Int J Pharm Bio Sci 2013 July; 4(3): (P) 739 - 747

Research Article Biotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

DNA BINDING AND ANTI-MICROBIAL STUDIES OF Ag(II) AND Cu(II) METAL COMPLEXES CONTAINING MIXED LIGANDS OF 1,10-PHENANTHROLINE AND 8-HYDROXYQUINOLINE.

VEENA V PILLAI AND B.SREEKANTH*

Department of Biotechnology, Acharya Institute of Technology, Bangalore -560 107

ABSTRACT

The two new complexes $Ag(L_1)^2L_2(PF_6)_2$[Complex **(1)** and $Cu(L_1)^2L_2(PF_6)_2$[Complex **(2)**] containing bioactive mixed ligand of typeL_1=8-Hydroxyquinoline and L_2=1,10-Phenanthroline have been synthesized and characterized by analytical and spectral methods. The binding constant of the complexes in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2, are 25.79 x 10^6 $M^{-1}$and 9.95 x 10^6 $M^{-1}$for complex **(1)** and **(2)** respectively. . The synthesized compounds have been tested against microorganisms such as Gram positive bacteria (*Staphylococcus aureus* and *Proteus vulgaris*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*). A comparative study of the Minimum Inhibitory Concentration(MIC) values of the ligands and their complexes indicates that the complexes exhibit moderate antimicrobial activity than the free ligand and control.

KEYWORDS: **Silver and Copper complexes, DNA Binding and Antimicrobial studies**

B.SREEKANTH
Department of Biotechnology, Acharya Institute of Technology, Bangalore -560 107,India.

Corresponding author

INTRODUCTION

There is a continuing interest in transition metal complexes of Schiff bases because of the presence of both hard nitrogen and oxygen and soft sulphur donor atoms in the backbones of these ligands. In the synthesis and characterization of metal complexes containing Schiff bases as ligands due to their importance as catalysts for many reactions [1-4].Tetradentate Schiff base complexes are also important for designing model complexes related to synthetic and natural oxygen carriers[5]. Many transition metal Schiff base complexes have been found to considerable interesting biological properties such as antibacterial, antitumour activity [6]. Catalysts for various organic reactions[7], models of reaction centers of metalloenzymes[8], outstanding magnetic properties[9], and nonlinear optical materials[10].The study of mixed ligand-complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity, sensitivity and has also great importance in the field of biological and environmental chemistry [11-12].The effect of the presence of methyl substituent in the phenyl rings of aromatic Schiff bases on their antimicrobial activity has been reported [13].Although many Schiff bases are known to beactive against a wide range of micro-organisms, for example; *E.coli, B.subtilis*, and *S.aureus* and fungi *R.stolonifer* and *C.ablican* , antibacterial activity has been studied more than antifungal activity, since bacteria are usually more resistant to antibiotics through biochemical and morphological modifications. Moreover, the incorporation of transition metal into Schiff bases enhances the biological activity of the ligand and decreases the cytotoxic effects of both the metal ion and ligand on the host[14].However the literature survey that the mixed ligands of 8-Hydroxyquinoline and 1-10 Phenanthroline derivatives with their transition metal complexes have not been reported and studied so far. Hence the present study aims for the Synthesis, Characterization, Antimicrobial and DNA binding studies of Ag(II) and Cu(II) complexes containing mixed ligands 8-hydroxyquinoline and 1-10 Phenanthroline.

MATERIALS AND METHODS

EXPERIMENTAL

All reagents and solvents were of AR grade, solvents were purified and used.Metal salts such as Silver nitrate and Copper Sulphate were purchased from Karnataka fine chemicals.Ligands such as 8-Hydroxyquinoline and 1,10-Phenanthroline was purchased from Himedia chemicals Ltd. (Bangalore).

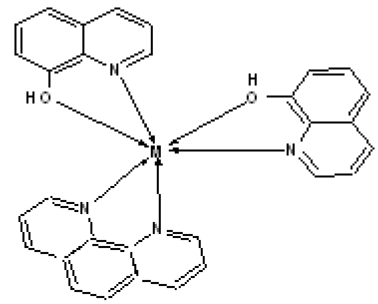
Synthesis of Metal Complexes

Synthesis of Silver complex $[Ag(L_1)^2L_2](PF_6)_2$[Complex (1)]

Silver nitrate (0.169 g, 1mmol) 8-hydroxyquinoline L_1 (0.29 g, 2mmol) 1,10-phenanthroline L_2 (0.234g, 1mmol) were dissolved in ethanolic solution and refluxed on the water bath for 4 hours.The contents were cooled to obtain precipitate. The complex was filtered and dried under vacuum before being recrystallized in acetone.

Synthesis of Copper complex $[Cu(L_1)^2L_2](PF_6)_2$[Complex (2)]

Copper sulphate (0.171 g, 1mmol), 8-hydroxyquinoline L_1 (0.29 g, 2mmol), 1,10-phenanthroline L_2 (0.234g,1mmol) were dissolved in ethanolic solution and refluxed on the water bath for 4 hours. The contents were cooled to obtain precipitate.The complex was filtered and dried under vacuum before being recrystallized in acetone.



Where M= Ag or Cu

FIGURE 1
Proposed structure of the metal complexes

Spectral Measurements
IR spectra were recorded with Shimadzu model FT-IR spectrophotometer UV-visible absorption spectra were recorded using ELICO model SL-159 UV-Vis Spectrophotometerat room temperature.

DNA Binding Studies
The concentration of CT-DNA per nucleotide [C(p)] was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1})[15]. Tris HCl-buffer [5mM Tris(hydroxymethyl) amino methane, pH 7.2, 50 mMNaCl] was used for the absorption, viscosity, and thermal denaturation experiments.Absorption titration experiments were carried out by varying the DNA concentration (0-100μM) and maintaining the metal complex concentration constant. Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 minutes). For both(**1**) and (**2**), the observed data were then fitted into Equation (1) to obtain the intrinsic binding constant K_b [16].$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_a - \varepsilon_f)$,where ε_a, ε_b, and ε_f are the apparent, bound, and free metal complex extinction coefficients, respectively, at 266 nm for (**1**) and 260 nm for (**2**). A plot of $[DNA]/(\varepsilon_b - \varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a *y* intercept equal to $l/K_b(\varepsilon_b - \varepsilon_f)$, where K_b is ratio of the slope to the *y* intercept.

Antimicrobial Studies
The ligands and its complexes were investigated for anti-bacterial and anti-fungal properties. Gram positive bacteria (*Staphylococcus aureus* and *Proteus vulgaris*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and one fungus (*Aspergillus niger*) were used in this study to assess their antimicrobial properties. The tested compounds were dissolved in DMSO and the solutions were serially diluted in order to find the MIC values. The antibiotic Chloramphenicol was used as standard reference in the case of Gram-negative bacteria, Amikacin was used as standard reference in case of Gram-positive bacteria and Clotrimazole was used as standard anti-fungal reference. The solvent DMSO was used as negative control. In a typical procedure, a well was made in the Muller Hinton agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plates were incubated at37 $^{\circ}$C at 72 h for fungi and 24 h for bacteria. During this period, the test solution diffused and affected the growth of the inoculated fungi and bacteria.Activity was determined by measuring the diameter of the zone of inhibition (mm).

RESULTS AND DISCUSSION

Characterization of Complexes

The elemental analysis data agree with the theoretical values within the limit of experimental error. These complexes are soluble in DMF, DMSO and in buffer(pH 7.2) solution.The IR spectra of the complexes were recorded in the range of 4000-400 cm^{-1} on KBr pellets. The spectra of the ligands 8-Hydroxyquinoline and 1,10-Phenanthrolineshowed bands at 1577 $cm^{-1}$and 1597 cm^{-1}assigned to νC=N aromatic hydrocarbon, 3045 $cm^{-1}$and 3024cm^{-1}assigned to νC-H group and 3600$cm^{-1}$and 3800 cm^{-1}assigned to νO-H group. The spectra of both the complexes showed a peak in the range 1510 $cm^{-1}$to 1630 $cm^{-1}$for νC=Ngroup are shifted slightly indicating that the coordination taken place through nitrogen atom.

Absorption Spectral Studies

The absorption spectra of complexes (1) and (2) in the absence and presence of CT-DNA are given in Figures 2 and 3, respectively. Figure 2 depicts a well-resolved band at 266 nm for complex (1) and in Figure 3; there exists a well-resolved band at 260 nm for complex (2) with increasing DNA concentrations (0–100 *μ*M).

The result shows that the absorbance (hypochromism) decreased by the successive addition of CT-DNA to the complex solution. The hypochromism observed for the bands of complexes (1) and (2) is accompanied by a small bathochromic shift of 2 and 1 nm, as shown in Figures 2 and 3, respectively. The hypochromism and bathochromic shift observed for the complexes suggest that binding is in intercalative mode. To compare quantitatively the DNA binding strengths of these complexes, the intrinsic DNA binding constants K_b are determined from the decay of the absorbance at 266 nm for complex (1) and 260 nm for complex (2) with increasing concentrations of DNA by using equation (1) given in our previous article[17,23]. The observed K_b values for complexes (1) and (2) are equal to the classical intercalators bound to CT-DNA. The K_b values for complexes (1) and (2) are 25.79× 10^6 $M^{-1}$and 9.95 × 10^6 M^{-1}, respectively. Thus, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

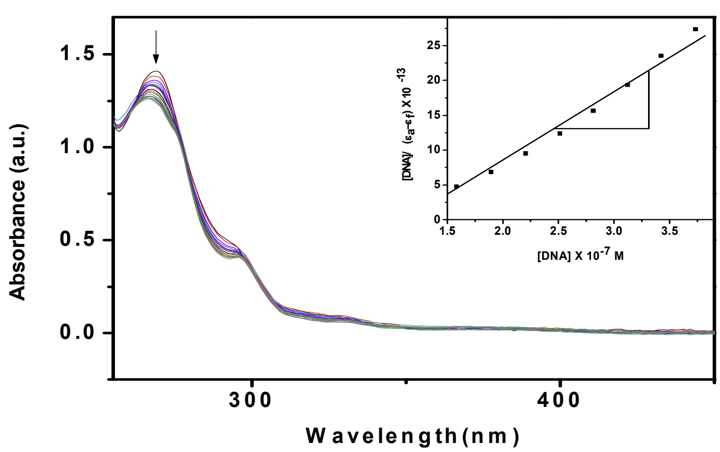


FIGURE 2

Absorption spectra of complex (1) in Tris-HCl buffer upon addition of DNA. [Ag] = 0.5 μM, [DNA] = 0–100 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
Inset: The plot of [DNA]/(εa − εf) versus [DNA] for the titration of DNA with Ag(II) complex.

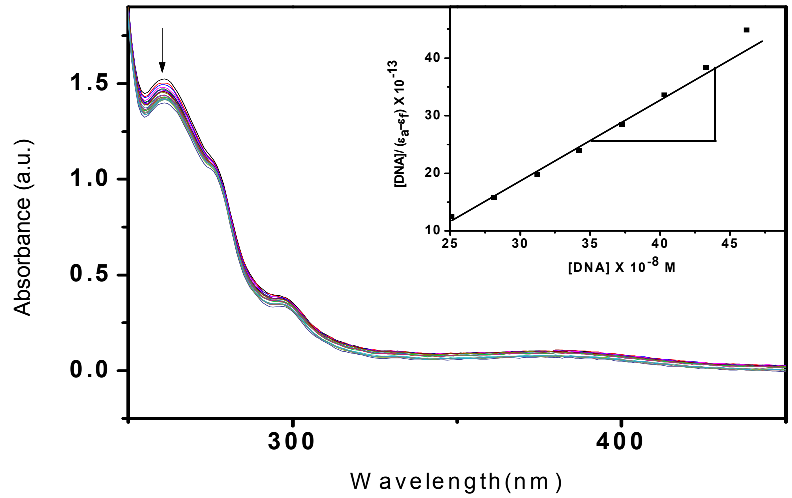


FIGURE 3

Absorption spectra of complex (2) in Tris-HCl buffer upon addition of DNA. [Cu] = 0.5 μM,[DNA] = 0–100 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
Inset: The plot of [DNA]/(εa − εf) versus [DNA] for the titration of DNA with Cu(II) complex.

Antimicrobial Studies

The in vitro antimicrobial activity of the compounds was tested against the bacterial species including *Staphylococcus aureus,Proteus vulgaris, Escherichia coli* and *Pseudomonas aeruginosa* and the fungus *Aspergillus niger* by well diffusion method.These complexes are inhibiting Gram-positive and Gram negative bacterial strains. The importance of this unique property of the investigated Schiff base complexes lies in the fact that, it can be applied safely in the treatment of infections and some common diseases e.g. Septicaemia, Gastroenteritis, Urinary tract infections and hospital acquired infections[25]. The ligand and their complexes have been tested for in vitro growth inhibitory activity against gram-positive microbe *Staphylococcusaureus,Proteus vulgaris* and gram-negative microbe's *Escherichia coli, Pseudomonas aeruginosa* by using well-diffusion method. As the test solution concentration increases, the biological activity also increases. The Minimum Inhibitory Concentration (MIC) values of the investigated compounds are summarized in Table 8. From the table, the observed MIC values indicate that the complexes have higher antibacterial activity. But the complexes shows no inhibition against fungi. (*Aspergillus Niger*). The metal complexes Cu (II) and Ag (II) have higher antibacterial activity than the legend are shown in Figs. 4(a), 4(b) and 4(c). The increase in antimicrobial activity is due to the faster diffusion of metal complexes as a whole.

Figure 4 ***Difference between the antimicrobial activity of ligand & metal complexes.***

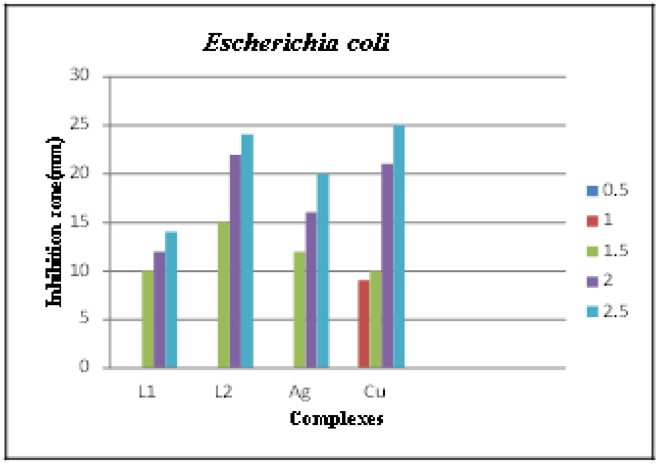


Figure 4 (a)
Activity of E.coliagainst Ligands and Complex (1) and (2)

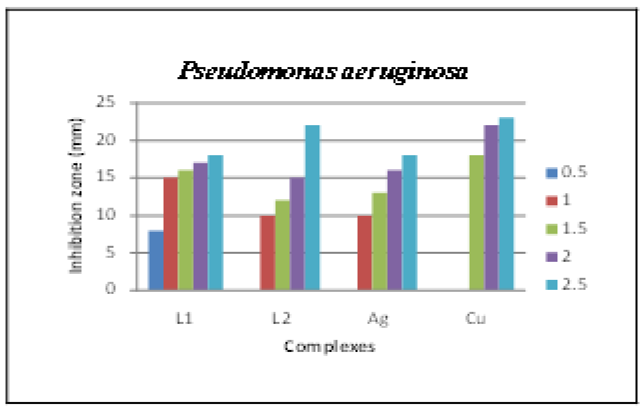


Figure 4 (b)
Activity of P.aeruginosaagainst Ligands and Complex (1) and (2)

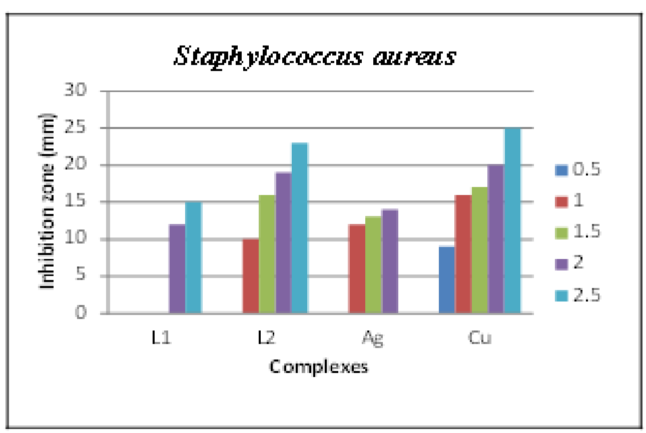


Figure 4(c)
Activity of S.aureusagainst Ligands and Complex (1) and (2)

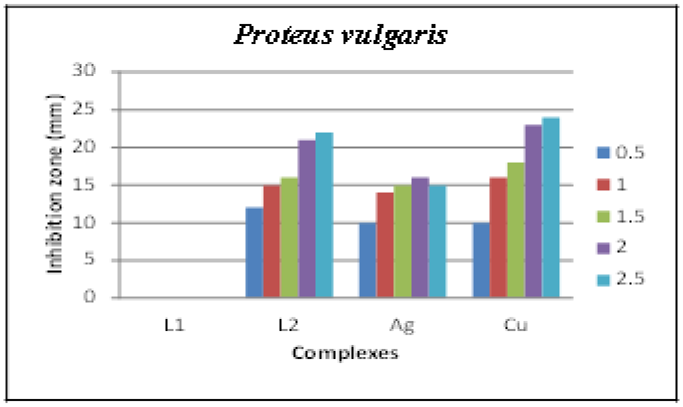


Figure 4 (d)
Activity of Proteus vulgaris against Ligands and Complex (1) and (2)

Table 1
Antimicrobial activity of the ligand and metal complexes

Sl.No	**Extract**	**Conc (mg/ml)**	***E.coli (mm)***	***P. aeruginosa (mm)***	***Staphylococcus aureus (mm)***	***Proteus vulgaris (mm)***	***A.niger (mm)***
1	**L_1**	0.5	0	8	0	0	0
		1	0	15	0	0	0
		1.5	0	16	0	0	0
		2	0	17	12	0	0
		2.5	0	19	15	0	0
2	**L_2**	0.5	0	0	0	12	0
		1	0	10	10	15	0
		1.5	15	12	16	16	0
		2	22	15	19	21	0
		2.5	24	22	23	22	0
3	**$Cu(L_1)^2L_2$**	0.5	0	0	9	10	0
		1	9	0	16	16	0
		1.5	10	18	17	18	0
		2	21	22	20	23	0
		2.5	25	23	25	24	0
4	**$Ag(L_1)^2L_2$**	0.5	0	0	0	10	0
		1	0	10	12	14	0
		1.5	10	13	13	15	0
		2	12	16	14	16	0
		2.5	14	18	15	17	0
5	**Antibiotic**	0.5	21	24	21	21	0
		1	21	22	20	22	0
		1.5	22	22	21	22	0
		2	24	24	24	24	0
		2.5	22	24	25	24	0
6	**DMSO**	0.5	0	0	0	0	0
		1	0	0	0	0	0
		1.5	0	0	0	0	0
		2	0	0	0	0	0
		2.5	0	0	0	0	0

Less than 10mm-- Inactive; Less than 10-15mm--Weakly active; Less than 15-20mm--Moderately active;More than 20mm—Highly active

Standard: Antibiotic Chloramphenicol for Gram negative bacteria, Amikacin for Gram-positive bacteria**,** Clotrimazole for fungi

Control: DMSO

CONCLUSION

In conclusion, we have synthesized and characterized two new complexes of the type [Ag $(L_1)^2L_2$] $(PF_6)_2$ **(1)** and [Cu $(L_1)^2L_2$] $(PF_6)_2$ **(2)**. Interactions of the new complexes with (double stranded) DNA were investigated by absorption spectra, viscosity and thermal denaturation studies. From the experimental results, it was confirmed that the complexes bound with the double stranded DNA with binding constant K_b=25.79 x 10^6 $M^{-1}$for (**1**) andK_b=9.95 x 10^6 $M^{-1}$for (**2**), respectively.The metal complexes have higher antimicrobial activity than the free ligand.

REFERENCES

1. Ullah M R and Bhattacharya P K. Study of the Effect of nature of Ligand on reproportionation constants of ternary complexes. *Proc. Indian Acad. Sci. (Chem. Sci.)*, 102: 513-519, (1990).
2. Enikolopyan N S, Bogdanova K A and Skarov K. Metal Complexes of Porphine and Azaporphine Compounds as Catalysts of Reactions Involving Oxidation by Molecular Oxygen *.A. Russ. Chem. Rev.* 52:13-26, (1983).
3. LeungW H and Che C M. Oxidation chemistry of ruthenium-salen complexes*. Inorg. Chem,* 28:4116- 4121, (1989).
4. El-Hendawy A M, Alkubaisi A H, El-Ghany A, El- Kourashy K and Sharab M N. Ruthenium(II) Complexes of O,N-donor Schiff base ligands and their use as catalytic organic oxidants. *Polyhedron.***,** 12: 2343-2350, (1993).
5. Norden B, Lincoln P, Akerman B and Tuite E. DNA interactions with substitution-inert transition metal ion complexes. In *Metal Ions in Biological Systems: Probing of Nucliec Acids by Metal Ion Complexes of Small Molecules*; Sigel, A., and Sigel, H. (Eds.),Marcel Dekker: New York., 33:177–252, **(**1996).
6. Bregant F, Pacor S, Ghosh S, Chattopadhyay S K and Sava G.Effects of some ruthenium chelates on Mca mammary carcinoma and TLX5 Lymphoma in mice.*Anti Cancer Res*.**,** 13:1007 -1010, (1993).
7. Li A H, Dai L X, and Aggarwal, V.K., Asymmetric Ylide reactions: epoxidation, cyclopropanation, aziridination, olefination, and rearrangement. *Chem. Rev.*, 97: 2341–2372, (1997).
8. Dhar S, Senapati D, Das P K, Chattopadhyay P, Nethaji M and Chakravarty A R. Ternary copper complexes for photocleavage of DNA by red light: direct evidence for sulfur-to-copper charge transfer and d-d band involvement. *J. Am. Chem. Soc*., 125: 12118–12124,(2003).
9. Costes J P,Dahan F and Dupuis AExperimental evidence of the acidic character of lanthanide ions in protic solvents*. Inorganica Chimica Acta* **,** 39:165-168, (2000).
10. Mishra L, Ajay K Y, Ratna S P, Choi C S and Koji A. Studies on some new Ru(iii) complexes using arylazo pentane-2,4-dione and 2,6-bis (2'-benzimidazolyl) pyridine as ligands: Synthesis, spectroscopic, luminescent, electrochemical and biological activities.*Metal Based Drugs*.,8: 65–7(2001).
11. Pyle A M,Barton J K and Lippard S J.Oxidative DNA damage through long-range electron transfer. *J. Am. Chem. Soc*.,114:2303-2311, (1992)
12. Tullius T D. Metal-DNA Chemistry. ACS Symposium Series, *American Chemical Society*, Washington, DC, 402, (2008).
13. Shivarama Holla B, Mahalinga M, Sithambaram Karthikeyan M, Poojary B, Mohammed Akberalib P and Suchetha Kumar N. Synthesis, characterization and antimicrobial activity of some substituted 1,2,3-triazoles, *Eur. J. Med. Chem*. 40:1173-1178,(2005).

14. Ameerunisha Begum M S, Saha S, Nethaji M and Chakravarty A R, Iron(III) Schiff base complexes of arginine and lysine as netropsin mimics showing AT-selective DNA binding and photonuclease activity, *J. Inorg. Biochem.* 104: 477-484,(2010).
15. Reichmann M E, Rice S A, Thomas C A and Doty P. A further examination of the molecular weight and size of deoxypentose nucleic acid. *J. Am. Chem. Soc.*, 76: 3047–3053, (1954).
16. Wolfe A, Shimer G H and Meehan T. Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry*, 26:6392–6396, (1987).
17. Sreekanth B, Krishnamurthy G, Bhojya Naik H S, Vishnuvardhan T K, Vinaykumar B and Sharath N. Synthesis, DNA binding, and oxidative cleavage studies of Fe (II) and Co (III) complexes containing bioactive ligands. *Nucleosides Nucleotides Nucleic Acids*, 30: 83–96, (2011).
18. Sreekanth B, Krishnamurthy G, Bhojya Naik H S, Vishnuvardhan T K and Prabhakar M C.Fe(II) complexes containing bioactive ligands: Synthesis, DNA binding, and cleavage studies. *Synth. React.Inorg. Met-Org. Nano-Met. Chem.*,40: 955–962, (2010).
19. Sreekanth B, Krishnamurthy G, Bhojya Naik H S, Vishnuvardhan T K, Lokesh M R, Shashikumar N D. DNA Interaction Studies of Cu (II) and Mn (II) Complexes containing Mixed Ligand of 1, 10-phenanthroline and 2-hydroxy-4-methyl-1, 8-naphthyridine *Res. J. of Pharm., Biol. and Chem. Sci.*,2: 201-213,(2011)
20. Sreekanth B, Krishnamurthy G, Bhojya Naik H S, Vishnuvardhan T K, Lokesh M R and Shashikumar N D.DNA Binding and Cleavage Studies of Fe(II) and Zn(II) Complexes containing Mixed Ligand of 1,10-phenanthroline and 2-hydroxy-4-methyl-1,8-naphthyridine. *J. of Chem. and Pharm. Res.*, 3: 407-419, (2011).
21. Sreekanth B, Krishnamurthy G, Bhojya Naik H S and Vishnuvardhan T K. Cu(II) and Mn(II) Complexes Containing Macroacyclic Ligand: Synthesis, DNA Binding, and Cleavage Studies.*Nucleosides, Nucleotides and Nucliecacids*., 31:1-13, (2012).
22. Sreekanth B, Krishnamurthy G, Bhojya Naik H S, Gopinath S M, Vishnuvardhan T K,Ismail Shareef M.Synthesis, Characterization, DNA Binding and Cleavage studies of Fe(III)and Zn(II) Complexes Containing Mixed Ligands. *International Journal of Pharma and Biosciences,* 4 (3): 257-265, (2013).
23. Natesan Mahalakshmi, Rajavel R. Binuclear Copper(II), Nickel(II) and Oxovanadium(IV) Schiff base complexes bearing N_2O_2 donors and their DNA cleavage and antibacterial activity.*International Journal of Pharmacy & Technology.*2: 1133-1157, (2010).