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MONONUCLEAR COPPER, NICKEL AND ZINC COMPLEXES POSSESSING BIO-POTENTIAL LIGAND PINOCEMBRIN: SYNTHESIS, CHARACTERIZATION AND DNA BINDING CLEAVAGE STUDIES

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Abstract:
Flavonoids are widely occurring polyphenol compounds of plant origin with multiple biological and chemical activities. DNA binding studies of flavonoids are needed to understand the reaction mechanism and improve drugs that target DNA. Due to the presence of carbonyl and hydroxyl groups they can coordinate metal ions and form complexes. Pinocembrin is a natural flavonoid compound which is capable of antioxidant, antibacterial, anti-inflammatory, and antineoplastic activities. Present research work reports the new metal [Cu(II), Ni(II) and Zn(II)] complexes of Pinocembrin. Their structural features and other properties has been characterized by FT-IR and Mass spectral analysis. Interactions of the synthesized complexes towards calf thymus-DNA were determined by emission, absorption, circular dichroism, and viscosity techniques. Detailed investigation reveals that these complexes bind with DNA via intercalation binding. The present effort shows the potential of spectroscopic analysis to characterize the nature of drug–metal complex and the effects of such interaction on the structure of biomolecule.
Key words: *Flavonoid, Pinocembrin, DNA binding, Intercalation, DNA–drug interaction*

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1. INTRODUCTION:

DNA plays a key role in the synthesis of proteins (gene expression) as well as its own replication making it a potential target for drugs, especially for antiviral, antibiotic and anticancer action. Thus, favorable DNA interaction patterns based on the study of small molecules that bind to nucleic acids is one of the most important parameters in the screening design for new drugs and development processes [1]. Over the last few decades structure of DNA and its interaction with different bioactive molecular moieties have gained a great interest in the field of organic synthesis and pharmacology. DNA is a nucleic acid that contains all the information necessary for specifying the biological development of all living bodies. It is a molecule that controls hereditary information transferred to the offspring. During reproduction, DNA is replicated and transmitted to the new trait. In this process, the sequence of DNA base pairs defines the characters of individuals ranging from physical traits to disease susceptibility. It is necessary to understand at molecular level gene expression and their mechanism of transfer to offspring [2]. Interaction of small molecules and DNA are mainly of two types. One is covalent interactions and another one is non-covalent interactions. Three common modes of non covalent interactions between DNA and small molecules have been identified: (1) electrostatic interactions with the negatively charged phosphate backbone,(2) binding interactions with the minor and major grooves of DNA double helix, and (3) intercalation between the stacked base pairs of double-stranded DNA leading to perturbation in DNA structure [3].

A new group of emerging anticancer agents is based on the naturally occurring flavonoids. Flavonoids that belong to the polyphenols family are secondary plant metabolites and one the most occurring groups of phytochemicals. They occur in fruits, seeds, flowers and vegetables among others and are of important in the human diet. These compounds are of high physiological and morphological importance in plants [4]. Flavonoids are polyphenolic compounds and due to their tremendous biological importance and broader range of pharmacological activities including antioxidant, anticancer, antitubercular, antibacterial, antiallergic, antimicrobial, anti-inflammatory, antiviral, antitumor, antimutagenic, antidiabetic, hepatoprotective and cardiovascular activities, flavonoids gained much attention and become a topic of interest for researchers in the last decades [5-7]. Recent studies indicated that metal-flavonoid complexes have good biological and pharmacological activities, and some of which have been successfully used in clinical practices [8]. A great number of flavonoids are able to chelate metal ions; and often the presence of a 3-hydroxyl group or a 5- hydroxyl group allows the complex formation involving the carbonyl function [9-11]. Therefore, research on the metal flavonoid complexes is very supportive in budding new medicines, based on these complexes and investigating new ways in screening, discovery and development of new drugs.

Pinocembrin (5,7-dihydroxyflavanone) is one of the primary flavonoids isolated from the variety of plants, mainly from Pinus heartwood, Eucalyptus, Populus, Euphorbia, and Sparattosperma leucanthum, in the diverse flora and purified by various chromatographic techniques. Pinocembrin is a major flavonoid molecule incorporated as multifunctional in the pharmaceutical industry. Its vast range of pharmacological activities has been well researched including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities. In addition, pinocembrin can be used as neuroprotective against cerebral ischemic injury with a wide therapeutic time window, which may be attributed to its antiexcitotoxic effects. Pinocembrin exhibits pharmacological effects on almost all systems, and our aim is to review the pharmacological and therapeutic applications of pinocembrin with specific emphasis on mechanisms of actions. The design of new drugs based on the pharmacological effects of pinocembrin could be beneficial [12]. Apart from natural extraction, pinocembrin has been successfully biosynthesized [13] and chemosynthesized [14].

Biological importance of drug pinocembrin allows focusing the attention on the importance of metal complexes of Pinocembrin with DNA interaction studies. In the present work, we have investigated the mode of Pinocembrin metal complex binding towards DNA double helix with the aid of emission, absorption, circular dichroism, and viscosity measurement analysis.

2 EXPERIMENTAL

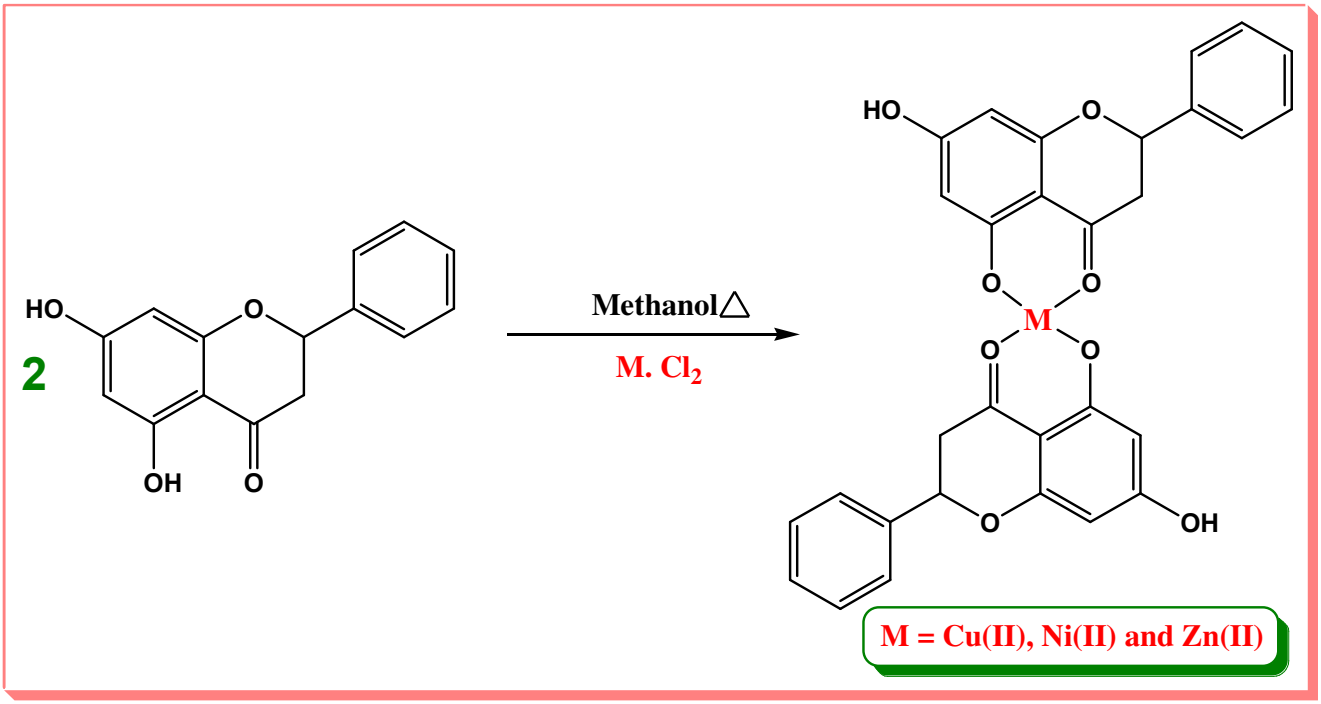
2.1 Materials and methods

All the chemicals used in the present investigation were of Analytical grade. Metal salts as chlorides obtained from Aldrich and were used as such without any further purification. Elemental analysis (C, H and N) of samples was carried out by using Carlo Erba Elemental analyzer Model 1106. The FT-IR spectra were recorded on Shimadzu IR affinity-I 8000 FT-IR spectrometer using KBr disc. The IR spectra were recorded on a Perkin Elmer FTIR spectrometer with samples prepared as KBr pellets in the range of 400-4000 cm^{-1}. UV-visible spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer

operating in the range of 200-1100 nm with quartz cells and are given in M^{-1} cm^{-1}. The emission spectra were recorded on a Perkin-Elmer LS-45 Fluorescence spectrometer. Electrospray ionization mass spectral measurements were done using a Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer. Circular dichroic titrations with CT-DNA were attained by using a JASCO J-715 spectro polarimeter outfitted with a Peltier temperature control device at 25 ± 0.1 °C with a 0.1 cm path length cuvette. Viscosity measurements were carried out in Brookfield Programmable LV DVII+ viscometer. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. DNA was purchased from Bangalore Genei (India). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared by means of deionized and sonicated triple distilled water. The entire glassware used was made of Pyrex or corning glass. The burettes, pipettes, and standard flasks were calibrated by the technique described by Vogel[15]. An analytical balance of 100µg sensitivity was used for weighing samples.

2.2. Synthesis of metal complexes

Methanolic solution of the metal (II) chloride (20 ml) was progressively added in a drop wise manner to the hot methanolic (25 ml) solution of the Pinocembrin in 1:2 molar ratio. The solution was continuously stirred for 4 h, during which the metal complex precipitated. The resulting coloured precipitates were filtered off, washed with methanol then with diethyl ether and finally dried under vacuo. The synthesized complexes are air stable in the solid state and are absolutely soluble in DMF and/or DMSO. The progress of reaction was monitored by TLC as represented in scheme 1.



Scheme 1 Synthesis of novel Pinocembrin metal complexes.

DNA binding experiments

The procedures for the DNA binding experiments are as same as discussed in our previous work [16].

RESULTS AND DISCUSSION:

3.1 Structural characterization of the Schiff base ligand (L) and their complexes.

3.1.1 FT-IR spectral analysis.

The FT-IR spectrum data in KBr with parent ligand pinocembrin and their complexes were compared as represented in Figure 1, 2 and S1 and S2. The absorption around 3380 cm^{-1} due to phenolic hydroxyl in the free ligand shows significantly spectral change in the complexes, indicating the chelate formation through hydroxyl group. The intense absorption bands due to $\upsilon_{(C=O)}$ of free ligands at 1678 cm^{-1} was also shifted to lower frequency and

overlapped with the band at 1618 cm^{-1} for the Cu(II) pinocembrin, 1616 cm^{-1} for the Ni(II) pinocembrin and 1617 cm^{-1} for the Zn(II) pinocembrin complex and was no more visible. The latter behavior can be interpreted as active evidence of the involvement of the carbonyl group in the metal binding. The $\upsilon_{(C\text{-}O\text{-}C)}$ at 1432 cm-1 shifted to 1286 cm^{-1}, thus it suggests that pinocembrin is coordinated with M(II) through oxygen atom of –OH and C=O group [17].

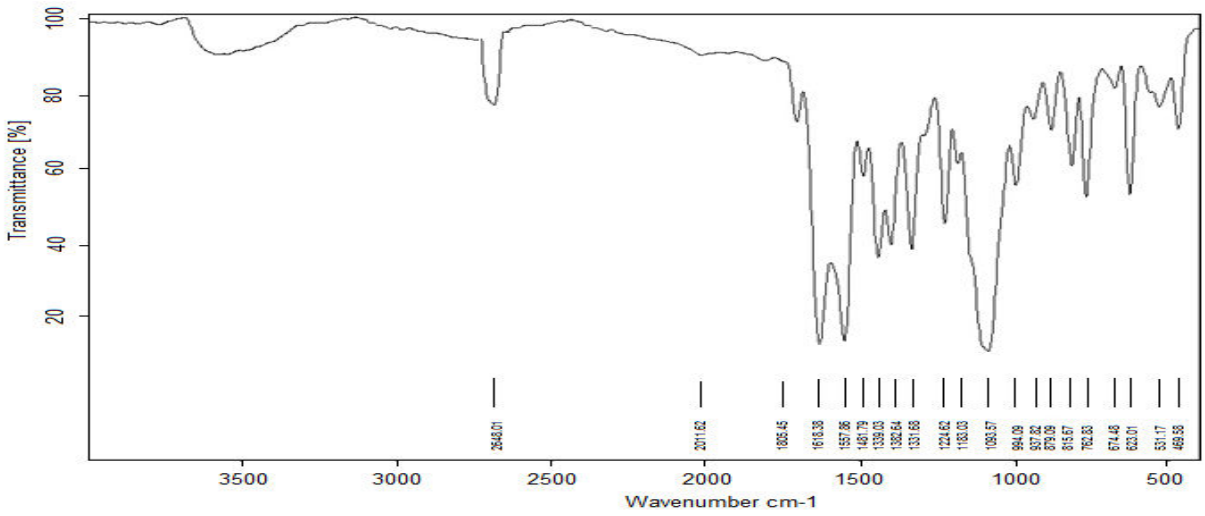


Figure 1 FT-IR spectrum of Free Pinocembrin.

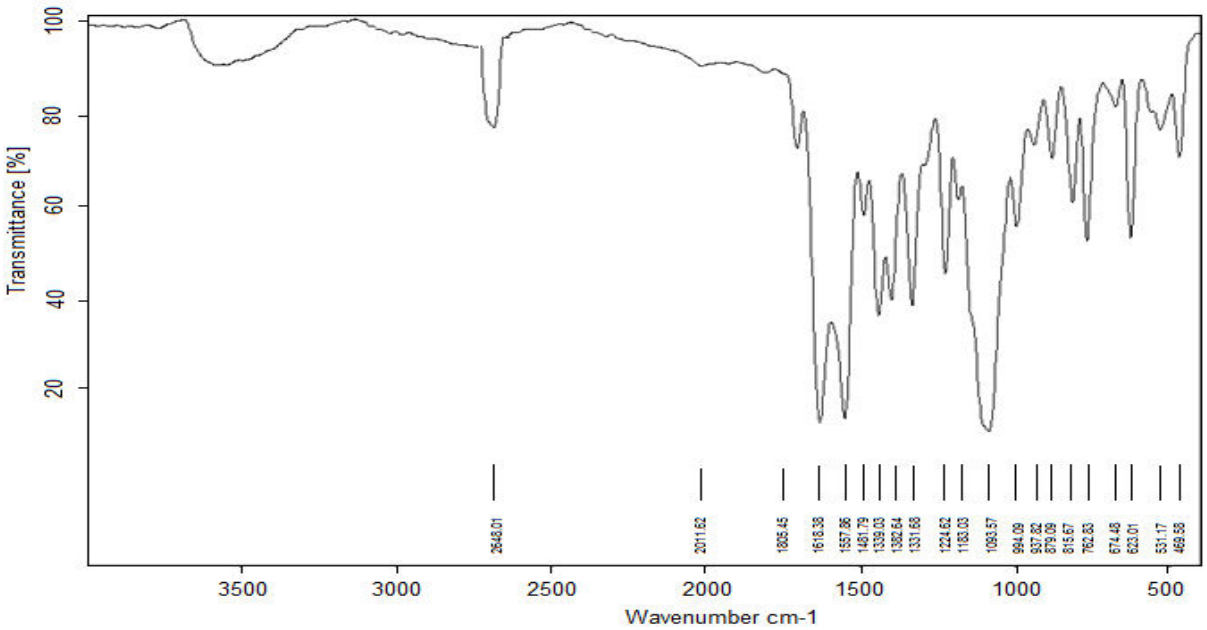


Figure 2 FT-IR spectrum of Cu(II) complex.

3.1.2 ESI-MS spectrum.

The molecular ion peak [M+] at m/z = 574 confirms the molecular weight of the Cu(II) pinocembrin complex $C_{30}H_{22}CuO_8$. The peaks at m/z = 542, 465, 389, 347, 305, 255, 205 and 185 corresponds to the various fragments like $C_{30}H_{22}CuO_6$, $C_{24}H_{18}CuO_6$, $C_{18}H_{14}CuO_6$, $C_{16}H_{12}CuO_5$, $C_{14}H_{10}CuO_4$, $C_{10}H_8CuO_4$, $C_6H_6CuO_4$ and $C_4H_{10}CuO_4$ respectively as shown in Figure 3 and Figure S3and S4. The molecular ion peak [M+] at m/z = 569 and 575 confirms the molecular weight of the macrocyclic Schiff base Ni(II) and Zn(II) complex $C_{30}H_{22}O_8M$. [M = Ni and Zn]. The type of fragmentation observed in Ni(II) and Zn(II) complexes was analogous with that of the Cu(II) complex.

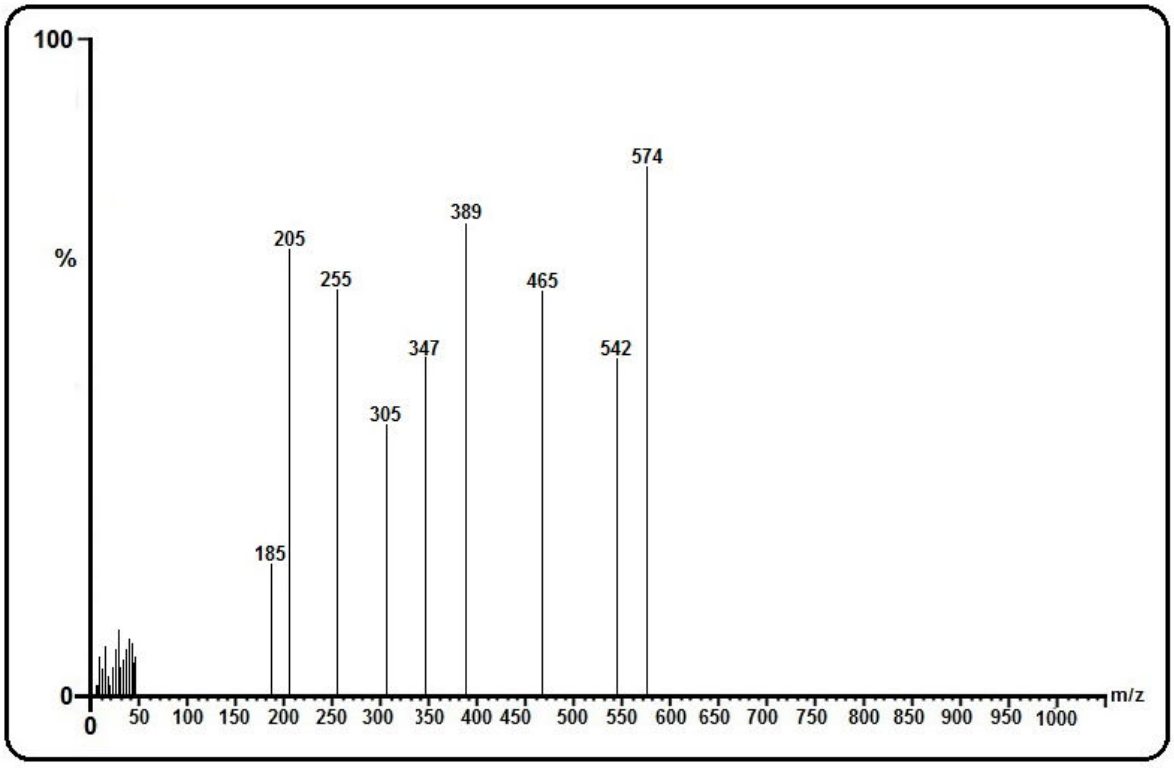


Figure 3 Mass spectrum of Cu(II) complex.

3.1.3 Electronic spectral analysis

The electronic spectra of the complexes have been measured in the range 200-800 nm in DMSO. The UV-Vis absorption bands of the complexes Cu(II), Ni(II) and Zn(II) are obtained in the range of 200-500 nm is given in Figure 4 (a), and the absorption bands obtained in the range of 500 to 800 nm is shown in Figure 4 (b). The spectrum shows absorption between 200-300 nm which is intra-ligand charge transfer transitions. The absorption band in the region 350-430 nm is attributed to the metal - ligand charge transfer. In the electronic absorption spectrum of the present Cu(II) complex, it shows one d–d transition at 695 nm which can be assigned to $^2A_{1g} \rightarrow {}^2B_{1g}$ transition. It reveals that the Cu(II) complex exists in square planar geometry. The electronic absorption spectrum of Ni(II) complex shows a low intensity band at 606 nm, which is assigned to ($^1A_{1g} \rightarrow {}^1B_{1g}$) transition reveal that this nickel complex exists in square-planar environment. The observed square-planar environment for the nickel(II) complex in conformity with the fact that all known square-planar complexes of nickel(II) are diamagnetic.Zn (II) ion which has a completely filled [10] electronic configuration is not expected to show any d-d electronic transition, and the complex is expected to have tetrahedral geometry with sp^3 configuration.

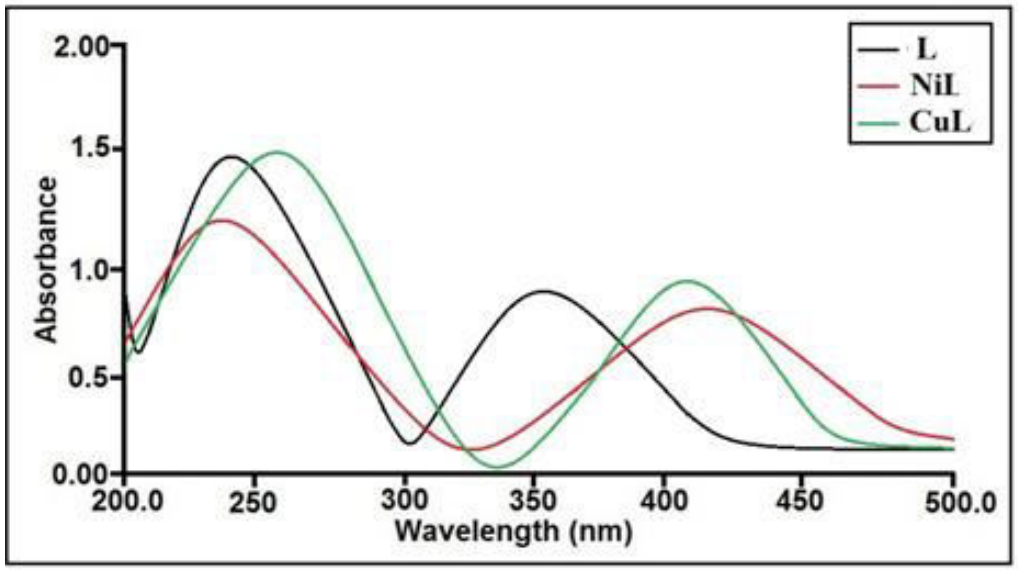


Figure 4 (a) Electronic spectrum of L, CuL & NiL complexes (200 to 500 nm).

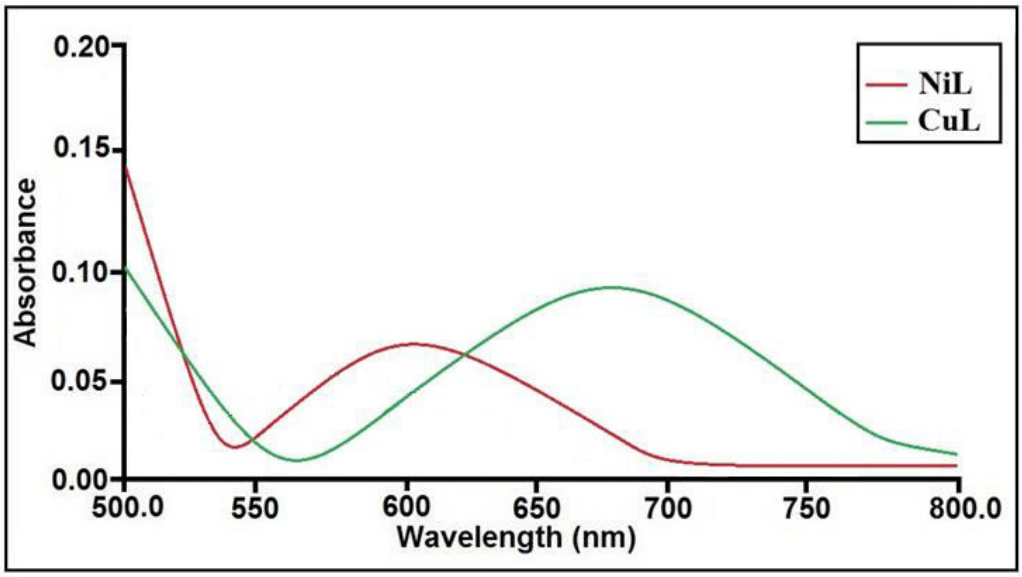


Figure 4 b Electronic spectra of NiL & CuL complexes (500 to 800 nm)

3.2 DNA binding experiments

3.2.1 Absorption spectral studies

The binding phenomenon is studied by electronic absorption spectral technique. Any change in the UV–Vis absorption spectra of metal complexes upon the addition of DNA serves as a proof for the existence of an interaction between them; in particular, hypochromism due the $\pi \rightarrow \pi^*$ stacking interaction with a red-shift (bathochromism) may appear in the case of an intercalative binding leading to stabilization of DNA duplex. The electronic absorption spectra of metal (II) complexes exhibit two well-resolved bands in the range of 250–400 nm [18]. Binding of the macromolecule leads to changes in the electronic spectrum of the metal complex. Base

binding is expected to perturb the ligand field transition of the metal complex. A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative mode involving a stacking interaction between the aromatic chromophore and the DNA base pairs. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction. On the other hand, metal complexes, which bind non-intercalatively or electrostatically with DNA may result in hyperchromism or hypochromism [19].

The interaction of the synthesized metal(II) complexes (Figure 5 and S5 and S6) towards DNA was investigated by electronic absorption titration to evaluate their binding affinities. The electronic absorption spectra of all complexes showed an intensive absorption band at 259.0 nm. Increasing the concentration of CT-DNA resulted in the bathochromic shift in the range 2-4 nm and significant hypochromicity deceitful in the range of 19–30%. The observed hypochromism could be credited to stacking interaction between the aromatic chromophores of the complexes and CT - DNA. The prominent shift in the spectra also suggests the tight complexation of synthesized molecule with DNA, which resulted in the change in the absorption maxima of the DNA. These results suggest an intimate association of the compounds with CT-DNA and it is also likely that compounds bind to the helix *via* intercalative mode. To categorize the obtained information on the binding affinity of the three complexes towards CT-DNA, the intrinsic binding constants (K_b, insets in Figure 5) were estimated using

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA, the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[complex], the extinction coefficient for the free metal complex and the extinction coefficient for the metal complex in the fully bound form, respectively. The K_b value is given by the ratio of the slope to the intercept. The obtained spectroscopic change implies that the intrinsic binding constants (K_b) of Cu(II), Ni(II) and Zn(II) complexes are 6.34 x 10^4 M^{-1}, 5.28 x 10^4 $M^{-1}$and 4.62 x 10^4 M^{-1}.

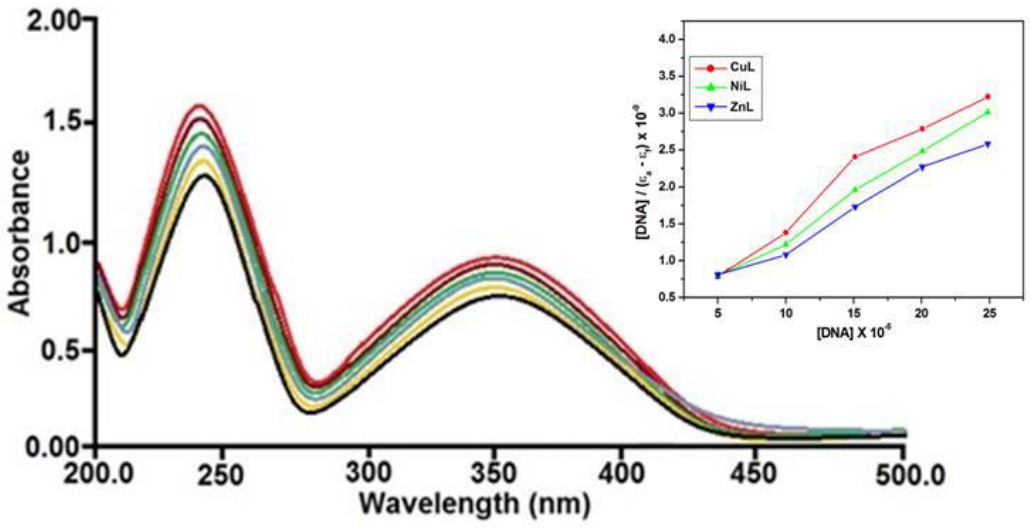


Figure 5 The absorption spectra of complex Cu(II) (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at normal temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). The Inset shows the plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

3.3.2. Fluorescence emission spectrometry

The relative binding of the synthesized metal complexes of pinocembrin towards CT DNA was investigated by fluorescence spectral method using EB (Ethidium Bromide) bound CT DNA solution in Tris–HCl/NaCl buffer (pH, 7.2). In common, the measurement of the ability of a complex to affect the EB fluorescence intensity in the EB-DNA adducts allows determination of the affinity of the complex for DNA, whatever the binding mode may be. If a complex can replace EB from DNA-bound EB, the fluorescence of the solution will be quenched due to the fact that free EB molecules are readily quenched by the surrounding water molecules. Two mechanisms have been proposed to account for this reduction in the emission intensity: the replacement of molecular fluorophores (EB in this case) and/or electron transfer [20].

The fluorescent intensity of EB bound CT-DNA at 612 nm with increasing concentration of the complexes steadily was recorded. The addition of the synthesized metal complexes to CT-DNA could result in the competitive displacement of EB and hence decrease in the emission intensity. The quenching of the emission spectra of EB bound to DNA by the complexes as shown in Figure 6, S7 and S8. For all the synthesized compounds, no emission was observed either alone or in the presence of CT-DNA in the buffer. The quenching plots (insets in Figure 6) illustrate that the fluorescence quenching of EB bound to DNA by Cu (II), Ni (II) and Zn (II) complexes in linear agreement with the Stern–Volmer equation, equation (2) which corroborates that the complexes bound to DNA. The Kapp values for Cu(II), Ni(II) and Zn(II) complexes are found to be 5.91 x 10^5 M^{-1}, 4.87 x 10^5 M^{-1} and 4.13 x 10^5 M^{-1} respectively.

$$I_0/I=1 + K_{svr}. \quad (2)$$

Where I_0, is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K_{sv} is a linear Stern – Volmer quenching constant and r is the ratio of the total concentration of quencher to that of DNA, [M] / [DNA]. A plot of I_0 / I_{vs}. [complex]/ [DNA], K_{sv} is given by the ratio of the slope to the intercept. The apparent binding constant (K_{app}) was calculated using the equation K_{EB}[EB] / K_{app}[complex], where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and K_{EB} = 1.0 x 10^7 M^{-1} ([EB] = 3.3 µM).

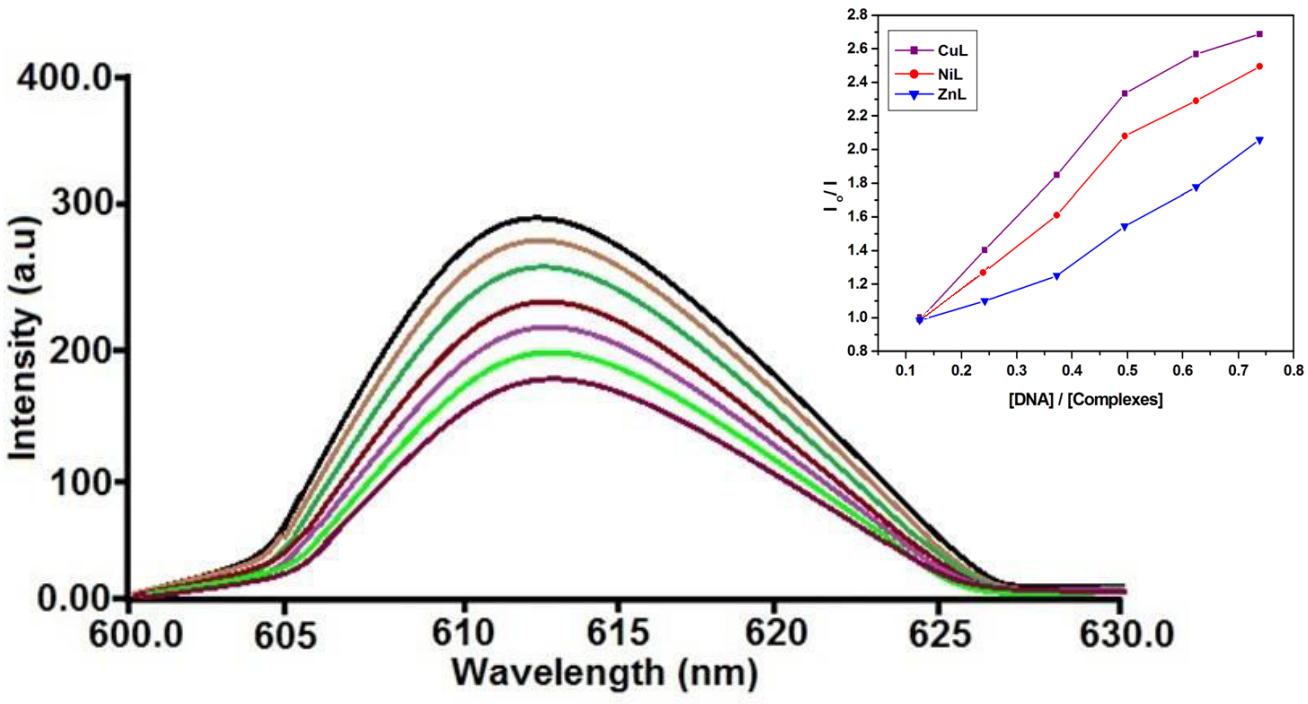


Figure 6 Emission spectrum of EB bound to DNA in the presence of Cu(II):([EB] =3.3 µM,[DNA] = 40 µM, [complex] = 0-25 µM, λ_{ex}= 440 nm). Inset shows the plots of emission intensity I_o / I *vs* [DNA] / [complex] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

3.2.3 Circular dichoric spectral studies

A solution of CT-DNA exhibits a positive band (275 nm) from base stacking interactions and a negative band (245 nm) from the right-handed helicity of DNA. Classical intercalation reactions tend to enhance the intensities of both bands due to strong base stacking interactions and stable DNA conformations (right-handed B conformation of CT-DNA), whereas simple groove binding and electrostatic interactions with small molecules show less of a perturbation or no perturbation whatsoever on the base stacking and helicity bands [21]. The intensity increases of the band at 286 nm were less pronounced for the synthesized complexes as shown in Figure 7, respectively. This indicates that the complexes interact with the DNA double strands by the intercalative mode between the base pairs of DNA strands without any significant change in the right-handed helicity of the DNA.

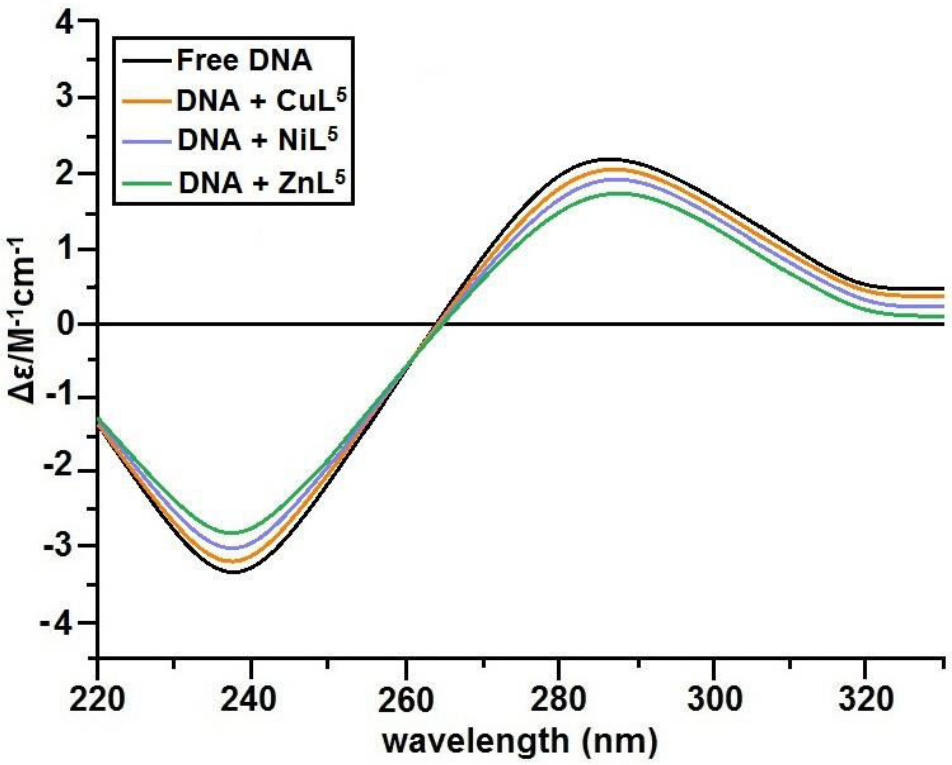


Figure 7 CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and mononuclear Cu(II), Ni(II) and Zn(II) complexes (100 µM).

3.3.4 Viscosity measurements

Viscosity is considered as least ambiguous and most critical test in predicting the nature of binding of the complexes to CT-DNA, in absence of crystallographic data. A classical intercalator causes significant increase in the viscosity of DNA solution due to the increase in the separation in overall DNA contour length. A partial/or non-classical intercalation of metal complexes causes a bend or kink in the DNA helix reducing its effective length and, as a result, DNA solution viscosity is decreased or remains unchanged [22]. The effects of all the complexes synthesized on the viscosity of CT DNA are shown in Figure 8. The viscosity measurements noticeably demonstrated that all the complexes can intercalate between adjacent DNA base pairs, origins a extension in the DNA helix and thus increasing the viscosity of DNA with an increasing concentration of the complexes. On the source of the entire the spectroscopic studies collectively with the viscosity measurements, we found that all the synthesized the metal complexes can bind to CT-DNA *via* an

intercalative mode.

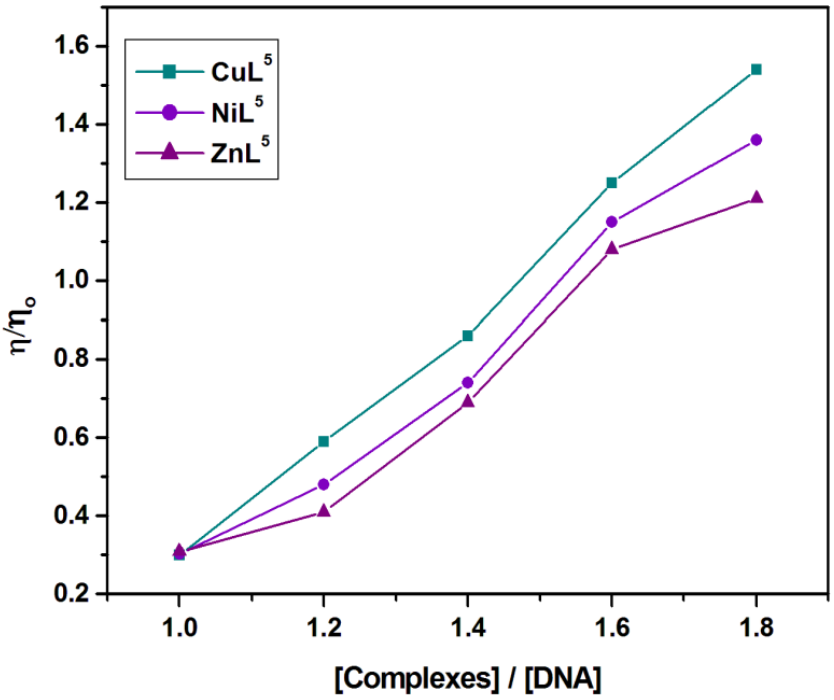


Figure 8 Viscosity measurements of the Cu (II), Ni (II) and Zn (II) complexes.

CONCLUSION:

In conclusion, the present paper reports on the synthesis, characterization of metal based pinocembrin complexes. The synthetic procedure in this work resulted in the formation of complexes in the molar ratio (1:2) (M:L), respectively. In this work, we explored the binding interaction of the synthesized Cu (II), Ni (II) and Zn (II) complexes with CT-DNA in physiological buffer using UV–Vis and fluorescence spectroscopic techniques. The intercalative binding of mentioned complex with DNA was deduced by taking account of relevant UV–Vis absorption spectra, circular dichroism, fluorescence spectra and viscosity measurements.

Abbreviations

DNA -	Deoxyribose nucleic acid
CT -	Calf Thymus DNA
Cu -	Copper
Ni -	Nickel
Zn -	Zinc
Tris-HCl -	Tris(hydroxymethyl)aminomethane
EB -	Ethidium Bromide
CD -	Circular Dichroism

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Supplementary Figure captions.

Figure S1. FT-IR spectrum of Ni(II) complex.

Figure S2. FT-IR spectrum of Zn(II) complex.

Figure S3. Mass spectrum of Ni(II) complex.

Figure S4. Mass spectrum of Zn(II) complex.

Figure S5. Absorption spectra of complexes Ni(II), (1 x 10-5 M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10-3 M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

Figure S6. Absorption spectra of complexes Zn(II), (1 x 10-5 M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10-3 M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

Figure S7. Emission spectrum of EB bound to DNA in the presence of Ni(II); ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm).

Figure S8. Emission spectrum of EB bound to DNA in the presence of Zn(II); ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm).

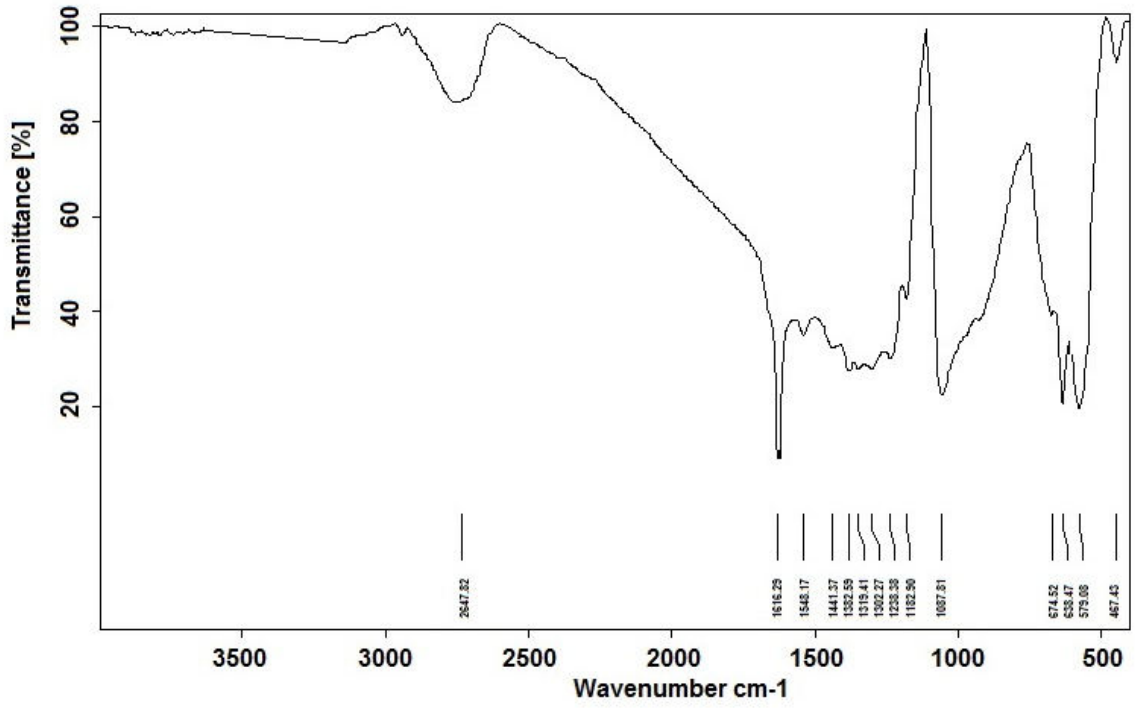

Figure S1: FT-IR spectrum of Ni(II) complex.

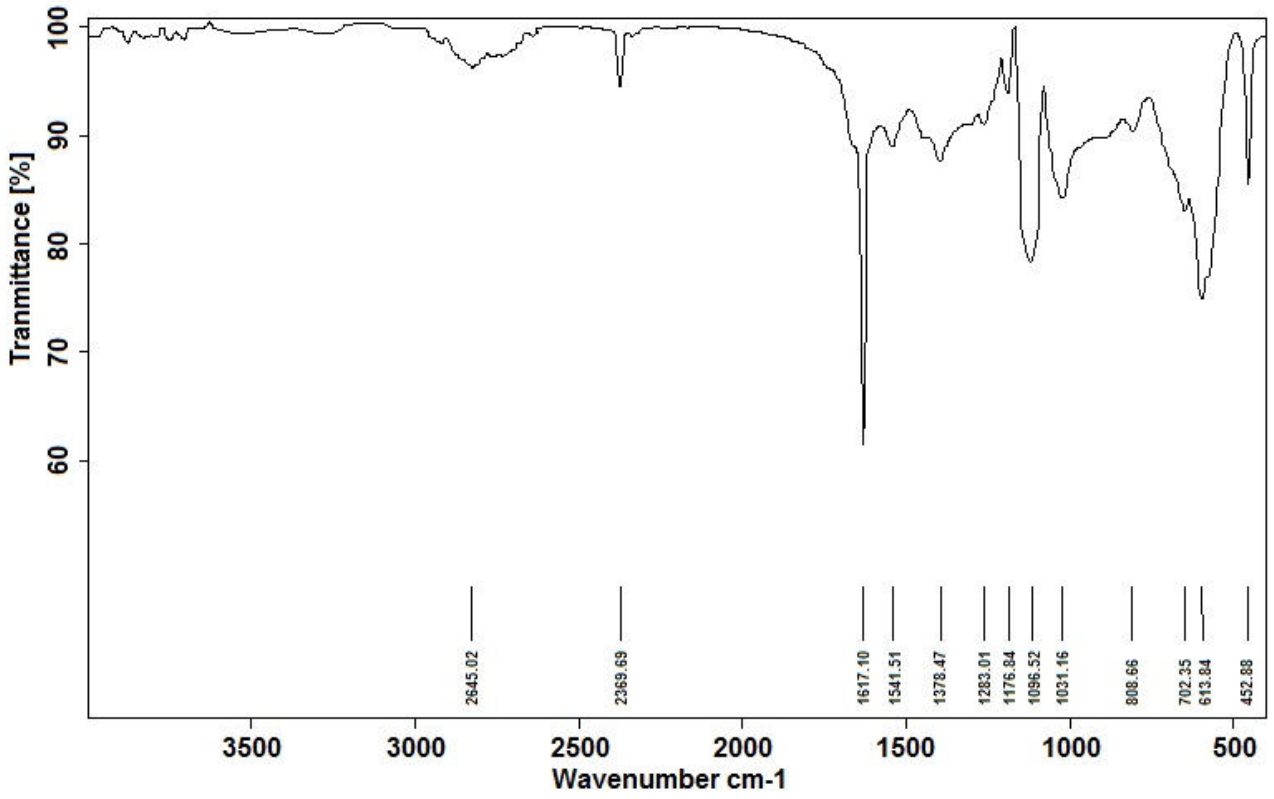

Figure S2: FT-IR spectrum of Zn(II) complex.

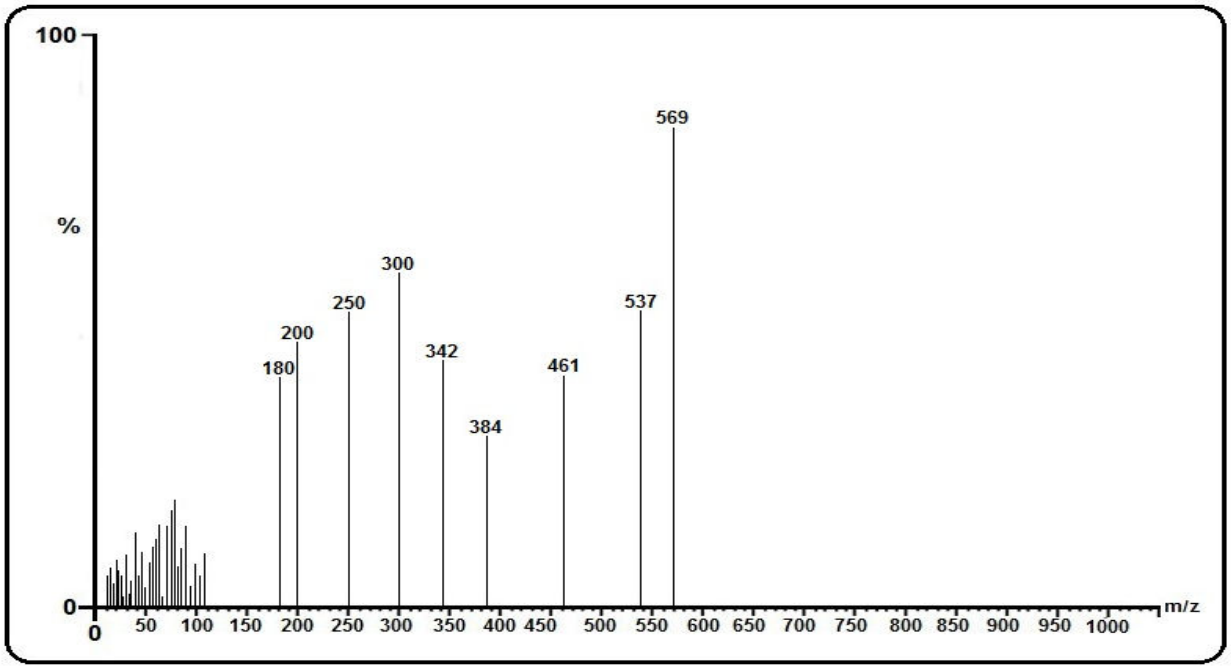

Figure S3: Mass spectrum of Ni(II) complex.

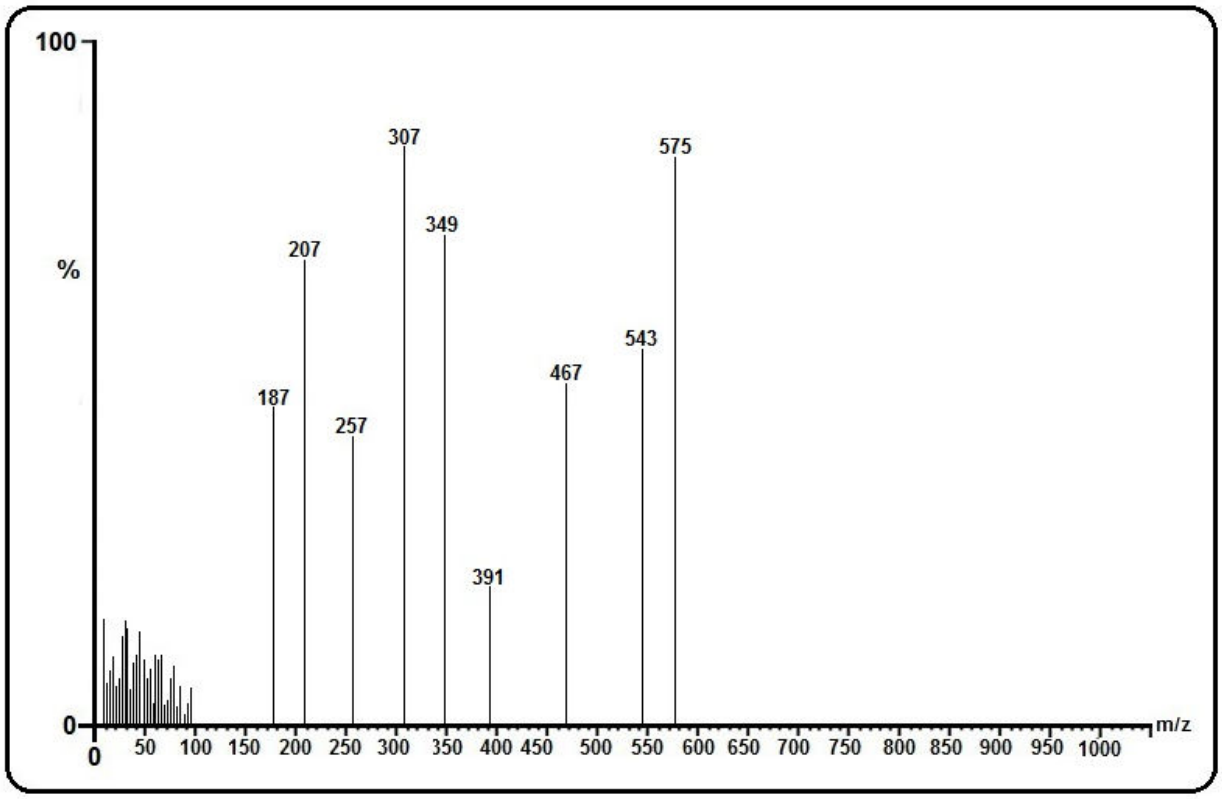

Figure S4: Mass spectrum of Zn(II) complex.

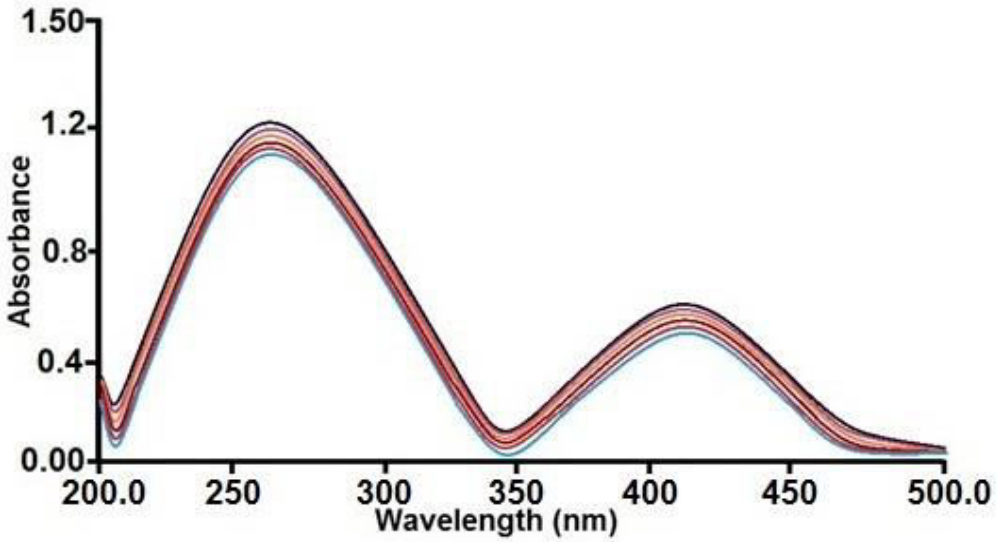

Figure S5. The absorption spectra of complex Ni(II)(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at normal temperature in 50 mM Tris-HCl / NaCl buffer

(pH = 7.5).

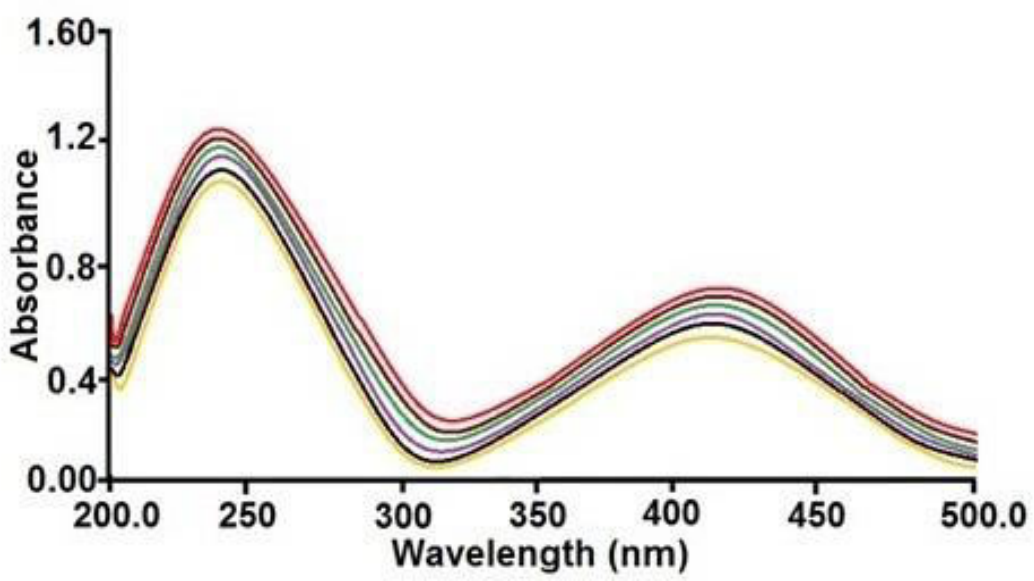

Figure S6 **The absorption spectra of complex Zn(II)(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{3} M) at normal temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).**

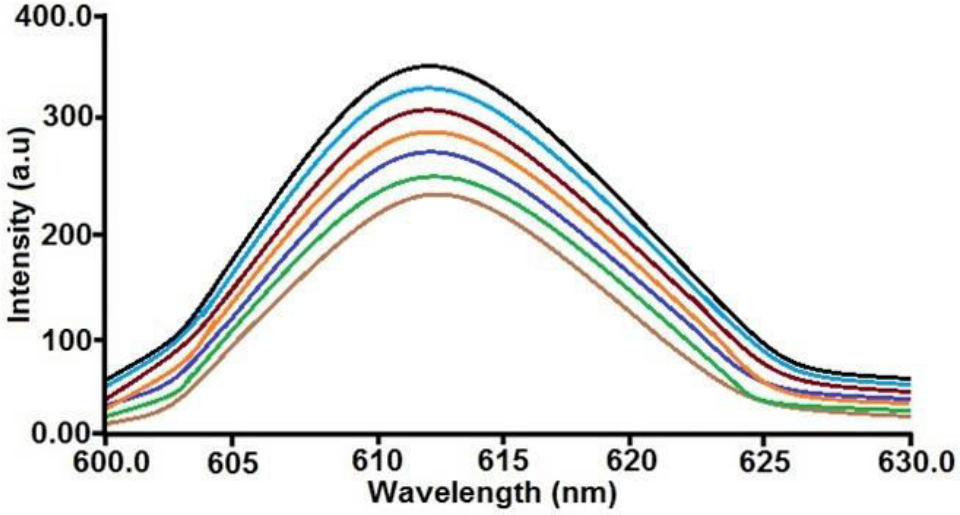

Figure S7 **Emission spectrum of EB bound to DNA in the presence of Ni(II):([EB] =3.3 µM,[DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm).**

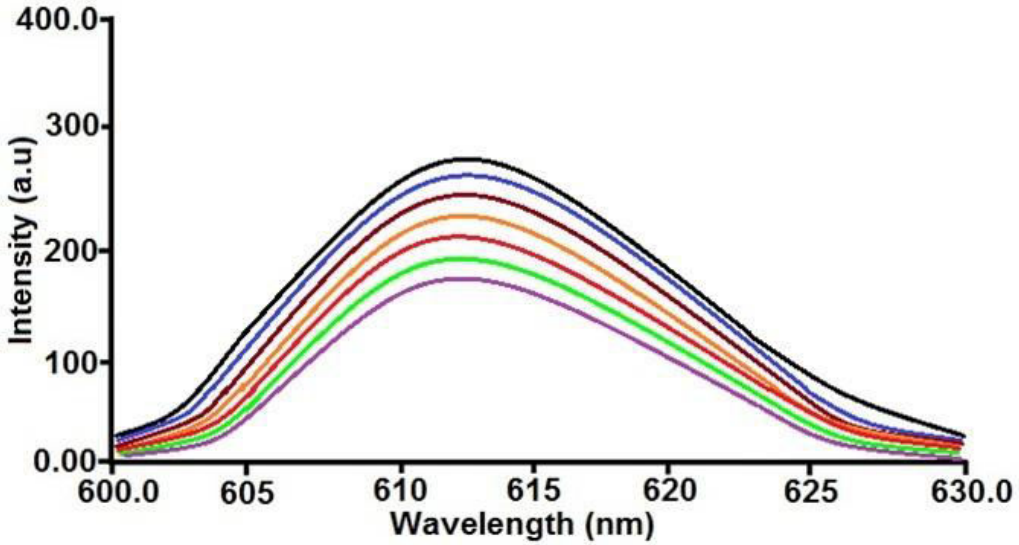

Figure S8 **Emission spectrum of EB bound to DNA in the presence of Zn(II):([EB] =3.3 µM,[DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm).**