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Research Article

PHARMACOLOGICAL ACTIVITY OF (E) 3-2-(1-(1-HYDROXYNAPHTHALEN-2-YL) METHYLENEAMINO) PHENYL) -2-METHYLQUINAZOLINE-4 (3H) -ONE SCHIFF BASE AND ITS TRANSITION METAL COMPLEXES

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ABSTRACT

Objectives: To evaluate the biological activity of newly synthesized Schiff base ligand (E) 3-2-(1-(1 hydroxynaphthalen-2-yl) methyleneamino) phenyl) -2 methylquinazoline-4 (3H) -One (HNMAPMQ) and its transition metal complexes.

Methods: The newly synthesized ligand (HNMAPMQ) was prepared by the condensation of 3-(2-aminophenyl) -2-methylquinazolin-4 (3H) -one and 2-hydroxy-1-naphthaldehyde. The spectral and structural characterization of the ligand (HNMAPMQ) and its metal complexes were attained by the aid of their elemental analyses and various spectral studies such as IR, ^{1}H NMR and Mass.

The biological activities such as antioxidant, antitubercular and antimicrobial of ligand (HNMAPMQ) and its metal complexes were also studied.

Results: According to the analytical and spectroscopic studies, the ligand (HNMAPMQ) acts as tridentate ONO donor towards divalent metal ions (Cu (II), Co (II), Ni (II) and Mn (II)) with the involvement of phenolic oxygen, azomethine nitrogen, carboxylato oxygen there by suggesting the octahedral geometry, whereas Zn(II) complex show tetrahedral geometry.

All the metal complexes shows promising antioxidant activity than the ligand (HNMAPMQ) as compared with Butylated hydroxy anisole (BHA), Tertiary Butylated hydroxyl quinoline (TBHQ) and Ascorbic acid (AA). Similarly the antitubercular activity of ligand (HNMAPMQ) and its metal complexes showed good results. The results obtained from the antimicrobial studies clearly reveals that all the metal complexes show superior activity than the ligand (HNMAPMQ).

Conclusion: The newly synthesized ligand (HNMAPMQ) and its metal complexes shows good antioxidant, antitubercular and antimicrobial activities thus, can be used as a new drug of choice in the field of pharmacy.

Keywords: Quinazoline, Antioxidant, Antitubercular, DNA Cleavage, Antimicrobial Activities.

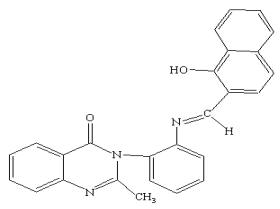
INTRODUCTION

In view of broad applications of Schiff bases and their metal complexes the research work in the field of coordination chemistry is improved, but still there is a lot of challenging work has been carried out on Schiff base metal complexes along with their different industrial and chemotherapeutic studies. Schiff base complexes are considered to be among the most important stereo chemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety [1]. However the incorporation of transition metal ions into these compounds have enormous wide applications in the field of the food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical along with biological activities and decrease in the cytotoxicity of both metal ion and Schiff base [2-3].

The Schiff base ligands also serve as a cation carrier in potentiometric sensors as they have shown excellent selectivity, sensitivity and stability towards specific metal ions such as Cu(II), Co(II), Ni(II) and Zn(II) [4]. In general ortho-substituted with a hydroxyl group have primarily arouse the researchers' interest, 2-Hydroxy-1-naphthaldehyde and its Schiff base have shown significant attention with regard to their chelating ability with the transition metal ions [5]. On the other hand Schiff bases derived from o-phenylenediamine and its transition complexes also possess variety of applications including biological, clinical and analytical. In addition to that they have been reported to exhibit photo-luminescence and catalytic activity [6-7]. The o-phenylenediamine acts as a key intermediate used in the production of fungicides, corrosion inhibitors, various pigments, pharmaceuticals compunds. Furthermore it was also used to remove sulfur from ores and coloration by aldehydes in polymeric products [8].

The extensive literature on heterocyclic compounds revels that there exists a strong connection between wide spectrums of biological activities with quinazoline-4(3H)-One containing pyrimidine moiety in their structure [9-10]. The compound containing pyrimidine ring system present in nucleic acids, several vitamins, coenzymes and antibiotics provides potential binding sites for metal ions and their coordination nature is important in the perceptive the role of metal ions in biological systems [11]. Majority of quinazoline-4(3H)-one derivatives possess diverse activities such as, anti-convulsant, anti-inflammatory, hypnotic, anti-tumor, anti HIV, CNS depressant, analgesic, anthelmintic, anti-allergic, anti-malarial and anti-oxidant [12-13], for this reason quinazoline-4 (3H) -one was considered to be a lead compound for designing potential bioactive agents and the stability of the quinazoline nucleus has inspired medicinal chemists to introduce many bioactive moieties in order to synthesize new potential medicinal agents [14].

In continuation of our work on the chemistry of quinazoline-4 (3H) -one Schiff base and its transition metal complexes [15]. The available literature survey on chemical, analytical and chemotherapeutic application of Schiff base, o-phenylenediamine and quinazoline-4(3H)-One stimulated our interest to synthesize a new (E) 3-2-(1-(1-hydroxynaphthalen-2-yl) methyleneamino) phenyl) -2-methylquinazoline-4(3H)-One (HNMAPMQ) Schiff base ligand (Figure 1) and its metal complexes. The newly synthesized compounds were screened for their anti-oxidant, antitubercular, DNA cleavage and antimicrobial potency.



(E)-3-(2-((1-hydroxynaphthalen-2-yl)methyleneamino)phenyl)-2-methylquinazolin-4(3H)-one

(HNMAPMQ)

Fig. 1: Structure of ligand (HNMAPMQ)

MATERIALS AND METHODS

Chemical and Solvents

All the chemicals and reagents were of AR grade and used without further purification. The organic chemicals such as o-phenylenediamine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Butylated hydroxy anisole (BHA), Tertiary Butylated hydroxyl quinoline (TBHQ), Ascorbic acid (AA) and the metal salts such as Copper (II), Nickel (II), Cobalt (II), Manganese (II) and Zinc (II) chlorides were procured from Merck/Aldrich (India).

Instruments

Elemental analysis (C, H and N) was carried out using Flash EA 1112 series elemental analyzer. IR spectra of the ligand (HNMAPMQ) and its complexes were performed by Perkin Elmer Spectrum one FT-IR spectrometer. The ^{1}H NMR spectra were recorded on AMX-400 NMR spectrometer, using TMS as internal standard. Mass spectra were recorded with a JEOL GCMATE II GC-MS mass spectrometer. The molar conductance data were recorded on the ELICO-CM-82T Conductivity Bridge. Magnetic susceptibilities were measured on a Guoy balance at room temperature using $Hg[Co(NCS)_4]$ as calibrant and Interaction of metal complexes with Calf thymus DNA was performed in 0.01M buffer (pH 7.2).

Preparation of 3-(2-aminophenyl)-2-methyl quinazolin-4(3H)-one

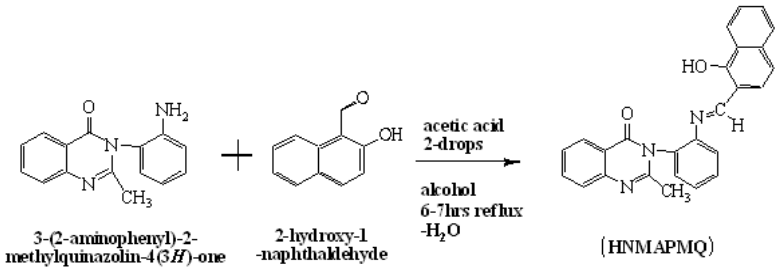
A homogeneous quantity of 2-methyl-aminobenzoate (1.61 g, 0.01 mol) and o-phenylenediamine (1.08 g, 0.01 mol) in ethanol (25 ml) was heated to reflux for 2-3 h on water bath. The resulting mixture was then concentrated and the precipitated solid thus separated, filtered off followed by washing with hot ethanol and finally recrystallized from toluene.

Preparation of Schiff base ligand (HNMAPMQ)

Scheme 1 shows the preparation of ligand (HNMAPMQ) as follows, 30 ml hot ethanolic solution of 3-(2-aminophenyl)-2-methylquinazolin-4(3H)-one (2.51 g, 0.01 mol) was mixed with 25 ml ethanolic solution of 2-hydroxy-1-naphtholdehyde (1.72 g, 0.01 mol), the resulting solution was stirred vigorously for few minutes and refluxed on a water bath for about 5-6 h. The progress of the reaction was monitored regularly by the TLC. The yellow colored precipitated thus formed on evaporation of the solvent was cooled, filtered followed by washing with methanol. The recrystallization of the ligand (HNMAPMQ) was carried out from hot methanol.

Preparation of metal (II) complexes

A general method has been used for the preparation of complexes using the reaction of metal salts and the corresponding Schiff-base in a molar ratio (M: L = 1:1 and 1:2). A methanolic solution of ligand (HNMAPMQ) (35ml, 0.01 mmol) and Cu(II), Ni(II) Co(II), Mn(II) and Zn(II) chlorides (10 ml, 0.01 mmol) both were mixed gently and refluxed for 3 h. The volume of the resulting solution was concentrated to one-half, by evaporating the solvent and 0.5 g of sodium acetate was added to adjust the p^H 7-8. The reaction mixture was further refluxed for 2 h more and then cooled to room temperature which solidified on cooling. The solid thus obtained was filtered, washed thoroughly with hot methanol to apparent dryness and dried in vacuum over fussed $CaCl_2$.



Scheme 1**: Synthetic route for the preparation of ligand (HNMAPMQ)**

Biological study

Antioxidant activity: Radical Scavenging Activity

The radical scavenging activity of ligand (HNMAPMQ) and its complexes were determined by using 1, 1-diphenyl-2, 2-picryl hydrazyl free radical (DPPH•) assay method [16]. DPPH• is a stable free-radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule and also contained an odd electron in its structure that frequently used for detection of the radical scavenging activity in chemical analysis [17]. The reduction capability of DPPH• radicals was determined by a decrease in its observance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was found at 517 nm, thus resulting from a color change from purple to yellow, the absorbance decreased when the DPPH• is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH• molecule. In the radical form, this molecule shows an absorbance at 517 nm, which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic spin-paired molecule [18]. The stock solutions (1mg/mL) of the ligand (HNMAPMQ) and its complexes were diluted to a final concentration of 25, 50, 75 and 100 µg/ml in methanol. DPPH• in methanol solution (1 ml, 0.1 mmol) was added to 2.5 ml of test solution of different concentrations and allowed to react at room temperature. After 30 min the absorbance was measured at 517 nm. A graph was plotted with concentration (µg/ml) on the x-axis and percentage scavenging effects on the y-axis. Radical scavenging activity was expressed as a percentage and calculated using the following formula.

$$\text{Scavenging effect (\%)} = \frac{\text{Ac (control)} - \text{As (sample)}}{\text{Ac(control)}} \times 100$$

Where As (sample) is the absorbance of the test sample and Ac (control) is the absorbance of the control. The scavenging capability of the ligand (HNMAPMQ) and its complexes were compared with standard drugs namely Butylated hydroxyl anisole (BHA) and Tertiary butylated hydroxyl quinoline (TBHQ) and ascorbic acid.

Antitubercular activity

The ligand (HNMAPMQ) and its complexes were evaluated in vitro for antitubercular activity against *M. tuberculosis* using Micro plate Alamar Blue Assay (MABA) [19]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method [20].

DNA cleavage study

Preparation of culture media for the DNA cleavage studies of metal complexes and the isolation of DNA were carried out according to the literature procedure [21].

Agarose gel electrophoresis

The DNA cleavage products were tested by agarose gel electrophoresis method [22]. The stock solution of complexes was prepared by dissolving 10 mg of the compounds in 10 ml of DMSO. The sample (25 µg/mL) was added to the isolated DNA of Calf-thymus (CT-DNA) and incubated for 2 h at 37 °C and then 20 µL of DNA sample (mixed with bromophenol blue dye at a 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with a

standard DNA marker in TAE buffer (4.84 g Tris base, pH 8.0; 0.5 M EDTA/1L) and finally loaded onto the agarose gel, then a constant electricity (50 V) was applied for about 30 min. Finally, the gel was removed and stained with 10 µg/ml of ethidium bromide for 10-15 min. The bands obtained was observed under the Vilberlourmate Gel documentation system followed by photographed to determine the extent of DNA cleavage as compared with standard DNA marker.

In vitro Antibacterial and Antifungal Bioassay

The newly synthesized ligand (HNMAPMQ) and its complexes have been subjected to their in vitro antimicrobial screening programmers by the serial tube dilution technique so as to find out their suitability as potential chemotherapeutic and medicinal agents [23]. All the MTCC cultures were collected from Chandigarh, the antibacterial and antifungal activities of the ligand (HNMAPMQ) and its complexes were tested against two gram-positive bacteria such as *Staphylococcus aureus* (MTCC No. 7443), *Bascillus subtilis* (MTCC No. 9878), one gram- negative *Escherichia coli* (MTCC No. 1698) and two fungi *Aspergillus niger* (MTCC No. 281) and *Aspergillus flavus* (MTCC No. 277) using cup plate method.

The antibacterial activities of all test organisms were evaluated on Mueller Hinton agar and antifungal activities on Potato dextrose agar (PDA) by making a lawn of test organisms (maintained at 10^6 cfu/ ml) with the help of cotton swabs, the holes of 6 mm diameter were punched carefully using a cork borer and then these holes were filled with different concentration of test solution. The compounds to be tested were dissolved in DMF at a concentration 0.25 mg/ml.. The MIC was measured after 24 h in case of antibacterial activity, 48 h for antifungal activity. The control containing only DMSO and the standard antibiotics (*Gentamicine and fluconazole*) was also kept for comparison.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) represents the lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganisms. In general the MIC methods are widely used in the comparative testing of new agents. In clinical laboratories they are used to establish the susceptibility of organisms that give oblique results in disk tests. The MICs of ligand (HNMAPMQ) and its complexes against *Staphylococcus aureus* (MTCC No. 7443), *Bascillus subtilis* (MTCC No. 9878), *Escherichia coli* (MTCC No. 1698) (bacterial strains), *Aspergillus niger* (MTCC No. 281) and *Aspergillus flavus* (MTCC No. 277) (fungal strains) were determined by liquid dilution method [24]. The stock solutions of ligand (HNMAPMQ) and its metal complexes with 4, 8, 16, 32, 64 and 128 µg/mL in DMSO solvent were prepared along with the standard drugs (*Gentamicine* and *fluconazole*) at the same concentration.

RESULTS AND DISCUSSION

The newly synthesized ligand (HNMAPMQ) and its complexes were colored, stable and non hygroscopic at room temperature. The complexes were sparingly soluble in common organic solvents while completely soluble in DMF and DMSO. The evidence drown for the formation of the ligand (HNMAPMQ) and its complexes has been done by using their analytical data, spectral techniques (IR, ^{1}H-NMR and Mass spectra's) and magnetic studies. Thus on the basis of above results the possible geometry around the metal ion was evidently confirmed in such a way that the Cu(II), Co(II), Ni(II) and Mn(II) complexes form an octahedral while Zn(II) complex form tetrahedral geometries. The observed molar conductance values of all the metal complexes in 10^{-3} M DMF solution was found in the range 16-27 Ohm^{-1} cm^2 mol^{-1} indicating the non-electrolytic nature. The metal content of the complexes was analyzed by decomposing the complexes with a mixture of HNO_3 and HCl followed by H_2SO_4 and chloride were determined as AgCl, respectively by following a standard procedure [25]. The elemental analysis (C, H and N) data of ligand (HNMAPMQ) and its complexes are in good agreement with proposed molecular formulas. In addition to this keeping in view of the increasing problems of antimicrobial resistance, the ligand (HNMAPMQ) and its complexes were screened for their antioxidant, antitubercular, DNA cleavage and antimicrobial potency, which may be used for formulating novel medicinal and chemotherapeutic agents.

Table 1: Elemental analysis, magnetic data of ligand (HNMAPMQ) and its complexes.

Ligand / Complex	Mol. Wt. (g/mol)	Color	Mp. (°C)	Yield (%)	Elemental analysis found and (calculated) %					μ_{eff} (BM)	Molar conductance (Ω cm^2mol^{-1})
					C	H	N	M	Cl		
$C_{26}H_{19}N_3O_2$ (HNMAPMQ)	405.45	yellow	247	82	76.92 (77.02)	4.60 (4.72)	10.24 (10.36)	-	-	-	-
$[Cu(C_{26}H_{18}N_3O_2)_2]$	872.43	Light green	282	87	71.42 (71.59)	4.08 (4.16)	9.47 (9.63)	7.14 (7.28)	-	1.84	27.15
$[Ni(C_{26}H_{18}N_3O_2)_2]$	867.57	Greenish	270	74	71.89 (72.01)	4.04 (4.18)	9.58 (9.69)	6.58 (6.77)	-	3.45	25.49
$[Co(C_{26}H_{18}N_3O_2)_2]$	867.81	Light Pink	287	80	71.83 (71.97)	4.05 (4.18)	9.56 (9.68)	6.63 (6.79)	-	4.52	24.37
$[Mn(C_{26}H_{18}N_3O_2)_2]$	863.82	White	274	79	72.21 (72.30)	4.14 (4.20)	9.58 (9.73)	6.22 (6.36)	-	5.78	22.64
$[Zn(C_{26}H_{18}N_3O_2)Cl)]$	505.30	Cream white	283	86	61.65 (61.80)	3.48 (3.59)	8.24 (8.32)	12.80 (12.94)	6.91 (7.02)	Diam	16.87

IR spectral data

The IR spectral data along with the possible assignments of the ligand (HNMAPMQ) and its complexes are presented in Table 2. The nature and possible mode of bonding of the ligand with different metal ion have been studied by comparing IR spectra of ligand and complexes. The coordination of metal with the ligand causes shifts of bands of the ligand to slightly lower or higher frequencies with different intensities [26]. The ligand (HNMAPMQ) exhibits the following bands in its IR spectrum, a broad band at 3408-3392 cm^{-1}due to ν(-OH) of naphthalene moiety. A band at 1582-1568 cm^{-1} corresponds to a characteristic high intensity due to azomethine group (>C=N). Furthermore a strong high intensity band in the region 1728-1712 cm^{-1} was observed due to the carboxyl group of quinazoline ring (C=O). Moreover, upon complexation of ligand (HNMAPMQ) with different metal ions it experiences subsequent changes in its spectrum as follows. The disappearance of broad band due to ν(-OH) indicating the participation of phenolic oxygen via deprotonation [27]. The band due to azomethine observed at 1578-1562 cm^{-1} showed a major downfield shift to lower wave number by 15-20 cm^{-1} suggesting the coordination of azomethine (>C=N) nitrogen with the metal ion [28] and the strong intensity band due to quinazoline ring (C=O) experiences a negative shift of 20–30 cm^{-1} indicates the participation of carboxyl group in complex formation [29].

In the low-frequency region, the spectra's of the metal complexes exhibited new bands which are not present in the spectrum of ligand (HNMAPMQ). These bands assigned due to *ν* (M–O) and *ν* (M–N) stretching vibrations observed at 554-542 and 460-448 cm^{-1} which interns support the involvement of oxygen and nitrogen atoms in coordination [30]. In addition to this a weak band observed in the region 356-350 cm^{-1}due to the formation of the M-Cl bond, which was characteristic of the chloride atom in [Zn ($C_{26}H_{18}N_3O_2$) Cl)] complex. Thus from the above results the evidence for the involvement of the tridentate donor site of ligand (HNMAPMQ) has

been confirmed via phenolic oxygen, azomethine nitrogen and carboxylato oxygen with different metal ions.

Table 2: The diagnostic infrared frequencies (in cm^{-1}) of ligand (HNMAPMQ) and its complexes.

Ligand/Complex	$\nu_{OH/H2O}$	$\nu_{C=O}$	$\nu_{C=N}$	ν_{C-O}	ν_{M-O}	ν_{M-N}	ν_{M-Cl}
$C_{26}H_{19}N_3O_2$ (HNMAPMQ)	3408	1728	1582	1315	-	-	-
$[Cu(C_{26}H_{18}N_3O_2)_2]$	-	1710	1570	1326	552	463	-
$[Ni(C_{26}H_{18}N_3O_2)_2]$	-	1706	1567	1324	547	454	-
$[Co(C_{26}H_{18}N_3O_2)_2]$	-	1714	1565	1323	544	450	-
$[Mn(C_{26}H_{18}N_3O_2)_2]$	-	1701	1571	1325	540	456	-
$[Zn(C_{26}H_{18}N_3O_2)Cl)]$	-	1707	1562	1322	546	452	358

^{1}H NMR Spectral study

The ^{1}H NMR spectra of the ligand (HNMAPMQ) and its diamagnetic $[Zn(C_{26}H_{18}N_3O_2)Cl)]$ complex were recorded in DMSO-d_6 using TMS as an internal standard. The ^{1}H NMR spectra of ligand (HNMAPMQ) exhibit signals at δ 9.82 ppm (s, 1H) assigned to phenolic-OH of naphthalene moiety, 8.45 ppm (s, 1H) assigned to -CH=N followed by signals at 2.50 ppm (s, 3H), 2.53 ppm (s, 3H) assigned to -2CH_3 protons and 6.84-8.87 ppm (m, 14H) assigned to multiplets aromatic protons.

In comparison with the spectrum of ligand (HNMAPMQ), the ^{1}H NMR spectrum of $[Zn(C_{26}H_{18}N_3O_2)Cl)]$ complex shows disappearance of signal due to -OH (9.82 ppm) proton via deprotonation [31]. The signal due to azomethine proton experiences a downfield shift and appeared at 8.51 ppm show the contribution of -CH=N nitrogen in coordination [32]. The methyl protons show a slight shift in the signals and resonated in the region of 2.54 ppm (s, 3H) and 2.58 ppm (s, 3H). The signals due to aromatic protons owing to quinazoline and phenyl rings were observed as a multiplet in the region 6.88-8.93 ppm (m, 14H).

Thus based upon above discussion it was concluded that the total number of protons calculated from the integration curves and those obtained from the values of their expected C, H and N analysis be in agreement with each other and it was also observed that DMSO did not have any coordinating effect neither on the legend (HNMAPMQ) nor on its [Zn ($C_{26}H_{18}N_3O_2$) Cl)] complex respectively.

Molar conductance and Magnetic Susceptibility Measurements

In order to obtain further structural information about the metal complexes their molar conductance on the Conductivity Bridge in DMF solution (10^{-3} M) and Magnetic Susceptibility on Guoy balance at room temperature were determined (Table 1). The conductivity measurement has frequently been used in structure elucidation of metal chelate i.e. the possible mode of bonding within the limit of their solubility, they also provide a method of testing the degree of ionization of the complexes. The measured molar conductance values of metal complexes are in the range 16-27 Ohm^{-1} cm^2 Mol^{-1} representing the non-electrolyte nature of complexes [33]. The effective magnetic moment values for Cu(II), Ni(II), Co(II) and Mn(II) complexes were lie in the range of 1.80–1.85 BM, 3.34–3.62 BM, 4.39–4.90 BM, and 5.62-5.90 BM respectively, which was characteristic of mononuclear, Cu(II) (d^9) one unpaired electron per Cu(II) ion with slight orbital contribution to the spin only value and the absence of spin–spin interactions in the complexes accounting for distorted octahedral geometry, Ni(II) (d^8) two unpaired electrons per Ni(II) ion, Co(II) (d^7) three unpaired electrons per Co(II) ion and Mn(II) (d^5) five unpaired electrons per Mn(II) ion respectively, suggesting consistency with their octahedral environment [34].

Mass spectral studies

The formation of ligand (HNMAPMQ) and its complexes were further investigated by their mass spectral analysis. The mass spectrum of ligand (HNMAPMQ) shows the formation of a molecular ion peak at m/z = 405 $[M]^+$corresponds to the empirical formula $[C_{26}H_{19}N_3O_2]^+$ which was also supported by the nitrogen rule, since the compound containing three nitrogen atoms. The mass spectrum of diamagnetic $[Zn(C_{26}H_{18}N_3O_2)Cl)]$ showed the formation of molecular ion peaks at m/z = 505 $[M]^+$and 507 [M+2]. This stoichiometry is further supported by the elemental data which are in close agreement with the values calculated from molecular formulae assigned to this complex. Thus, the mass spectral data reinforce the conclusion drawn from the physical and analytical values.

Biological results

Antioxidant activity

The antioxidant activity of ligand (HNMAPMQ) and its complexes were investigated by comparing with the standard drugs such as BHA, TBHQ and Ascorbic acid using spectrophotometer at 517 nm. From the investigation it was clearly observed that metal complexes scavenge DPPH· effectively than ligand (HNMAPMQ), suggesting their stronger free radical scavengers and excellent antioxidant properties. In the present study the Cu(II), Ni(II), Co(II) and Zn(II) complexes shows greater activity while Mn(II) complexes are modest activity as a radical scavenger compared with standards as shown in Figure 2. Consequently these results are in agreement with earlier studies of metallic complexes in which the ligand has antioxidant activity and it is expected that the metal ion will increase its activity extensively [35]. Their for due to the greater scavenging activity of ligand (HNMAPQ) and its metal complexes may possibly contribute to their claimed antioxidant property and lead to chemical entities with potential for clinical chemotherapeutic usage [36].

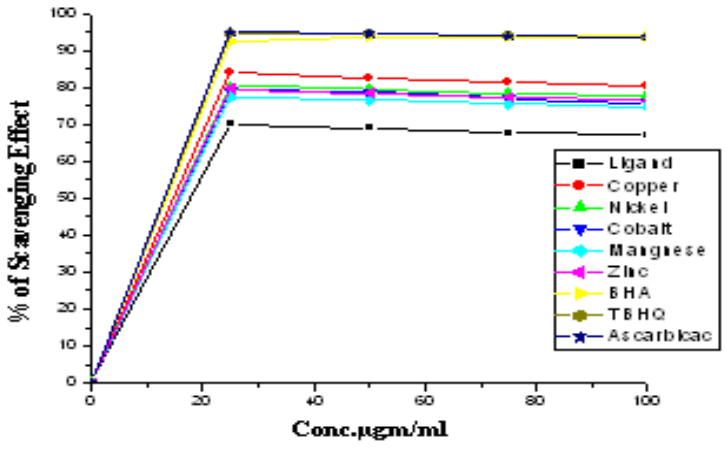


Fig. 2: Antioxidant data of ligand (HNMAPMQ) and its complexes

Antitubercular activity

The encouraging results obtained from the antimicrobial studies prompted us to attempt preliminary screening of ligand (HNMAPMQ) and its complexes for their in vitro antituberculosis activity, expressed as MIC. Compounds were assayed for their inhibitory activity toward *M. tuberculosis H37Rv* and the results were compared with standard anti-TB drugs namely *Pyrazinamide* and *Streptomycin*. The results of the analysis clearly reveal that ligand (HNMAPMQ) shows inhibition at concentrations with MIC 12.5µg/mL. While Cu(II), Co(II) and Ni(II) complexes shows superior activity with MIC 6.25 µg/mL similar to that of *Streptomycin* but less than *Pyrazinamide* drug, whereas Mn(II) and Zn(II) complex registered the MIC at 12.5 µg/mL showed average activity as shown in Table 3. The superior antitubercular activity Cu(II), Co(II), Ni(II), Mn(II) and Zn(II) complexes mainly due to slow release of the ligand inside the mycobacterium which can be explained on the basis of chelation and Tweedy's theory [37]. On chelation, the polarity of the central metal ion will be reduced to a greater extent results in the overlap of the ligand orbital and also involve the partial sharing of the positive charge of the metal ion with donor groups, which in turns increases the delocalization of the π-electrons over the whole chelate ring and increases the lipophilicity of the metal complexes as a result the metal complexes can easily penetrate into the lipid membranes and blocks the metal binding sites of enzymes of the microorganisms. Furthermore these metal complexes also affect the respiration process of the cell and consequently block the synthesis of proteins, which restrict further growth of the organism [38].

DNA cleavage activity

The metal (II) complexes synthesized from tridentate ligand (HNMAPMQ) were subjected to their DNA cleavage activity by gel electrophoresis method. The Cu(II) (lane I), Ni(II) (lane II), Co(II) (lane III) and Zn(II) (lane V) complexes shows complete DNA cleavage activity whereas Mn(II) (lane IV) show partial cleavage as presented in Figure 3. A difference in the bands of complexes (lanes I-V) was observed as compared to that of control Calf-thymus DNA. The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability [39]. Hence it was accomplished that the control DNA alone does not show any apparent cleavage, whereas its complexes show promising activity, along with this it explain the fundamental role of metal ions in these isolated DNA cleavage reactions. However, the nature of the reactive intermediates involved in the DNA cleavage by the complexes is not clear. Thus from the above, it was clear concluded that as the complexes was observed to cleave the DNA, therefore inhibits the growth of the pathogenic organism by cleaving the genome [40].

Table 3: In vitro antitubercular activity of the ligand (HNMAPMQ) and its metal complexes.

Ligand/complexes	Concentration of MIC (µg/mL)					
	100	50	25	12.5	6.25	3.125
$C_{26}H_{19}N_3O_2$ (HNMAPMQ)	S	S	S	S	R	R
$[Cu(C_{26}H_{18}N_3O_2)_2]$	S	S	S	S	S	R
$[Ni(C_{26}H_{18}N_3O_2)_2]$	S	S	S	S	S	R
$[Co(C_{26}H_{18}N_3O_2)_2]$	S	S	S	S	S	R
$[Mn(C_{26}H_{18}N_3O_2)_2]$	S	S	S	S	R	R
$[Zn(C_{26}H_{18}N_3O_2)Cl)]$	S	S	S	S	R	R
Pyrazinamide	S	S	S	S	S	S
Streptomycin	S	S	S	S	S	R

Pyrazinamide- 3.125µg/mL, *Streptomycin*- 6.25µg/mL, S – Sensitive, R – Resistant.

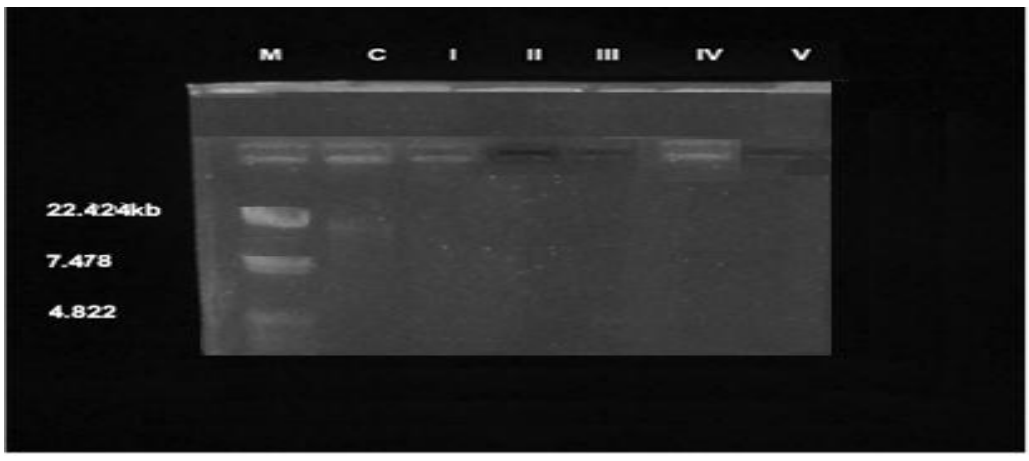


Fig. 3: DNA Cleavage study Calf-thymus (CT) -DNA with Cu (II), Ni (II), Co (II), Mn (II) and Zn (II) Complexes. M: Standard Molecular weight Marker; Calf-thymus – Control DNA; Lane 1: Cu (II) (I); Lane 2: Ni (II) (II) ; Lane 3: Co (II) (III); Lane 4: Mn (II) (IV); Lane 5: Zn (II) (V)

Antimicrobial activities

Antibiotics are well known to produce from microorganisms like *Streptomyces coelicolor* [41-42] but the major drawback of these processes is that these processes are time consuming. In the present study we report simple and rapid synthesis of antimicrobial agent. The in vitro antimicrobial evaluation results of the ligand (HNMAPQ) and its complexes were compared with those standard drugs (*Gentamicine* and *Fluconazole*) to check their biological potency. The results obtained from the antibacterial studies clearly indicates that, the ligand (HNMAPMQ) show superior active against *Staphylococcus aureus* (MTCC No. 7443), *Bacillus subtilis* (MTCC No. 9878) and moderately active towards *Escherichia coli* (MTCC No. 1698) whereas its metal complexes such as Cu(II), Ni(II), Co(II) and Mn(II) (27-35 mm) were more potent bactericides exhibiting greater

activity and Zn(II) complex shows moderate activity than the respective ligand (HNMAPMQ) as presented in Table 4.

In case of antifungal activity, the ligand (HNMAPMQ) shows promising activity against *A. niger* (MTCC No. 281) than *A. flavus* (MTCC No. 277). The Cu(II), Ni(II) and Co(II) (24-29 mm) complexes were more potent fungicides exhibiting higher activity against fungal strain while Mn(II) and Zn(II) complexes shows reasonable activity than ligand (HNMAPMQ) as compared with *fluconazole*.

In the present study the $[Cu(C_{26}H_{18}N_3O_2)_2]$ complex shows evidence of marked increase in the bacteria and fungi activities as compared with ligand (HNMAPMQ) and other complexes which was in agreement with the previously reported antimicrobial properties of Cu(II) complexes screened against a number of pathogenic fungi and bacteria. In addition for past many years it was believed that a trace of Cu(II) destroys the microbe however, a more recent mechanism is that activated oxygen in the surface of Cu(II) kills the microbe [43].

The antimicrobial activity of ligand (HNMAPMQ) and its complexes were found to be increased as the concentration of these compounds increases thereby indicating the important role of concentration in increasing the degree of inhibition [44]. The enhanced antimicrobial activity of the Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) complexes than ligand (HNMAPMQ) can also rationalized due to presence azomethine (C=N) group this imports in elucidating the mechanism of transamination and resamination reactions in biological system. In addition to this the formation of hydrogen bonds through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes [45]. It has also been suggested that the ligands with nitrogen and oxygen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation [46]. Their fore from the above observations it was evidently confirms that the Cu(II), Ni(II), Co(II) and Mn(II) complexes shows potential antimicrobial activities, hence they may be used in the field of medicinal and therapeutic applications as potent agent.

Minimum inhibitory concentration

MICs values of ligand (HNMAPMQ) and its complexes are depicted in Table 5. It was observed that the MICs of these compounds were varied from 16 to 128 µg/mL against *Staphylococcus aureus* (MTCC No. 7443), *Bascillus subtilis* (MTCC No. 9878), *Escherichia coli* (MTCC No. 1698) (bacterial strains), *Aspergillus Niger* (MTCC No. 281) and *Aspergillus flavus* (MTCC No. 277) (fungal strains) as compared with standard drugs (*Gentamicine and Fluconazole*).

Table 4: Antimicrobial results of ligand (HNMAPMQ) and its complexes.

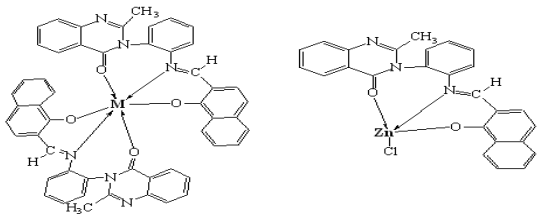
Compounds	Zone of Inhibition in (mm)				
	S.aureus	***B. subtilis***	***E. coli***	***A. flavus***	***A. niger***
$C_{26}H_{19}N_3O_2$ (HNMAPMQ)	20	24	19	19	22
$[Cu(C_{26}H_{18}N_3O_2)_2]$	35	33	35	27	29
$[Ni(C_{26}H_{18}N_3O_2)_2]$	31	30	30	24	27
$[Co(C_{26}H_{18}N_3O_2)_2]$	31	29	30	24	26
$[Mn(C_{26}H_{18}N_3O_2)_2]$	28	28	29	22	24
$[Zn(C_{26}H_{18}N_3O_2)Cl)]$	25	25	26	21	24
Gentamicine	37	35	37	-	-
Fluconazole	-	-	-	29	31

Table 5: MIC (µg/ml) of ligand (HNMAPMQ) and its metal complexes.

Compounds	***S.aureus***	***B. subtilis***	***E. coli***	***A. flavus***	***A. niger***
$C_{26}H_{18}N_3O_2$ (HNMAPMQ)	128	128	128	128	128
$[Cu(C_{26}H_{18}N_3O_2)_2]$	16	16	16	16	16
$[Ni(C_{26}H_{18}N_3O_2)_2]$	32	32	32	32	32
$[Co(C_{26}H_{18}N_3O_2)_2]$	32	32	32	32	32
$[Mn(C_{26}H_{18}N_3O_2)_2]$	32	32	32	64	64
$[Zn(C_{26}H_{18}N_3O_2)Cl)]$	64	64	64	64	64
Gentamicine	4	8	4	-	-
Fluconazole	-	-	-	4	4

CONCLUSION

The title compound (HNMAPMQ) acts as a tridentate ligand coordinating to metal ion through azomethine nitrogen, carboxylato oxygen and phenolic oxygen atom. Based on their analytical, magnetic and spectral data, the appropriate bonding and stoichiometry of ligand (HNMAPMQ) with metal ions were confirmed along with their preferred geometry, in such a way that Cu(II), Co(II), Ni(II) and Mn(II) complexes form an octahedral geometry while Zn(II) complex exhibit tetrahedral geometry as shown in Figures 4 and 5. Thus ^1H NMR spectra showed that the calculated number of protons for each functional group in the ligand (HNMAMPQ) and its diamagnetic $[Zn(C_{26}H_{19}N_3O_2)Cl]$ complex is equal to the number predicted from their molecular formula without exhibiting additional resonances, consequently reflects the purity. The measured molar conductance values (16-27 Ohm^{-1} cm^2 Mol^{-1}) of all the metal complexes confirmed their non-electrolytic behaviour. The radical scavenging activity of ligand (HNMAPMQ) and its complexes were found to be more potent as compared with the earlier reports on Schiff base metal complexes. In the present study the ligand (HNMAPMQ) and its Cu(II), Ni(II) and Zn(II) complexes shows promising antioxidant activity as compared with BHA, TBHQ and AA due to the presence of hydroxyl group in them, their fore these compounds may contribute to their claimed antioxidant property and may be used as potent antioxidant agents in the field of biology applications. The antitubercular activity of CuII), Co(II) and Ni(II) complexes show superior activity toward M. tuberculosis H37Rv assay by registering the MIC at 6.25 µg/mL. The DNA cleavage study evidently inferred that the Cu(II), Co(II), Ni(II) and Zn (II) complexes shows complete DNA cleavage. The antimicrobial analysis results indicates that Cu(II), Co(II), Ni(II) and Mn(II) (27-35 mm) complexes are superior active against all bacterial strains and Cu(II), Co(II), Ni(II) (24-29 mm) complexes were found to be potentially active against fungal strain as compared with standards *Gentamicine* and *Fluconazole*. The MICs of ligand (HNMAPMQ) and its complexes were found to be in the range 16 to 128 µg/mL.



Figs. 4 and 5: Proposed structures of Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) complexes

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REFERENCES

1. Keypour H, Rezaeivala M, Valencia L, Lourido PP, Khavasi HR, Synthesis and characterization of some new Co(II) and Cd(II) macroacyclic Schiff-base complexes containing piperazine moiety, Polyhedron 2009; 28(17): 3755-3758.
2. Sonmez M, Berber I, Akbas E, Synthesis, antibacterial and antifungal activity of some new pyridazinone metal complexes, Eur. J. Med. Chem 2006; 41(1): 101–105.
3. Katwal R, Kaur H, Kapur B, Applications of Copper – Schiff's base complexes: A Review, Sci. Rev. Chem. Commun 2013; 3(1): 1-15.
4. Ashraf MA, Wajid A, Mahmood K, Maah MJ, Yusoff I, Spectral Investigation of the Activities of Amino Substituted Bases, Int. J. Chem. Eng. Appl 2011; 2(4): 252-255.
5. Chavan VL, Mehta BH. X-ray, Thermal and Biological studies of Ru(III), Rh(III) and Pd(II) Schiff base metal complexes, Res. J. Chem. Environ 2011; 15(2): 57-61.
6. Raman N, Pitchaikani YR, Kulandaisamy A, Synthesis and characterization of Cu(II), Ni(II), Mn(II), Zn(II) and VO(II) Schiff base complexes derived from o-phenylenediamine and acetoacetanilide, Proc. Indian Acad. Sci. (Chem Sci) 2001; 113(3): 183–189.
7. Fasina TM, Ogundele O, Ejiah FN, Dueke-Eze CU, Biological activity of Copper(II), Cobalt(II) and Nickel(II) Complexes of Schiff base Derived from o-phenylenediamine and 5-bromosalicylaldehyde, Int. J. Biol. Chem 2012; 6(1): 24-30.
8. Uhlig E, Non-innocent bidentate nitrogen-ligands in electron-rich complexes of 3d-elements, Pure. Appl. Chem 1988; 60(8) 1235-1240.
9. Ganguli S, Panigrahi MK, Singh P, Shukla PK, One-pot synthesis of novel quinazoline derivatives and their antimicrobial activity, Int J Pharm Pharm. Sci 2012; 4(4): 434-440.
10. Hemalatha K, Girija K, Synthesis, characterization, preliminary QSAR studies and in vitro antioxidant activity of some novel 2, 3-disubstituted quinazolinone derivatives, Int J Pharm Pharm Sci 2012; 4(3): 99-103.
11. Hill JO, Chirawongaram S, Thermal analysis studies of tin dithiocabmate- a short review, J. Therm. Anal 1994; 41(2-3): 511-518.
12. Saad HA, Osman NA, Moustafa AH, Synthesis and analgesic activity of some new pyrazoles and triazoles bearing a 6, 8-dibromo-2-methylquinazoline moiety, Molecules 2011; 16(12): 10187-10201.
13. Rajveer Ch, kumaraswamy D, Sudharani S, Stephen BR, Synthesis of some 6-bromo quinazolinone derivatives for their pharmacological activities, Int J Pharm Biol Sci 2010; 1(3) 1-10.
14. Shweta T, Vikas M Mujalda1, Sharma1 V, Saxena1 P, Shrivastava M, Synthesisand in-vitro anti-fungal evaluation of 6-bromo, 3, 4 substituted-4-one-1, 3 thiazolidin-3-yl benzylquinazoline-4(3H)-one, Int. J. Drug. Des. Discovery 2013 4(1): 998-1002.
15. Siddappa K, Chandrakant PR, Mallikarjun K, Tukaram R, Mahesh T. Mallikarjun M, Synthesis, spectral, powder X-Ray diffraction and antimicrobial studies of some transition metal (II) complexes of Schiff base derived from 3-[(2-hydroxy-6-methylquinolin-3-ylmethylene) -amino] -2-methyl-3H-quinazoline-4-one, Asian J. Chem 2011; 23(10): 4511-4516.
16. Chen D. Martell E, Dioxygen affinities of synthetic cobalt Schiff base complexes, Inorg. Chem 1987; 26(7): 1026–1030.
17. Soares JR, Dinis TCP, Cunha AP, Almeida LM, Antioxidant activities of some extracts of thymus zygis, Free Radical Res 1997; 26(5): 469-478.
18. Matthaus B, Antioxidant activity of extracts obtained from residues of different oilseeds, J. Agric. Food. Chem 2002; 50(12): 3444–3452.
19. Collins L, Franzblau S, Microplate Alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium, Antimicrob. Agents Chemother 1997; 41(5): 1004–1009.
20. Reis RS, Neves IJ, Lourenço SLS, Fonseca LS, Lourenco MCS, Comparison of flow cytometric and Alamar Blue tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid, J. Clin. Microbiol 2004; 42(5): 2247-2248.
21. Patil SA, Naik VH, Kulkarni AD, Badami PS, DNA cleavage, antimicrobial, spectroscopic and fluorescence studies of Co (II), Ni (II) and Cu (II) complexes with SNO donor coumarin Schiff bases, Sperctrochim. Acta, Part A 2010; 75(1): 347-354.
22. Brown, T. A. Essential Molecular Biology: A Practical Approach. Vol. I., Oxford University Press: New York, 1990.
23. Gould JC, The laboratory control of antibiotic therapy, Br. Med. Bull 1960; 16(1): 29-34.
24. Kapoor P, Fahmi N, Singh RV, Microwave assisted synthesis, spectroscopic, electrochemical and DNA cleavage studies of lanthanide (III) complexes with coumarin based imines, Spectrochim. Acta, Part A 2011; 83(1): 74– 81.
25. Vogel AI. A Text Book of Quantitative Inorganic Analysis. 3rd ed., ELBS, Longman, London: UK, 1969.
26. Sindhu Y, Athira CJ, Sujamol MS, Selwin RJ, Mohanan K, Synthesis, characterization, DNA cleavage, and antimicrobial studies of some transition metal complexes with a novel Schiff base derived from 2-aminopyrimidine, Synth. React. Inorg. Met-Org. Nano-Met. Chem 2013; 43(3): 226-236.
27. Usharani M, Akila E, Ramachandran S, Velraj G, Rajavel, R, Synthesis, composition, geometry and antibacterial assay of binuclear Schiff base metal complexes derived from benzene-1,4-dicarbaldehyde, 2-hydroxy-benzaldehyde and pyridine-2,6-diamine, Int J Pharm Pharm Sci, 2013; 5 (2): 639-647.
28. Raman N, Jeyamurugan R, Rajkapoor B, Mitu L, Novel metal-based pharmacologically dynamic agents of transition metal (II) complexes: Designing, synthesis, structural elucidation, DNA binding and photo-induced DNA cleavage activity, Spectrochim. Acta, Part A 2010; 75(1) 88-97.
29. Muthukumar M, Viswanathamurthi P, Synthesis, spectral characterization and catalytic studies of new ruthenium (II) chalcone thiosemicarbazone complexes, Cent. Eur. J. Chem 2010; 8(1) 229-240.
30. Maurya RC, Rajput S, Oxovanadium (IV) complexes of bioinorganic and medicinal relevance: synthesis, characterization and 3D molecular modeling and analysis of some oxovanadium (IV) complexes involving O, O-donor environment, J. Mol. Struct 2004; 687(1): 35–44.
31. Saydam S, Synthesis and characterization of the new thiazole Schiff base 2-(2-hydroxy) naphthylideneaminobenzothiazole and its complexes with cobalt (II), copper (II) and nickel (II) ion, Synth. React. Inorg. Met-Org. Nano-Met. Chem 2002; 32(3): 437-447.
32. Alam MM, Begum R, Rahman SMM, Saiful ISM, Synthesis, spectroscopic and electrochemical studies of mononuclear Fe (II) and Ni (II) complexes containing a macrocyclic ligand derived from Pyridine-2, 6-dicarboxaldehyde and 1,2-Bis (2-aminoethoxy) Ethane, J. Sci. Res 2011; 3(3): 599-607.
33. Geary WJ, The use of conductivity measurements in organic solvents for the characterization of coordination compounds, Coord. Chem. Rev 1971; 7(1): 81-122.
34. Patel KS, Patel JC, Dholariya HR, Patel VK, Patel KD, Synthesis of Cu (II), Ni (II), Co (II), and Mn (II) complexes with Ciprofloxacin

and their evaluation of antimicrobial, antioxidant and anti-tubercular activity, O J Metal 2012; 2 (3): 49-59.

35. Bukhari SB, Memon S, Mahroof-Tahir M, Bhanger MI, Synthesis, characterization and antioxidant activity of copper-quercetin complex, Spectrochim. Acta, Part A 2009; 71(5): 1901-1906.
36. Pawar V, Joshi S, Uma V, Antibacterial and antioxidant properties of macrocyclic Schiff bases with vanadium (V) complexes, Biokemistri 2011; 23(1): 21-27.
37. Raman N, Kulandaisamy A, Thangaraja C, Synthesis, structural characterization and electrochemical and antibacterial studies of Schiff base copper complexes, Transition Met. Chem 2004; 29(2): 129–135.
38. Natarajan R Ramaraj J, Mariyyappan S, Raja B, Chithu RY, Synthesis, DNA binding, and antimicrobial studies of novel metal complexes containing a pyrazolone derivative Schiff base, Chem. Pap 2010; 64(3): 318–328.
39. Puja K, Nighat F, Singh RV, Microwave assisted synthesis, spectroscopic, electrochemical and DNA cleavage studies of lanthanide (III) complexes with coumarin based imines, Spectrochim. Acta, Part A 2011; 83(1): 74–81.
40. Kulkarni A, Patil SA, Badami PS, Synthesis, characterization, DNA cleavage and in vitro antimicrobial studies of La (III), Th (IV) and VO (IV) complexes with Schiff bases of coumarin derivatives, Eur. J. Med. Chem 2009; 44(7): 2904-2912.
41. Deene Manikprabhu, Lingappa K, Antibacterial activity of silver nanoparticles against methicillin-resistant *Staphylococcus aureus* synthesized using the model *Streptomyces* Sp. Pigment by photo-irradiation method, J. Pharm. Res 2013; 6: 255-260.
42. Deene Manikprabhu, Lingappa K, γ Actinorhodin a natural and attorney source for synthetic dye to detect acid production of fungi, Saudi J. Biol Sci 2013; 20: 163–168.
43. Ran X, Wang L, Cao D, Lin Y, Hao J, Synthesis, characterization and in vitro biological activity of cobalt (II), copper (II) and zinc (II) Schiff base complexes derived from salicylaldehyde and D, L-selenomethionine, Appl. Organomet. Chem 2011; 25(1): 9-15.
44. Alka Choudharya, Renu Sharmaa, Meena Nagar, Synthesis, characterization and antimicrobial activity of mixed ligand complexes of Co (II) and Cu (II) with N,O/S donor ligands and amino acids, Int Res J Pharm Pharmacol 2011;1(6):172-187.
45. Dharmaraj N, Viswanathamurthi P, Natarajan K, Ruthenium (II) complex containing bidentate Schiff bases and their antifungal activity, Transition. Met. Chem 2001; 26(1-2): 105-109.
46. Chohan ZH, Arif M, Akhtar MA, Supuran CT, Metal-based antibacterial and antifungal agents: synthesis, characterization, and in vitro biological evaluation of Co (II), Cu (II), Ni (II), and Zn (II) complexes with amino acid-derived compounds, Bioinorg. Chem. Apple 2006, Volume 2006 (2006), Article ID 83131, 13 pages, doi: 10.1155/BCA/2006/83131.