Interaction of Catechu Dye with DNA: Spectroscopic and In Silico Approach

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ABSTRACT
Catechin, a yellow colored molecule obtained from the wood of Acacia catechu was analyzed for its interaction with synthetic DNA duplexes using spectroscopic analysis. UV-Visible spectroscopic analysis revealed the non-intercalative binding mode. Fourier Transform Infrared spectroscopy (FTIR) analysis expose chemical shift indicated by various vibrational stretches and an increase in the intensity of base stacking was observed by Circular Dichroism (CD), respectively. This inference was further confirmed through nuclear staining technique and also in electrophoretic technique; the dye quenches the fluorescent intensity of ethidium bromide. The result of fluorescence spectroscopy was in concordance with the electrophoretic technique. In addition, the spectroscopic results were in accordance with the molecular docking studies of specific catechin compound from the catechu dye with CT-DNA. This kind of site specificity is a gain in the medicinal field as the drug can be DNA targeted for cancer therapeutics. The present work reveals that catechu dye has a noteworthy application in the field of medical bioscience.

Introduction
The ubiquitous distribution of flavonoids, a polyphenolic has shown several biological activities, such as antioxidant, antimicrobial and anticancer.[1,2] The bioflavonoid catechin is a yellow color component obtained from the heartwood of Acacia catechu.[3] Catechin forms the major coloring component for Acacia catechu dye.[4] Apart from catechin, it also contains tannin, flavotannin, gallotannin and phloratannin.[5] Due to the presence of five hydroxyl group and one electron donation property, catechin has the ability to repair vitamin E.[6] Catechin has been used for the treatment of skin disease such as leucoderma and also act as an anti-hormonal agent.[7,8] The water extract of catechu was consumed as a health drink for various beneficial purpose such as blood purifier, increasing the immune system and improvement of skin texture.[9,10] Many studies have
reported that non-toxic, antipyretic, antidiarrhoeal, hypoglycaemic, hepatoprotective, anti-inflammatory and anticancer activities of *Acacia catechu*.\[4,11,12\] The presence of polyphenols in catechu extract protects DNA damage from free radicals.\[12\] There are reports that catechin from green tea directly interacts with nucleic acid and also inhibits the bacterial DNA gyrase by binding to ATP binding site of the gyrase B subunit, respectively.\[13,14\] The focus on natural dyes is successively increasing due to non-toxic, non-allergic and environmental safety aspects.\[6,15\] We have already reported that the interaction studies of CT-DNA with morindone, brazilin, anthraquinone.\[16–19\] Here in this report, we are discussing the interaction of catechu dye with DNA through various spectroscopic and molecular docking studies.

DNA carries genetic information for the development of various physiological and biochemical functions. If the change occurs in 141 arrangement of deoxynucleotide it can lead to cascade formation of disease.\[20\] Moreover, the interaction of small molecules with DNA plays an imperative role in pharmacology. In addition, the mechanism of interaction of molecules with DNA can provide the information on the effect caused by the molecules towards the physical and structural properties of DNA which shows the way for designing specific novel drugs targeted towards DNA.

The drug interacts with DNA by different ways, such as by binding to the exterior of the helix, groove binding interaction and intercalation. Exterior binding of DNA is by electrostatic attraction. Groove binding engages direct interaction of the bound molecule with edges of base pairs either at major or minor grooves. Among two other modes of interaction, the intercalation of drug between the aromatic base pairs of DNA is an effective mode, which results in lengthening, unwinding of helical DNA and formation of Π–Π overlapping.\[21–23\] The present study is centered towards the mode of interaction of catechu dye and the component in catechu dye responsible for interaction with CT-DNA which were analyzed through UV-Vis spectrophotometer, fluorescence spectroscopy, FTIR, CD and then complemented by histological staining, electrophoretic analysis. The molecular docking studies provide further support, that particular compound catechin from catechu dye forms CT-DNA-catechin adducts. The investigation on catechu dye interaction with DNA provides fruitful information for the discovery of DNA directed drug therapy.

**Materials and methods**

**Chemicals**

\((\pm)-\text{Catechin (C1251–5G), sodium-calf thymus DNA (CT-DNA) (D1501–100MG) purchased from Sigma Chemical Co. (St. Louis, MO). Agarose, gel loading dye and ethidium bromide purchased from Himedia, India. All the solvents used were of analytical grade.}\)
Figure 1. UV-Vis spectrum of varied concentration of CT-DNA interacting with constant concentration of *Acacia catechu* dye (50 µg/3 mL). Insert – (a) Plot of $[\text{CT-DNA}] / \varepsilon_a$ – $\varepsilon_f$ versus CT-DNA (b) Spectrum of increasing concentration of free CT-DNA.

**Processing of CT-DNA**

Five grams of CT-DNA was de-proteinated by adding up of CHCl₃ and isoamyl alcohol in NaCl solution. The stock solution of CT-DNA was equipped by dissolving CT-DNA (1 mg/mL) in TE buffer at 4°C for 24 hours with intermittent stirring to ensure the formation of homogenous solution. The concentration of the calf-thymus DNA solution was resoluted from the UV absorbance at 260 nm using molar extinction coefficient $\varepsilon_{260} = 6600$ M⁻¹ cm⁻¹. The absorbance at 260 and 280 nm was recorded with the aim to check the protein content of CT-DNA solution (Figure 1 insert). The $A_{260}/A_{280}$ ratio was 1.85, showing that the CT-DNA was adequately free from protein. Different concentration of the CT-DNA was used to interact with a constant concentration of dye to obtain a diverse molar ratio of the CT-DNA-dye adduct in the course of the study.

**Preparation of the dye**

Catechu dye was extracted from the heart wood of *Acacia catechu* (100g) which was subjected to complete extraction in a soxhlet apparatus using methanol as a solvent at 45°C. The extract acquired was then evaporated to dryness at 40°C under reduced pressure (337 mbar) in a rotavapor R-215 (BUCHI Labortechnik AG, Switzerland). The dye extract thus obtained is stored at 4°C and used for the further studies. The composition of the dye were analysed by UV-Vis spectrophotometer, FTIR and
HPLC (Figs. S1–S3). The major presence of specific component catechin in the dye extract was proved through UV-Vis spectrophotometer and HPLC analysis method (Figs. S1–S3).

**UV-Vis spectrophotometer**

The absorption spectra were measured using Hitachi U-2910 UV–Vis Double beam spectrophotometer 1 cm quartz cell in the range of 200–400 nm. The stock concentration of catechu dye (1 mg/mL) was dissolved in 0.1% DMSO and catechin (1 mg/mL) was dissolved in MilliQ water. The interaction of catechu dye and catechin with CT-DNA was studied with a constant concentration of the catechu dye (50 µg/3 mL) and standard catechin (50 µg/3 mL), respectively, with varying concentrations of CT-DNA (10–80 µg/mL).

**Fluorescence studies**

The competitive interaction of catechu dye, catechin and well known intercalator ethidium bromide (EtBr) with CT-DNA was studied using Hitachi F-700 Fluorescence instrument. The constant concentration of CT-DNA (40 µg/mL) was pre-treated with EtBr (20 µg/mL). The EtBr-CT-DNA complex was titrated with different concentrations of catechu dye (10–50 µg/mL) and catechin (10–50 µg/mL). The reaction mixtures were mixed gently, the fluorescence emission spectra were carried out over the wavelength range of 500–800 nm with an excitation wavelength at 280 nm.

The fluorescence quenching data were plotted as the fluorescence intensity against the different concentrations of catechu dye and catechin. Fluorescence quenching was described by the Stern-Volmer equation.[24]

\[
\frac{F_0}{F} = 1 + K_{SV}[Q]
\]

where \(F_0\) and \(F\) are the fluorescence intensities of EtBr-CT-DNA complex before and after the addition of the quencher (catechu dye and catechin), respectively, \([Q]\) is the concentration of the quencher, and \(K_{SV}\) is the Stern-Volmer quenching constant. Hence, the above equation is applied to determine the \(K_{SV}\) using linear regression of a plot of \(F_0/F\) against \([Q]\).

**FTIR spectroscopy**

Infrared spectra were recorded on a Shimadzu IR Affinity-1 Fourier Transform Infrared spectrophotometer (DTGS detector, Ni-Chrome source and KBr beam splitter) with a total of 100 scans and a resolution of 4 cm\(^{-1}\). Spectra were collected and treated using the OMNIC software supplied by the maker of the spectrophotometer. Solution spectra were recorded after 1 hour of incubation, using AgBr windows. In the present investigation, the binding property of molar concentration
ratio of catechu dye and catechin to CT-DNA (5:3, 1:1) was examined through FTIR spectrum.

**Circular dichroism (CD) spectroscopy**

CD spectra for the credible interaction between CT-DNA with catechu dye and catechin compound were recorded on a Jasco J-715 spectropolarimeter in a rectangular cuvette with 3 cm optical path length at 25 ± 0.2°C temperature was synchronized by a Peltier type temperature control system. Additionally, the difference in the spectra was noted that adducts of CT-DNA-dye formed. All spectra were accumulated for five times with a bandwidth of 1.0 nm and a resolution of 0.5 nm at a scan speed of 100 nm min⁻¹.

**Histological staining**

The histological staining of onion tissue was carried out as per the protocol of Bhakta et al.[16] Fine sections of *Allium cepa* inner peel were dipped in isotonic solution for a span of ten min. The sections were stained with catechu dye (10 mg/mL) and then allowed to stand for 5 min. The excess stain was washed away with double distilled water and the slide was thereafter observed under light microscope adjusted at 10× magnification.

**Agarose gel electrophoresis**

The reaction mixture containing a constant concentration of CT-DNA (1 µg) with the different concentration of catechu dye (1, 2, 3, 4, 5 µg) was incubated at 37°C for 30 min with vortexing from time to time. To the 10 µL of reaction mixture, 5 µL of gel loading dye (bromophenol blue) was added. The samples were subjected to 0.8% agarose gel at a constant voltage of 100 V for 1 hour. After electrophoresis, the gel was stained for 1 hour by soaking it in a 0.5 µg/mL ethidium bromide solution. The stained gel was illuminated under a UV transilluminator and then photographed using Image Quant 300 Gel Imaging System.

**Insilico docking simulation**

The three dimensional structure of target octamer complexed with actinomycin D (PDBID: 1DSC) was retrieved from protein data bank (www.rcsb.org) and further modified for docking calculations. For docking (Auto Dock4.0) calculations, the co-crystallized ligands were identified and removed from the structure of 1DSC. The receptor DNA (1DSC) and the ligand bioflavonoid catechin were taken for insilico docking studies. The UCSF Chimera program (http://www.cgl.ucsf.edu/chimera) was used to prepare the structures for input to AutoDock4.0 (http://mgltools.scripps.edu) by adding Gasteiger charges (computed
using ANTECHAMBER) and running 10,000 steps of energy minimization. Docking was performed to obtain a possible conformations and orientations for the ligand at the binding site. Using the software, polar hydrogen atoms were added to the DNA and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for DNA, which is kept ridged and legend being flexible throughout the docking program were done using the Lamarckian Genetic Algorithm (LGA). The grid box with a dimension of $50 \times 50 \times 50$ points were used around the DNA to cover the entire binding site and provide place to the ligands to move freely. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interaction of complex DNA with bioflavonoid catechin conformation was analyzed using python molecular viewer.

**Result and discussion**

In recent years, the interaction of drugs or small molecules with DNA has created more attention towards the discovery of DNA targeted therapeutics. The mechanism of interaction of drugs with DNA can be used to design model DNA-targeted drugs. In this present investigation, the binding of catechu dye to CT-DNA exhibited the change in the absorbance and spectral shift which was compared with free dye by multi spectroscopic analyses. The molecular docking studies provide in depth binding affinity of specific catechin compound with CT-DNA. For further confirmation, the histological staining of the *Allium cepa* and electrophoretic analysis were performed to know the binding of the catechu dye to the nucleus which also quenches the fluorescent intensity of ethidium bromide, respectively.

**DNA binding studies**

**UV-Vis spectroscopy**

The interaction catechu dye with CT-DNA was evaluated by effective UV-Vis absorption technique (Figure 1). The spectral shift indicates the interaction and complex formation. The binding of molecule to CT-DNA exhibited through hypochromism and hyperchromism in the absorption spectrum. The hypochromism originates from the intercalative binding of the molecules with DNA. Meanwhile the hyperchromism originates from the disruption of secondary structure of DNA. The catechudye spectrum displayed absorption maximum wavelength of 280 nm which indicates the presence of catechin compound in catechu dye. One of the usual peak of catechu dye 280 nm get shifted to longer wavelength 290 nm with increase in absorbance upon addition of increasing concentration of CT-DNA. Similar wavelength shift of 280 nm to 290 nm (red shift) and increased absorbance was exhibited upon the interaction of catechin with increasing concentration of CT-DNA (Figure 2). This mode of interaction is due to the stacking of the catechin compound forming higher-order aggregates on to CT-DNA. Shahabadi et al., has reported that stabilization of DNA is denoted by red shift. The binding constant $K_b$ of catechu dye and catechin with CT-DNA was determined
Figure 2. UV-Vis spectrum of varied concentration of CT-DNA interacting with constant concentration of standard catechin (50 µg/3 mL). Insert (a) Plot of [CT-DNA]/(ε_a − ε_f) versus CT-DNA. (b) Spectrum of increasing concentration of free CT-DNA.

through the linear equation:[24]

$$\frac{[\text{DNA}]}{(\epsilon_a - \epsilon_f)} = \frac{[\text{DNA}]}{(\epsilon_b - \epsilon_f)} + \frac{1}{K_b(\epsilon_b - \epsilon_f)}$$  \hspace{1cm} (2)

where [DNA] is the concentration of CT-DNA and absorption coefficients ε_a, ε_b, and ε_f are adduct, fully bound to DNA form and free complex. In the plot of [DNA] / (ε_a − ε_f) versus [DNA], K_b is obtained by the ratio of slope to intercept. The binding constant of catechu-CT-DNA and catechin-CT-DNA adducts is 1.546 $\times$ 10$^5$ M$^{-1}$ and 1.71 $\times$ 10$^5$ M$^{-1}$, respectively. Finally, the result suggested that binding mode of the catechu dye and catechin with CT-DNA was not a intercalative binding which was indicated through hyperchromism with red shift.

**Competitive binding studies**

The interaction of drugs with DNA was investigated through sensitive fluorescence spectroscopy technique. The fluorescence spectra of the CT-DNA-EtBr complex in the absence and presence of catechu and catechin was shown in the (Figures 3 and 4). EtBr is a well known intercalator which exhibits low fluorescence intensity, but the fluorescence efficiency was gradually increased upon interaction with CT-DNA.[32] In our present study, catechu and catechin decreased the fluorescence intensity of the CT-DNA-EtBr complex without any peak shift. This result indicated that catechu and catechin displays the EtBr into aqueous solution through competitive binding with the CT-DNA. The $K_{sv}$ value of catechu dye was 1.013 $\times$ 10$^{-5}$ and 0.96 $\times$ 10$^{-5}$,
Figure 3. Fluorescence emission spectra of Ethidium bromide bound CT-DNA with increasing concentration of Acacia catechu dye (10–50 µg/mL), Insert Plot of $F_0/F$ versus concentration of *Acacia catechu* dye.

respectively. Based on the $K_{sv}$ value, catechu dye shows good quenching effect of CT-DNA-EtBr complex than catechin.

**FTIR analysis**

The chemical binding of the catechu dye with CT-DNA was studied by FTIR spectroscopy (Figure 5). The vibrational bands of CT-DNA at 1710 cm$^{-1}$, 1663 cm$^{-1}$, 1608 cm$^{-1}$ and 1491 cm$^{-1}$ are assigned to nitrogenous bases guanine, thymine,
Figure 5. FTIR spectrum of (i) Acacia catechu dye (ii) Acacia catechu- CT-DNA adduct (5:3) ratio (iii) Acacia catechu- CT-DNA adduct (1:1) ratio.

Adenine and cytosine, respectively.\textsuperscript{[33]} The spectral region between 2000–750 cm\(^{-1}\) indicates the vibration band of nitrogenous bases, PO\(_2\) stretch and deoxyribose stretch of CT-DNA.\textsuperscript{[34]} No major spectral changes were observed in the molar concentration ratio of catechu, catechin to CT-DNA 5:3 and 1:1 ratio. Upon addition of catechu dye to DNA, the formation of positive peak at 1631 cm\(^{-1}\) and 1452 cm\(^{-1}\) were due to the increase in the intensity of thymine and cytosine, respectively. As like the catechu dye, catechin-CT-DNA adducts (Figure 6) show vibration band at 1631 cm\(^{-1}\) and 1471 cm\(^{-1}\) which indicates the interaction of catechin with thymine and cytosine, respectively. The vibration stretch at 1225 cm\(^{-1}\) and 1088 cm\(^{-1}\) denotes phosphate asymmetric and symmetric, respectively.\textsuperscript{[33]} The catechu dye-CT-DNA adduct spectrum shows a positive peak at 1236 cm\(^{-1}\), 1089 cm\(^{-1}\), 1059 cm\(^{-1}\) are due to the binding of the dye to phosphate. Ghosh et al., stated that the disappearance of certain characteristic peak indicates the probable interaction of dye-DNA adduct formation.\textsuperscript{[18]} The disappearance of a characteristic spectrum vibration stretch of dye at 2227 cm\(^{-1}\), 2017 cm\(^{-1}\), 1207 cm\(^{-1}\) and 1112 cm\(^{-1}\) shows the plausible interaction between dye and DNA. The appearance of certain peak such as 2765 cm\(^{-1}\), 2654 cm\(^{-1}\), 2333 cm\(^{-1}\) and 1323 cm\(^{-1}\) in the catechin-DNA adducts which indicates the interaction formed between catechin
and DNA (Figure 6). On addition of DNA to dye, the replacement of a peak at 3,421 cm$^{-1}$ by 3441 cm$^{-1}$ shows the complex formation between the NH group of DNA and dye. Kanakis et al., reported that sharp peak at 968 cm$^{-1}$ is due to the deoxyribose C–C and C–O stretching vibration.$^{[33]}$ The spectral change upon dye-CT-DNA complex 972 cm$^{-1}$ denotes the binding of the dye to deoxyribose of CT-DNA.

**Circular dichroism spectroscopy**

CD is a sensitive technique used to determine the conformation changes occurring in the macromolecules such as DNA and protein when it interacts with drugs. The CD spectrum of CT-DNA shows a positive peak at 275 nm (i.e.,) due to the base stacking (Figure 7 insert). If the molecule exhibits groove binding and electrostatic interaction with DNA that shows less disorder on the base stacking and helicity bands. Moreover, the intercalation of molecules with DNA was indicated through the weak intensity of base stacking of CD bands.$^{[35]}$ The effect of catechu dye and catechin on the conformation of secondary structure of CT-DNA was studied by keeping the constant concentration of dye and catechin, while the variable concentration of CT-DNA was used. The catechu dye and catechin strongly interacts with the base stacking of the CT-DNA, which was expressed as the increased intensity of CD band upon addition of CT-DNA (Figure 7).
Figure 7. (a) CD spectrum of varied concentration of CT-DNA interacting (10–50 µg) with constant concentration of Acacia catechu dye (b) CD spectrum of varied concentration of CT-DNA interacting (10–50 µg) with constant concentration of standard catechin. Insert—CD spectrum of free CT-DNA.

Nuclear staining

The histological staining towards specific organelle in the cell has permitted the cytologist to visualize and quantitate the cellular constituents.\textsuperscript{[36]} The non-ionic force of binding of the dye with tissue leads to the formation of equilibrium between dye and tissue.\textsuperscript{[37]} The various synthetic nuclear stains such as ethyl violet, geimsa interacts with DNA.\textsuperscript{[38]} The interaction of dye with macromolecular DNA can infer to study the cell nucleus morphology. Histological staining of Allium cepa cells enables us the direct image of the nucleus using catechu dye. Figure 8(b) indicates the specificity of Acacia catechu dye binding with the nucleus seen under the microscope in 10× magnification.

Figure 8. (a) Tissue section of Allium cepa peel without staining of Acacia catechu dye (blue arrow denotes nucleus) under 10× magnification; Histological staining of Allium cepa peel with Acacia catechu dye (black arrow denotes stained nucleus) under 10× magnification.
Electrophoretic studies

The DNA damage caused by molecule can be determined by agarose gel electrophoresis. Electrophoretic pattern of interaction of dye with CT-DNA have shown increased quenching of fluorescence of ethidium bromide with the increasing concentration of catechu dye (Lane 2–5; Figure 9) and it is compared with the control free CT-DNA (lane 1; Figure 9). The compound has the ability to interfere with the intercalation of ethidium bromide with CT-DNA. Vujcic et al., has reported similar result in case of quinone avarone. The result suggests that the protection of CT-DNA from the intercalator ethidium bromide by catechu dye. Thus, the competitive binding studies of catechu dye with CT-DNA-EtBr complex were in concordance with the electrophoretic technique.

In silico studies of DNA with bioflavonoid catechin

The binding pattern was analysed using AutoDock4.0 to predict the interaction of catechin with the receptors of known 3D structure. The ligand and target DNA were given as input and the flexible docking was performed to find out the conformational energy of catechin (Figure 10(b,c)). Each autodock run here not only predicted a binding conformation but also produced a value for the free energy of binding (FEB) in Kcal/mol and an estimated inhibitory concentration (Ki) in µM. The negative value of ΔG bind indicates strong favourable bonds between DNA and the novel ligand to ensure that the ligand orientations and positions obtained from the docking studies were likely to represent valid and reasonable potential binding modes of the inhibitors. Therefore, the binding energy value of bioflavonoid catechin is −4.2. The docking results illustrated that Van der waals (VDW) force were in association with the hydrogen bond. Moreover, the positive value of VDW energies indicated that the ligand is not fitting well into the active site. But the result of the
study shows that VDW force energies are considerably negative for bioflavonoid catechin, which fits well into the DNA molecule. The result suggests that bioflavonoid catechin can be used as an indicative probe and DNA directed therapeutics.

**Conclusion**

In summary, the binding property between catechu dye and catechin with CT-DNA were examined through spectroscopic, staining and molecular docking analysis. The binding of dye and catechin with CT-DNA showed significant change in the spectrum. Hyperchromism with red shift were observed in absorption spectrum that reveals the non-intercalative binding of dye and catechin to CT-DNA. The FTIR analysis shows the spectral shift after the addition of CT-DNA to dye that indicates the probable interaction between dye and catechin with CT-DNA. CD spectrum showed conformation changes in the CT-DNA (i.e,) increase in the intensity of base stacking upon addition of catechu dye. Furthermore, the histological staining and an electrophoretic technique prove that catechu dye binds only to the DNA of the nucleus and prevent the CT-DNA from the intercalator, respectively. In addition, the results of molecular docking were proved the investigation of spectroscopic techniques. This study of interaction between dye and CT-DNA shows the
pharmacological nature of dye and also helps in the development of natural colorant as a new novel drug in future.

**Acknowledgments**

The authors are thankful to management of VIT University for their constant support. We wish to thank VIT-SIF lab, SAS, chemistry division for spectroscopy analysis.

**References**


