 10^{-3} M DMSO): 5.6 ohm^{-1}cm^{2}mol^{-1}; Magnetic susceptibility, μ_{eff}: 3.79; Stability constant: 11.289 logK (L/mole); Selected IR (KBr, *v*, cm^{-1}): (S=O): 1028; (C=N)$_{bnz}$: 1559; (C=N)$_{8HQ}$: 1623; (CO): 1727; *v*(H_2O): 3600-2700; *v*(M-O): 483; *v*(M-N): 449. ^{1}H NMR (400 MHz, δ, DMSO-d_6): 11.23 (s, 1H); 8.05, 8.44 (s, 2H); 7.02-7.71 (m, 7H); 6.91 (d, 1H); 4.68 (s, 2H, *CH$_2$-pyridine*); 3.59 (s, 6 H); 3.42 (s, 4H, $2H_2O$); 2.16 (d, 6H). Electronic spectra (λmax in nm): 343, 227 Anal. Calc. for $C_{26}H_{29}N_4O_6SCu$ (MW: 589.14 g/mol): C, 53.01; H, 4.96; N, 9.51; S, 5.44; O, 16.29; Cu, 10.79% and found: C, 53.28; H, 5.02; N, 9.49; S, 5.52; O, 16.19; Cu, 11.01%.MS (*m/*z, (relative abundance, %)): 589 [M$^+$, 13.7].

Magnetic susceptibility

The mixed ligand metal complexes had magnetic moments in the range of 1.71-3.79 B.M. Zn(II) complexes usually have distorted square planer configuration and a magnetic moment was 1.71 B.M. The Ni(II), Co(II) and Cu(II) complexes also support their octahedral geometry with magnetic moments of 3.42, 3.21 and 3.79 B.M, respectively.

FT-IR studies

The two main regions are of interest in mixed ligand complexes because they give valuable information about the coordination behavior of ligands to metals. These are C=N and S=O (sulphonic acid group). In the IR spectra of OMZ, a peak appeared at 3560 cm^{-1}. This peak was due to the stretching vibration of *v*(N-H) in benzimidazole. The stretching vibration of C=N occurs at 1586 cm^{-1} while that of S=O occurs at 1010 cm^{-1}. In the case of 8-hydroxyquinoline (HQL) molecules, the stretching vibration of *v(*CO) appears in medium peak at 1712 cm^{-1} whereas *v*(C=N) and *v(*OH) stretching vibrations occur at 1643 cm^{-1} and 3453 cm^{-1}, respectively.

In the FTIR spectra of the mixed ligand metal complexes, the stretching vibration of *v*(N-H) of the benzimidazole appeared at ~3060 cm^{-1}, indicating its non-involvement in coordination with metal ions. The peaks for *v*(S=O) (sulphonic acid group) appeared between 1023 and 1032 cm^{-1}, with a shift of 13-22 cm^{-1} to the higher wave number relative to OMZ, indicating the involvement of S=O group in the coordination of the complexes. The region between 1559-1534 cm^{-1} was allocated to the *v*(C=N)$_{bnz}$ vibrations of all the complexes with shift of 27-52 cm^{-1} to the lower wave number, than C=N bond become weak due to dπ-pπ (metal to ligand) back donation and it shifts to lower wave number which indicating its involvement in coordination with metal ions. In the case of stretching vibrations, *v*(C=N)$_{HQL}$ appeared at 1623-1637 cm^{-1} but it also shift to lower wave number of 6-20 cm^{-1}.

The stretching vibration of *v(*CO) (HQL) appeared between 1723-1733 cm^{-1}, with shift of 11-21 cm^{-1} to the higher wave number, confirming attachment to metal ions. The shifting of peaks of the C=N, S=O and CO on complexation gives valuable information about the point of attachment of metal ions to OMZ and 8- HQL; this also confirms that OMZ and HQL behave as bidentate ligands. In the case of HQL there is a considerable shift in the frequencies of O and C=N group and also acting as a bidentate ligand due to coordination with metal in all the complexes. The broad-stretching vibration bands found within the range 3600-2700 cm^{-1} was assigned to the coordination of water molecules in the Cu(II) and Ni(II) complexes. New absorption bonds of M-O stretching mode of vibration were assigned 479-488 cm^{-1} of the synthesized complexes, while M-N stretching vibration mode was assigned within the range of 435-449 cm^{-1}.

Electronic absorption spectra

The absorption spectra of 2.5 x 10^{-3}M solutions of OMZ, HQL and synthesized complexes in DMSO were recorded from 200 to 800 nm at room temperature. UV spectra showed high absorption intensity in ultraviolet (UV) region due to presence of benzimidazole chromosphere and these complexes are inactive in the visible region. Two strong bands were observed for OMZ at 257 and 300 nm, and for 8-hydroxyquinoline at 263 and 293 nm. These bands were attributed to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ electronic transition in the framework of the synthesized complexes. In all the complexes, bands that commonly appeared in- between 289-343 nm were attributed to intra ligand transitions consistent with electronic transition of C=N group. Upon complexation, the absorption bands of the complexes were slightly shifted to higher wavelength (red shift) compared to those of the free ligand omeprazole as well as 8-hydroxyquinoline. These modifications of the shifts and intensities of the absorption bands indicate the coordination of the ligand to the metal ions. A square-planar arrangement around Zn (II) complex is supported due to occurrence of intermolecular charge transfer and ligand-to-metal charge transfer (LMCT) at 289 and 229 nm, respectively. However in Co (II), Ni (II) and Cu (II) complexes, the structural geometry is octahedral due to attachment of water molecule. This was confirmed by FT-IR spectra of these complexes.

Morphological and structural properties of synthesized metal ligand complexes

The morphological and structural properties of the mixed ligand metal complexes were evaluated using scanning electron microscopy (SEM). The SEM micrographs are shown in Figure 2. Zn (II) and Co (II) complexes showed particles with sizes in the nanometer range, and their structures are homogenous. Some Ni (II) and Cu (II) complexes were irregularly-shaped and well-separated from each other, while most are present in agglomerated forms. Thus, these SEM results confirmed the nano-structured behavior of the synthesized metal complexes.

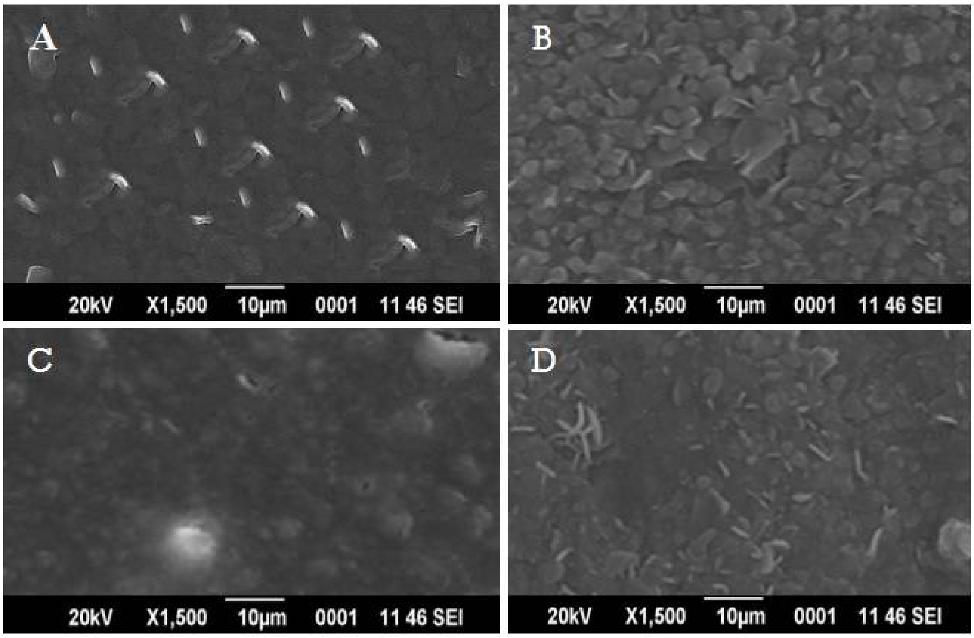

Figure 2: SEM images of mixed ligand metal complexes. (A) = Zn(II); (B) = Co(II); (C) = Cu(II) and (D) = Ni(II)

^{1}H NMR Spectra

The ^{1}H NMR spectra of the synthesized mixed ligand complexes was obtained in deuterated DMSO-d_6. In ^{1}H NMR spectrum, the signal that appeared around the δ 3.42-3.51 ppm was assigned to O-CH_3, while methylene (-CH_2) protons were responsible for the doublet peak at δ 4.64-4.69 ppm in all the synthesized complexes. The NMR signals for CH group were observed downfield in comparison to normal position because of the electron-withdrawing effect of neighboring atoms like nitrogen and oxygen in five-membered rings which make it more de-shielded.

There was a weak signal at 2.16 ppm which may be assigned to the methyl (-CH_3) protons but its integration showed otherwise. It may be that it was unusually merged with the DMSO peak. Due to non-involvement of NH proton in coordination, the singlet peak was observed at 11.23 ppm in all the synthesized complexes. The aromatic hydrogens were observed at δ 6.916-8.446 ppm. Figure 3 shows the ^{1}H NMR spectra of Zn (II) mixed ligand complex.

Mass spectra

The mass spectra were recorded for synthesized mixed ligand complexes at 300 °C and 70 eV. All the spectra exhibited parent peaks due to molecular ions. The proposed molecular formulae of these complexes were confirmed by comparing their molecular formula weights with m/z values. Molecular ion peaks were observed in complexes of Zn (II), Ni (II), Cu (II) and Co (II) at m/z 554, m/z 584, m/z 589, m/z 584, respectively. This was in line with the composition of synthesized complexes. Mass spectra of Zn (II) and Ni (II) complexes are shown in Figure 4 and Figure 5.

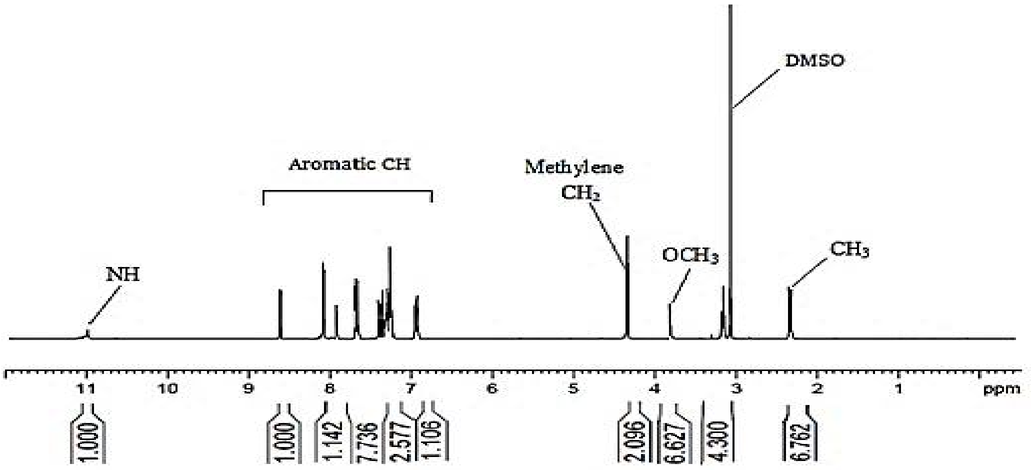

Figure 3: ^{1}H NMR spectra of Zn (II) mixed ligand complex

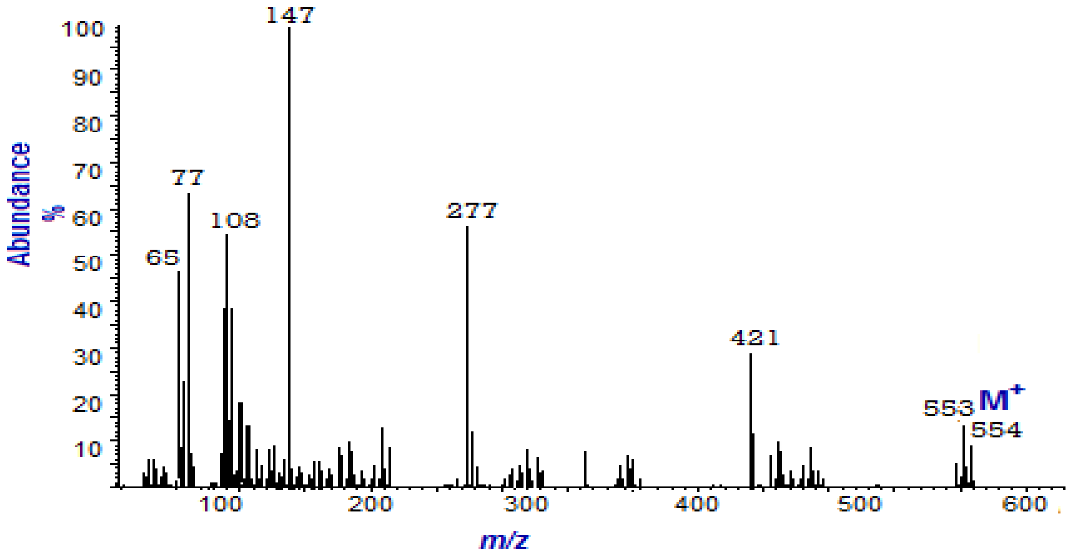

Figure 4: Mass spectra of Zn (II) mixed ligand Complex

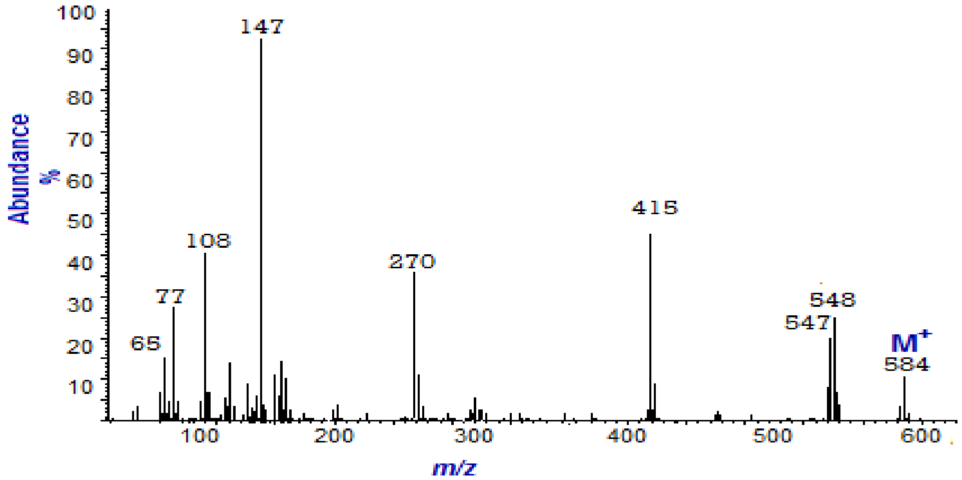

Figure 5: Mass spectra of Ni (II) mixed ligand complexes

Inhibitory effects on ALPs activity

OMZ, HQL and the mixed ligand complexes were examined for their inhibition against ALPs. All the synthesized complexes exhibited concentration–dependent inhibitory activities against ALPs. The exact mechanism involved in this inhibition is not clear. ALPs assay was carried out at different specimen concentrations (0.24, 0.48, 0.73 and 0.96 mM). Graphical representation of the effects of OMZ, HQL and synthesized complexes on ALPs is shown on Figure 6. It was also found that at a concentration of 0.96 mM, ALPs inhibition ranged from 80 to 98 % for all the complexes. Among the bimetallic complexes, the highest ALPs inhibition was observed with Ni (II) complex, followed by OMZ, HQL and other complexes. The order of inhibition of ALPs by the synthesized compounds was Zn (II) > Ni(II) > Co(II) > Cu(II) > OMZ > HQL.

DPPH radical scavenging activity

DPPH is a stable organic compound and it is extensively used for oxidative assay of the proton-donation ability of the samples. The results of free radical scavenging ability of the tested compounds are shown in Figure 7. All the complexes showed good antioxidant activity with respect to DPPH scavenging potential. Ni (II) complex (IC_{50} = 30.48±0.30) showed higher DPPH scavenging activity than Zn (II), Co (II) and Cu (II) complexes. Ni (II) complex was also better than BHT (IC_{50} = 40.31±0.4166 µM). The Cu (II) mixed ligand complex (IC_{50} = 36.11±0.37) was active but had lower DPPH radical–scavenging activity than other synthesized complexes and BHT. The order of DPPH scavenging was Ni (II) > Co(II) > Zn(II) > Cu(II) > OMZ > BHT > HQL.

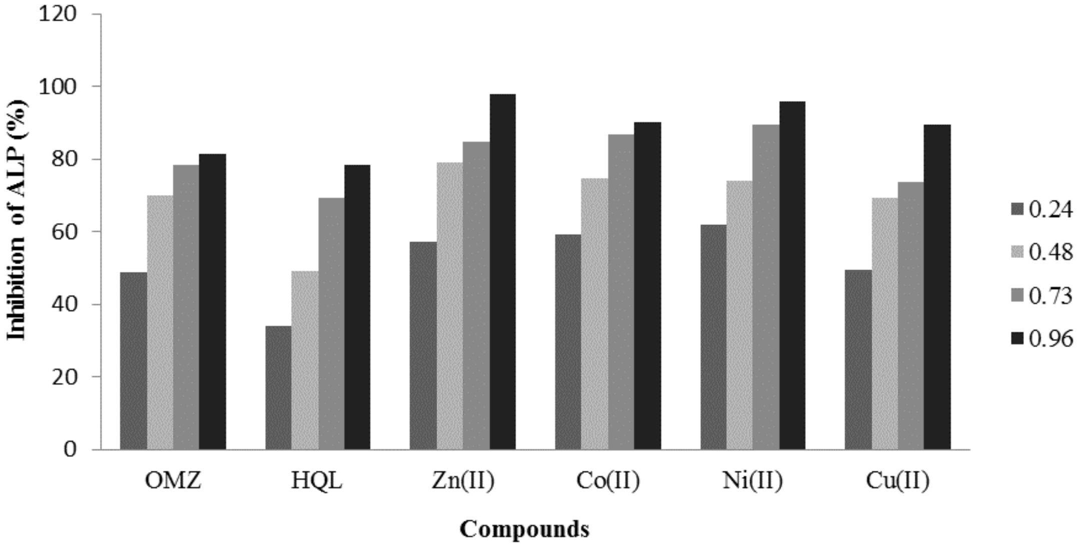

Figure 6: Inhibition of ALPs by OMZ, HQL and synthesized complexes

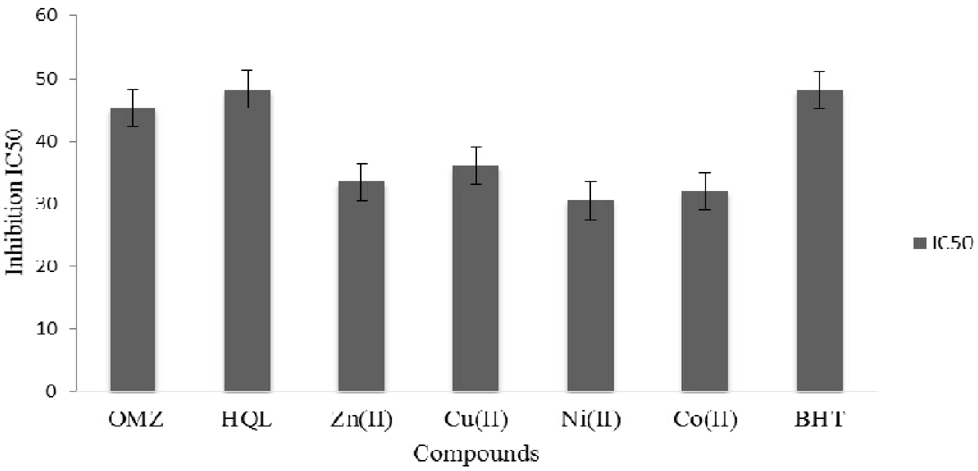

Figure 7: IC_{50} values in DPPH radical scavenging activities of ligands and synthesized complexes

Antibacterial activities of synthesized metal ligand complexes

The antibacterial activity of OMZ, HQL and synthesized complexes were investigated against three gram-negative strains*, E. coli, P. aeruginosa, and K. pneumoniae,* as well as three gram-positive strains, *A. viscosus, S. aureus and B. subtilis*. Cu(II) complex had the largest zones of inhibition against *E. coli*, *S. aureus* and *A. viscosus.* Ni(II) complex produced the largest antibacterial activity against *P. aeruginosa and E. coli.* Zn (II) complex higher inhibition zones for *K. pneumoiae* than any of the other complexes or Ampicillin. The order of antibacterial activity of the compounds was Cu(II) > Ni(II) > OMZ > Zn(II) > HQL > Ampicillin. The antibacterial effects of OMZ, HQL and synthesized complexes against both gram-negative and gram-positive microscopic organisms are shown in Table 1.

Antifungal activity of synthesized complexes

The antifungal activities of OMZ, HQL and synthesized complexes were determined against three parasitic strains: *C. albicans*, *A. flavus*, and *A. niger*. The complexes showed highest antifungal activity against fungal strains due to presence of S=O and C=N groups in their structures. Zn (II) complex showed the highest zones of inhibition against *C. albicans* and *A. niger,* while Co (II) complex had the highest activity against *C. albicans* and *A. flavus*. Cu (II) complex was more active against *A. niger* but lower in activity than OMZ. The order of antifungal activity of the compounds was Zn(II) >Co(II) >Ni(II) >Cu(II) >OMZ > HQL > Fluconazole. The antifungal activity of OMZ, HQL and synthesized complexes are shown in Table 2.

DISCUSSION

In the present study, new and mixed ligand metal complexes were synthesized, and characterized by elemental analysis, conductance measurements, UV-visible, Mass spectra, SEM, IR and ^{1}H-NMR.The major absorption bands in FTIR spectrum appeared at υ_{max}cm^{-1}1023-1032 (S=O, str.), 1559-1534 (C=N, str.)$_{bnz}$ and 1623-1637 for (C=N, str.)$_{HQL}$. Stretching vibration of

Table 1: Zones of inhibitions of OMZ, HQL and synthesized mixed ligand complexes (100 µg/mL) against bacterial strains

Compound	**Zone of Inhibition (mm)**					
	Gram positive bacteria			***Gram negative bacteria***		
	Bacillus subtilis	***Staphylococcus aureus***	***Actinomyces viscosus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Escherichia coli***
OMZ	23±0.21	21±0.03*	19±0.34	19±0.36	21±0.23	23±0.26
HQL	18±0.14	19±0.46	16±0.61	16±0.41*	14±0.72*	17±0.63
Zn (II)	21±0.25*	19±0.23*	18±0.46	17±0.37	19±0.31	21±0.27
Co (II)	19± 0.37	24±0.32*	17±0.61	24±0.21*	19±0.34	23±0.23
Ni (II)	19±0.32	20±0.33*	19±0.56	29±0.14	24±0.20	28±0.09*
Cu (II)	21±0.25*	30±0.11	29±0.34*	27±0.16	25±0.23	28±0.06*
*Ampicillin	14±0.43	16±0.37	15±0.46	14±0.52	13±0.73	15±0.41

**P<0.05,^{1}Standard drug; the diameter of inhibition zones are means of triplicate determinations± SEM and p < 0.05 were compared with negative control*

Table 2: Zones of inhibition of OMZ, HQL and synthesized mixed ligand complexes (100 µg/mL) against the fungal strains

Compounds	**Zone of inhibition (mm)**		
	Aspergillus nigar	***Aspergillus flavus***	***Candida albicans***
OMZ	18±0.33	17±0.52	21±0.24
HQL	14±0.54	16±0.63	13±0.52
Zn (II)	21±0.25	25±0.13	27±0.13
Co (II)	21±0.23	19±0.41	16±0.53
Ni (II)	24±0.13	23±0.24	18±0.36
Cu (II)	13±0.72	16±0.54	19±0.33
[1]FLuconazole	12±0.68	14±0.50	11±0.61

[1] *standard drug; the diameter of inhibition zones are means of triplicate determinations ± SEM.*

v(CO)$_{HQL}$ appeared at 1723-1733 cm^{-1}. This confirms coordination behavior with metal ions [19-22]. FTIR spectroscopy showed that OMZ and HQL acted as neutral bidentate ligands. The λ_{max} of OMZ was observed at 257 and 300 nm, and at 263 and 293 nm for 8-hydroxyquinoline. These absorption peaks are due to n→π* andπ→π* electronic transition [23]. The electronic spectra of the synthesized mixed ligand complexes were in the range of 300-350 nm. The presence of aliphatic and aromatic protons in the mixed ligand complexes was observed within the expected areas of the proton NMR spectra. The proposed structures of these complexes were confirmed by comparing their molecular formula weights with m/z values. The magnetic moment of all the synthesized complexes were observed in the range of 1.71-3.79 B.M. Spectroscopic studies used to verify the chemical composition of products (as well as geometry of the complexes and molar conductance) indicated that the synthesized metal complexes were non-electrolytes in nature. The complexes, at 0.96 mM exhibited 80-98 % inhibition of ALPs [17]. Zn(II) complex was more active against the enzyme than OMZ, HQL and other complexes. All the complexes showed higher antioxidant activity when compared to OMZ, HQL and BHT. The DPPH radical scavenging activity of Ni(II) complex (IC$_{50}$ = 30.48±0.3031) was highest when compared with those of Ni(II), Co(II) and Cu(II) complexes, as well as BHT (IC$_{50}$ = 40.31±0.42 µM) [24]. Cu (II) complex had the highest antibacterial activity against the bacterial strains, while Zn(II) complex had the highest antifungal activity against the fungal species.

CONCLUSION

A new series of mixed ligand complexes have been successfully synthesized and characterized by spectroscopic techniques. All the synthesized metal complexes possessed good antioxidant and antimicrobial properties. All the mixed ligand complexes demonstrated higher antioxidant, ALPs-inhibitory and antimicrobial activities than their corresponding ligands. This indicates their enormous therapeutic potential as future drug candidates for different diseases.

DECLARATIONS

Acknowledgement

This work was supported by the Department of Chemistry, School of Science, University of Management and Technology, Lahore, Pakistan.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

REFERENCES

1. *Bruni AT, Ferreira MRMC. Theoretical study of omeprazole behavior: Racemization barrier and decomposition reaction. Int J Quantum Chem 2008; 108: 1097-1106.*
2. *Souney PF, Matthews SJ. In Comprehensive Pharmacy Review, pp. 765-777, Harwal, 2nd edition, 1994.*

3. Munson K, Garcia R, Sachs G. Inhibitor and ion binding sites on the gastric H,K-ATPase. Biochemistry 2005; 44: 5267-5284.
4. Massoomi F, Savage J, Destache CJ. Omeprazole: a comprehensive review. Pharmacotherapy 1993; 13: 46-59.
5. Wallmark B, Brandstrom A, Larsson H. Evidence for acid-induced transformation of omeprazole into an active inhibitor of H+, K+-ATPase within the parietal cell. Biochim Biophys Acta 1984; 778: 549-558.
6. Kobayashiv K, Yamamoto T, Chiba K, Tani M, Ishizaki T, Kuroiwa Y. The effects of selective serotonin reuptake inhibitors and their metabolites on S-mephenytoin 4'-hydroxylase activity in human liver microsomes. Br J Clin Pharmacol 1995; 40: 481-485.
7. Gata L, Perna F, Figura N, Ricci C, Holton J, Danna L, Miglioli M.D, Vaira. Antimicrobial activity of esomeprazole versus omeprazole against Helicobacter pylori. J Antimicrob Chemother 2003; 51: 439-444.
8. Jonkers D, Stobberingh E, Stockbrugger R. Omeprazole inhibits growth of Gram-positive and Gram-negative bacteria including Helicobacter pylori in vitro. J Antimicrob Chemother 1996; 37: 145-150.
9. Russo MG, Hissi EGV, Rizzi AC, Brondino CD, Ibanez AGS, Vega AE, Silva HJ, Mercader R, Narda GE. Synthesis, physicochemical characterization, DFT calculation and biological activities of Fe(III) and Co(II)-omeprazole complexes. Potential application in the Helicobacter pylori eradication. J Mol Struct 2014; 10(61): 5-13.
10. Bosch ME, Sanchez AJR, Rojas FS, Ojeda CB. Analytical methodologies for the determination of omeprazole: an overview. J Pharm Biomed Anal 2007; 44(4): 831-844.
11. Cheatum CM, Heckscher MN, Crim FF, Excited-state dynamics in 8-hydroxyquinoline dimmers. Crim Chem Phys Lett 2001; 349: 37-42
12. Malik MR, Vasylyeva V, Merz K, Metzler-Nolte N, Saleem M, Ali S, Ahmad S. Synthesis, crystal structures, antimicrobial properties and enzyme inhibition studies of zinc(II) complexes of thiones. Inorg. Chim Acta 2011; 376: 207-211.
13. Ogunniran KO, Tella AC, Alensela M, Yakubu MT. Synthesis, physical properties, antimicrobial potentials of some antibiotics complexed with transition metals and their effects on alkaline phosphatase activities of selected rat tissues. Afr J Biotechnol 2007; 6(10): 1202-1208.
14. Khan SA, Rasool N, Riaz M, Nadeem R, Rashid U, Rizwan K, Zubair M, Bukhari IH, Gulzar T. Evaluation of Antioxidant and Cytotoxicity Studies of Clerodendrum inerme. Asian J Chem 2013; 13: 7457-7462.
15. Noipa T, Srijaranai S, Tuntulani T, Ngeontae W. New approach for evaluation of the antioxidant capacity based on scavenging DPPH free radical in micelle systems. Food Res Int 2011; 44(3): 798-806.
16. Khan SA, Shahid S, Jameel M, Ahmad A. In vitro Antibacterial, Antifungal and GC-MS Analysis of seeds of Mustard Brown. Int J Pharm Chem 2016; 6(4): 107-115.
17. Hussain S, Ali S, Shahzadi S, Shahid M. Heterobimetallic complexes containing Sn(IV) and Pd(II) with 4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid: Synthesis, characterization and biological activities. Cogent Chemistry 2015; 1: 1-13.
18. Nakao M, Tada M, Tsuchimori K, Uekata M. Antibacterial properties of lansoprazole alone and in combination with antimicrobial agents against Helicobacter pylori. Eur J Clin Microbiol Infect Dis 2005; 14: 391-399.
19. Das S, Malliik S, Jaiin B. Physicochemical characterization and synthesis of transitions metal complex of omeprazole. Int J Ad Sci Techni Res 2014; 4(4): 519-525.
20. Krishnakumar V, Ramasay R. DFT studies and vibrational spectra of isoquinoline and 8-hydroxyquinoline. Spectrochim Acta A 2005; 61(4): 673-683.
21. Mohamed GG, Nour-el-dien FA, Khalil SM, Mohammad AS, Metal complexes of omeprazole. Preparation, spectroscopic, thermal characterization and biological activity. J Coord Chem 2009; 62(4): 645-654.
22. Verma S, Shrivastva S, Rani P. Synthesis and physico-chemical studies of newly formed complexes of transition and inner transition metlas with (5-methoxy-2-{{(4-methoxy-3,5- dimethy-1-pyridiny) methyl}sulfinyi}-IH-benzimidazole). Der Chemica Sinica 2011; 2(5): 12-21.
23. Malik S, DAS S, Jain B. First- row transition metal complexes of omeprazole as anti-ulcerative drugs. Indo J Chem 2010; 10(3): 382-389.
24. Noipa T, Srijaranai S, Tuntulani T, Ngeontae W. New approach for evaluation of the antioxidant capacity based on scavenging DPPH free radical in micelle systems. Food Res Int 2011; 44(3): 798-806.