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# Spectroscopic and Molecular Docking Studies on the Interaction of Aloe-emodin with GC and AT specific DNA Oligonucleotides

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**Abstract:** Naturally occurring anthraquinone compounds have gained much attention as anticancer agents due to their distinguishing binding properties with nucleic acids. However, the binding profile with sequence specific DNA sequences have been less explored. Therefore, for present study Aloe-emodin was selected to study the complexation with short sequence specific oligonucleotides to determine its sequence specificity. The techniques employed to determine the binding behaviour are UV-Vis Spectroscopy, Fluorescence Spectroscopy and Molecular Docking. Effect of pH and temperature has also been studied. The thermodynamic properties of Aloe-emodin-DNA complex were determined using Spectroscopic methods. The four DNA sequences were chosen which are DNA-1, DNA-2, DNA-3 and DNA-4. DNA-1 and DNA-2 are GC specific while DNA-3 and DNA-4 are AT specific central core. The binding affinity of Aloe-emodin with DNA sequences was found in the following order: DNA-1>DNA-2>DNA-3>DNA-4. From absorption and emission studies, the binding constant values were estimated in the range of $10^4$-$10^5$ $M^{-1}$. Hence, Aloe-emodin has shown complementary binding with all DNA sequences. It was found that the aloe-emodin has shown intercalation mode of binding via π-π stacking interactions. Also, Aloe-emodin has shown prominent binding toward GC core specific DNA compared to AT-specific DNA. Potassium iodide (KI) quenching study confirmed the GC preferred intercalation binding mode of Aloe-emodin with these DNA sequences. The change in pH medium from acidic to basic medium revealed the noticeable change in the binding constant values. The thermodynamic studies suggested that the complex formation is spontaneous, endergonic, feasible and energy driven process. Molecular docking results revealed that the complexation involves H-bonding, van der Waals and electrostatic forces for the π-π stacking interactions. On comparison of computational data with experimental data, intercalation mode of binding was confirmed.

## Introduction:

Anthraquinone falls under the category of quinone compounds which are widespread among living organisms. They are known to exert their influence in various physiological and biochemical processes. Chemically, anthraquinone are 9, 10-dioxanthracene derivatives having common occurrence in medicinal plants (e.g. *Aloe vera*, *Senna*

*obtusifolia*, etc.) and food materials (Garden Rhubarb – *Rhubarb rhabarbarum*) [1-4]. Aloe-emodin is a natural anthraquinone compound and reported to show diverse biological activities. Chemically, Aloe-emodin is 1, 8-dihydroxy-3-hydroxymethyl-anthraquinone (Figure 1) found in traditional Chinese medicinal (TCM) herbs like *Aloe vera*, *Rheum palmatum*, *Cassia occidentalis*, etc [5-7]. Recently, it has drawn attention due to its significant activities on cancer cells exhibiting anti-cancer properties [8, 9]. The other reported activities of Aloe-emodin are anti-virus, anti-bacterial, anti-inflammatory, neuroprotective, hepatoprotective and anti-parasitic [10]. They exhibit their action either by affecting Topoisomerase II activity or by interaction with DNA at specific base pair rich sites [11]. Aloe-emodin has been reported to show minor grove and intercalation mode of binding with natural DNA like calf thymus-DNA (CT-DNA) sequences and herring sperm DNA [12-15]. Different spectroscopic techniques have been employed to study the interactions of Aloe-emodin with natural DNA. However, interaction of Aloe-emodin with short sequence specific oligonucleotides has not been explored yet. The reported studies suggested the varied mode of binding of Aloe-emodin-DNA complex, but little is known about the orientation of Aloe-emodin between specific bases in the DNA sequences. Therefore, sequence specific binding of Aloe-emodin needs to be investigated. Hence, a set of four DNA oligomer sequences were chosen.

1. DNA-1: 5'-d$(G_1A_2T_3\underline{G_4G_5C_6C_7}A_8T_9C_{10})_2$
2. DNA-2: 5'-d$(G_1A_2T_3\underline{C_4C_5G_6G_7}A_8T_9C_{10})_2$
3. DNA-3: 5'-d$(G_1G_2C_3\underline{A_4A_5T_6T_7}G_8C_9C_{10})_2$
4. DNA-4: 5'-d$(G_1G_2C_3\underline{T_4T_5A_6A_7}G_8C_9C_{10})_2$

These selected DNA sequences possess specific base pair central core. The unique feature of each DNA sequence is the presence of six GC base pairs. The number of GC base pairs is same i.e. six in all DNA structures while position is different. The DNA-1 and DNA-2 are GC specific while DNA- 3 and DNA-4 are AT specific containing central core [16, 17].

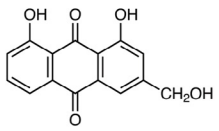


**Figure 1: Chemical Structure of Aloe-emodin**

The present study is focused on interaction of Aloe-emodin with the proposed specific DNA sequences (DNA-1 to DNA-4) using Absorbance and Fluorescence Spectroscopic techniques. The stability and thermodynamic aspects of Aloe-emodin-DNA complex is also investigated for gaining the insight into structural data and energies involved. Molecular Docking studies were also carried out to supplement the knowledge of structural data and to obtain the binding model for Aloe-emodin-DNA complex. The sequence specific binding of Aloe-emodin was explored using the above mentioned four custom designed DNA oligonucleotides sequences. These binding studies with DNA might be helpful in synthesizing and designing new drugs with enhanced biological properties.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Proposed DNA oligomers were purchased from Sigma Aldrich Chemicals Pvt. Ltd. New Delhi. Aloe-emodin (AE) was acquired from Tokyo Chemical Industry Co. Ltd. Tokyo, Japan. The phosphate buffer salts such as sodium dihydrogen phosphate, disodium hydrogen phosphate for preparing buffer solution and potassium iodide (KI) were received from Merck, Pvt. Ltd. Mumbai. The analytical grade reagents and freshly prepared solutions were used in all experiments. All the experimentations were accomplished at 25 °C in 10 mM sodium phosphate buffer at physiological pH of ~7.4.

### 2.2. Sample Preparation

Aloe-emodin was dissolved in a mixture of ethanol : water (1:1). The concentration of drug was fixed at 50 μM and 1 mM for Fluorescence and UV-Vis studies respectively. DNA samples were prepared by dissolving small amount of oligomers in 10 mM phosphate buffer at physiological pH (7.4). ds-DNAs were obtained by standard renaturation experiments [17]. The concentration of DNAs were determined spectrophotometrically with their corresponding molar coefficient (ε) values $\varepsilon_{260}$ = 96600 $Lmol^{-1}cm^{-1}$ for DNA-1, $\varepsilon_{260}$ = 95000 $Lmol^{-1}cm^{-1}$ for DNA-2, $\varepsilon_{260}$ = 93200 $Lmol^{-1}cm^{-1}$ for DNA-3, $\varepsilon_{260}$ = 92600 $Lmol^{-1}cm^{-1}$ for DNA-4 [19]. The purity of nucleic acids was checked by examining the ratio of UV-Vis absorption (A) at 260 and 280 nm. The ratio of $A_{260}/A_{280}$ was > 1.8, which signifies that nucleic acids were sufficiently purified and further used in all experiments without any purification.

## 2.3. Absorption Studies

The Absorption spectra of free Aloe-emodin (50 μM) and bound with DNA oligomers recorded on Cary 60 UV-Vis Spectrophotometer (Agilent Technologies) equipped with a Cary Single Cell Peltier temperature controller in 10 mM phosphate buffer solution. The absorption spectra of native Aloe-emodin (AE) and AE : DNA oligomer complexes were recorded in the range of 300 – 600 nm. Small aliquots of increasing concentration of DNA (0-50 μL) were added in the fixed concentration of the drug (20 ml of 50 μM) [18] in a quartz cuvette of 1 cm path length measuring the absorbance.

## 2.4. Emission Studies

Fluorescence measurements were performed on Varian Cary Eclipse Spectrofluorometer (Agilent Technologies) associated with Cary Varian Temperature Controller and a Xenon lamp using fluorescence free quartz cuvette of 1cm path length. Samples were excited at 480 nm and yielded the emission maxima ($\lambda_{max}$) at 553 nm. Both excitation and emission slits were adjusted at 10 nm. For emission titration studies, a fixed concentration of AE (50 μL) solution was titrated with an increasing concentration of DNA oligomers as reported [19, 20].

### 2.4.1. pH Studies

The role of pH medium on binding of AE with DNA decamers was studied by conducting the fluorescence titration in phosphate buffer of pH 6.0, 7.4 and 8.0. Binding constant values of AE with all four DNA decamers were calculated at proposed pH values ranging from acidic to basic at room temperature.

### 2.4.2. Iodide Quenching Studies

KI quenching studies were also performed by Fluorescence Spectrofluorimeter. In this study, the changes in emission spectra of Aloe-emodin were measured with increasing concentrations of anionic quencher in the absence and presence of DNA oligomers. The results were determined by the Stern-Volmer (equation **1**):

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

whereas, the fluorescence intensity of the complexes in the absence and presence of nucleic acid is $\mathbf{F_0}$ and **F** respectively. The concentration of nucleic acid and Stern–Volmer quenching constant is denoted as **[Q]** and $\mathbf{K_{sv}}$ respectively [21, 22].

## 2.5. Quantitative Analysis and Calculation of Binding Constants

Quantitative analysis of Aloe-emodin binding with DNA oligomers (DNA-1 to DNA-4) were performed using absorption and emission studies. The Benesi-Hildebrand plot / double reciprocal plot method was utilized, to estimate the non-specific ligand interactions with DNAs.

The Drug-DNA binding was calculated with the help of double-reciprocal method i.e. plot between 1/ [DNA] vs 1/ [$A_0$-A] or 1/[$E_0$ – E], where $A_0$ is the absorbance value of the drug in the absence of DNA, A is the absorbance value in the presence of DNA. Similarly, $E_0$ and E are the emission values of the drug in the absence and presence of DNA respectively [17, 18]. Straight line with a positive slope was achieved in all case and binding constant for all complexes was calculated by the ratio of intercept to the slope.

### 2.6. Thermodynamic Study: Effect of Temperature

To evaluate the effect of temperature between Aloe-emodin and proposed DNA oligomers (DNA-1 to DNA-4), fluorescence titrations were carried out at different temperatures (10 °C – 40 °C). The titrations were carried out by keeping the drug concentration fixed and varying the DNA concentration in increasing manner. From experimental data, the thermodynamic parameters were determined by Van't Hoff plot i. e. a plot between ln K vs. 1/T at above mentioned temperatures [23].

The gradient of the straight line obtained from Van't Hoff plot is equal to -ΔH/R which suggests the value of enthalpy change (ΔH = equation 2). Gibbs free energy change (ΔG = equation 3) and entropy change (ΔS = equation 4) can be calculated by using the following thermodynamic equations:

$$\ln K = -\frac{\Delta H}{RT} \quad (2)$$

$$\Delta G = -RT \ln K \quad (3)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (4)$$

### 2.7. Molecular Docking Studies

Molecular Docking studies of Aloe-emodin with nucleic acids were carried out with the AutoDock version 4.2 software [24]. The crystal structures of DNA 1 and DNA 2 (PDB ID: 4BZV and PDB ID: 1G3X) were obtained from Protein Data Bank (http://www.rcsb.org/pdb).

The 3D structure of drugs was constructed by ACD Labs Freeware ChemSketch and BIOVIA Discovery Studio. Hydrogen atoms and Gasteiger charges were added with the help of Autodock tools. The full grid size was set to 120 x 120 x 120 for X-, Y- and Z-axes, respectively. Other necessary parameters were assigned to the default values by the Autodock program [25]. After the course of the docking procedure, most favorable configurations were generated and analyzed using DiscoveryStudio visualizer. The theoretical binding constants were calculated with the help of equation (3).

## 3. Results & Discussion

### 3.1. Absorption Study: UV-Vis Spectroscopy

The absorption study of Aloe-emodin (AE) with four DNA oligomers was carried out using UV-Vis spectroscopy. Aloe-emodin showed its absorption maxima at 425 nm. On addition of aliquots of DNA oligomers (DNA-1 to DNA-4), the decrease in peak intensity was observed with an apparent shift in the peak position of Aloe-emodin in the UV spectra.

Figure 2 (a – d) shows the effect of increasing concentration of DNAs (1.5 - 15 x $10^{-6}$ M) on the spectral peak of fixed concentration of Aloe-emodin (6.67 x $10^{-6}$ Mol $L^{-1}$). Resultant hypochromic effect with a bathochromic shift in the Aloe-emodin : DNA complex indicated that AE complexed through intercalation mode of binding facilitated by stacking interactions. There was an isobestic point in between 325 - 350 nm and 475 - 500 nm. The isobestic points suggested the presence of intercalation mode of binding of AE with DNAs [26, 27]. From

titration study, the maximum hypochromism was found in the complexation with DNA-1.

The spectral data were utilized to calculate the binding constant for all the drug : DNA complexes. This was calculated with the help of Benesi-Hildebrand plots i. e. a plot between 1/ [DNA] vs [1/ $A_0$-A]. The Benesi-Hildebrand plots for Aloe-emodin with all nucleic acids are given in Figure 2.1 (a – d) and binding constants have been reported in Table 1. From Table 1, it was found that the calculated binding constant values of Aloe-emodin with four DNA oligomers were estimated in the range of $10^5$ $M^{-1}$. On analysis of all binding constant, it was observed that AE has shown complementary binding with all DNA oligomer. In view of the fact that DNA sequences contain equal numbers of GC base pairs (six) including different sequence specific central core as discussed before, it appears that the site of GC base pairs on the DNA molecule influences the AE : DNA binding. On the basis of above findings, it was revealed that Aloe-emodin has the prominent binding toward GC core-specific DNA-1: 5'-d$(GATGGCCATC)_2$, compared to other oligomers (DNA-2, DNA-3 & DNA-4).

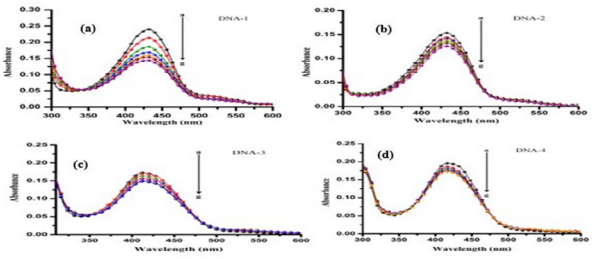


**Figure 2: Absorbance Titration Spectra of Aloe-emodin with a) DNA-1 b) DNA-2 c) DNA-3 d) DNA-4.**

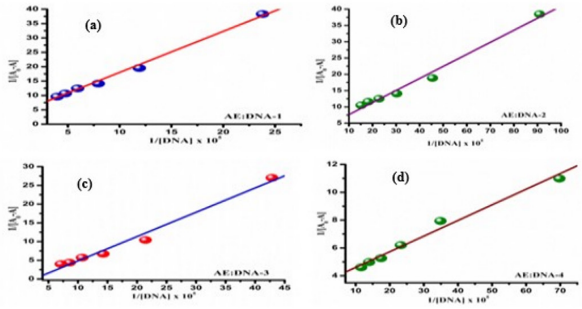


**Figure 2.1: Benesi-Hildebrand Plots for Aloe-emodin with a) DNA-1 b) DNA-2 c) DNA-3 d) DNA-4.**

**Table 1: Calculated Binding Constant (K) for Aloe-emodin with DNA Oligomers Using Absorption Spectral Data**

| Complexes | Calculated Binding Constants (K) $M^{-1}$ |
|---|---|
| Aloe-emodin:DNA-1 | $6.61 \times 10^5$ |
| Aloe-emodin:DNA-2 | $5.23 \times 10^5$ |
| Aloe-emodin:DNA-3 | $3.12 \times 10^5$ |
| Aloe-emodin:DNA-4 | $2.55 \times 10^5$ |

### 3.2. Emission Studies: Fluorescence Spectroscopy

Fluorescence emission titration of Aloe-emodin was performed with proposed DNAs to determine the base specificity and binding mode of Aloe-emodin with DNA. The emission study of all complexes was furnished in a buffer mixture of pH 7.4 at room temperature.

Figure 3 (a-d) illustrates the emission titration of Aloe-emodin with proposed oligomers. A fixed concentration of Aloe-emodin ($3.34 \times 10^{-6}$ Mol $L^{-1}$) was excited at 480 nm yielding emission maxima ($\lambda_{max}$) at 553 nm. On addition of each DNA aliquot to the AE solution, a consistent quenching in fluorescence emission of Aloe-emodin was observed. A bathochromic shift was also observed in the complex formation of Aloe-emodin with DNAs. The changes observed in emission spectra confirmed intercalative mode of binding of Aloe-emodin with nucleic acids

[14, 28] while the binding is predominantly favoured by stacking interactions. Preliminary analysis of spectra proved the excellent binding of Aloe-emodin with DNA-1 and DNA-2 containing GC specific central core and a moderate binding with AT specific DNA. To quantify the interaction affinity, binding constants were calculated by double reciprocal plot (Benesi-Hildebrand plots) and results are presented below in Figure 3.1 (a-d). The binding constant (K) values for all complexes are further given in Table 2.

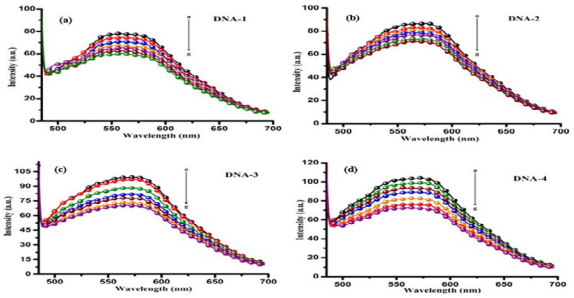


**Figure 3: Fluorescence Emission Spectra of Aloe-emodin with a) DNA-1 b) DNA-2 c) DNA-3 d) DNA-4.**

The calculated binding constant for Aloe-emodin with all the nucleic acids was estimated in the range of $10^5$ $M^{-1}$ (Table 2). It was revealed that Aloe-emodin showed complementary binding with four DNAs. Aloe-emodin showed the prominent binding with GC rich DNA-1. There were very slight differences obtained in the experimental K (binding constant) values with GC and AT rich DNA. Obtained binding constants and binding order showed a good agreement with values obtained through absorption studies.

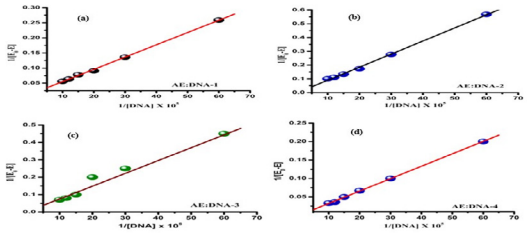


**Figure 3.1: Double Reciprocal Plots of Aloe-emodin with a) DNA-1 b) DNA-2 c) DNA-3 d) DNA-4.**

**Table 2: Calculated Binding Constant (K) for Aloe-emodin with Nucleic Acids using Emission Spectral Data**

| Complexes | Calculated Binding Constants (K) $M^{-1}$ |
|---|---|
| Aloe-emodin:DNA-1 | 8.38 x $10^5$ |
| Aloe-emodin:DNA-2 | 5.75 x $10^5$ |
| Aloe-emodin:DNA-3 | 3.15 x $10^5$ |
| Aloe-emodin:DNA-4 | 1.05 x $10^5$ |

### 3.2.1. Effect of pH on Aloe-emodin and DNA Interactions

The effect of pH on Aloe-DNA complexation was evaluated by Fluorescence Spectroscopy. The Fluorescence measurements were performed in a phosphate buffer solution of pH ranges from acidic to basic (pH 6.0, 7. 4 and 8.0) at room temperature. Table 3 outlines the effect of pH on Aloe-emodin binding with selected DNAs. There was a distinct variation observed in binding constant values in acidic medium to basic medium. With the help of pH study, it can be concluded that the alteration in pH of the solution had demonstrated a significant effect on the binding of Aloe-emodin with DNA oligomers.

**Table 3: Binding Constant Values for Aloe-emodin : DNA Oligomer Complexes at pH 6.0, 7.5 and 8.0**

| Complexes | pH 6.0 | pH 7.5 | pH 8.0 |
|---|---|---|---|
| Aloe-emodin:DNA-1 | 17.35 x $10^5$ $M^{-1}$ | 8.39 x $10^5$ $M^{-1}$ | 3.91 x $10^5$ $M^{-1}$ |
| Aloe-emodin:DNA-2 | 9.49 x $10^5$ $M^{-1}$ | 5.75 x $10^5$ $M^{-1}$ | 2.26 x $10^5$ $M^{-1}$ |
| Aloe-emodin:DNA-3 | 12.51 x $10^4$ $M^{-1}$ | 3.15 x $10^5$ $M^{-1}$ | 4.59 x $10^5$ $M^{-1}$ |
| Aloe-emodin:DNA-4 | 15.37 x $10^4$ $M^{-1}$ | 1.05 x $10^5$ $M^{-1}$ | 4.47 x $10^4$ $M^{-1}$ |

### 3.2.2. Potassium Iodide (KI) Quenching Study of Aloe-emodin with DNA Oligomers

To explain the intercalation, the binding of Aloe-emodin with DNA oligomers was studied using KI as a quencher. In this study, the fluorescence spectral characteristics of Aloe-emodin were compared with spectra of Aloe-emodin : DNAs (DNA-1 to DNA-4) complex during addition of KI aliquots. The change in the emission of Aloe-emodin was recorded in the presence and absence of proposed oligomers [19]. The result was examined by obtaining quenching constant with the help of Stern-Volmer plots. The Stern-Volmer Plot for KI quenching of Aloe-emodin in the presence and absence of DNAs has been shown in Figure 4 and $K_{sv}$ values for all nucleic acids are listed in Table 4.

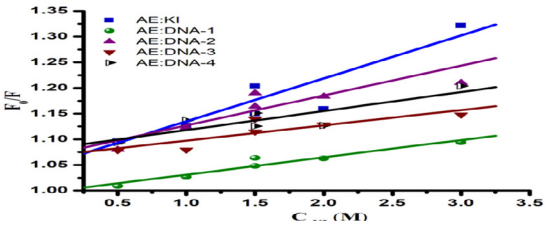


**Figure 4: Stern-Volmer Plots of Aloe-emodin (AE) by KI in the Absence and Presence of Nucleic Acids**

**Table 4: Stern-Volmer Constants ($K_{sv}$) for Aloe-emodin : Nucleic Acid Complexes**

| Complexes | Stern-Volmer Constant ($K_{sv}$) $M^{-1}$ |
|---|---|
| Aloe-emodin:KI | 178.23 |
| Aloe-emodin:DNA-1 | 126.23 |
| Aloe-emodin:DNA-2 | 33.67 |
| Aloe-emodin:DNA-3 | 85.69 |
| Aloe-emodin:DNA-4 | 29.75 |

Stern-Volmer Constant ($K_{sv}$) value of Aloe-emodin by potassium iodide (KI) was 178.23 $M^{-1}$. From Table 4, it was revealed that Stern-Volmer Constant values decreased in the presence of nucleic acids which confirmed the intercalation binding mode of Aloe-emodin with nucleic acids [21].

### 3.3. Thermodynamic Study: Effect of Temperature

In the present study, to evaluate the thermodynamic profile of the Aloe-emodin : DNA interactions, the temperature study was performed using Fluorescence Spectroscopy. The effect of temperature on fluorescence intensity was monitored for all the complexes of Aloe-emodin at various temperatures (10, 20, 30, 40 °C). Binding constant values obtained at different temperatures for Aloe-emodin with four DNA oligomers have been summarized in Table 5. The binding constant values were indirectly utilized to draw the Van't Hoff plots (plot of 1/T vs ln K), and further used to calculate the thermodynamic parameters [19, 29].

There was a straight line obtained in Van't Hoff plots for all the Aloe-emodin : DNA complexes revealed that the complexation is temperature dependent [30, 31]. The calculated values for Enthalpy change (△H), Gibbs free energy (△G) and entropic change (△S) was compiled in Table 6.

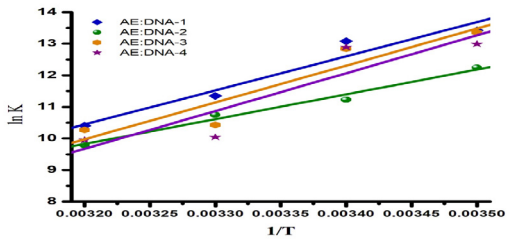


**Figure 5: Van't Hoff Plots for Complexes of Aloe-emodin with DNA:1 DNA:2, DNA:3, DNA:4 at four temperature 283, 293, 303 and 313 K using Fluorescence Spectroscopy.**

**Table 5: Binding Constant Values of Aloe-emodin with Nucleic Acids at Different Tempratures**

| Complexes | Binding Constants $M^{-1}$ | | | |
|---|---|---|---|---|
| | Temperatures | | | |
| | 10 °C | 20 °C | 30 °C | 40 °C |
| Aloe-emodin:DNA-1 | $6.7 \times 10^5$ | $4.8 \times 10^5$ | $8.5 \times 10^4$ | $3.3 \times 10^4$ |
| Aloe-emodin:DNA-2 | $2.1 \times 10^5$ | $7.6 \times 10^4$ | $4.7 \times 10^4$ | $1.8 \times 10^4$ |
| Aloe-emodin:DNA-3 | $6.4 \times 10^5$ | $3.8 \times 10^5$ | $3.1 \times 10^4$ | $2.9 \times 10^4$ |
| Aloe-emodin:DNA-4 | $4.4 \times 10^5$ | $4.0 \times 10^5$ | $2.3 \times 10^4$ | $2.1 \times 10^4$ |

Binding constant values decreased with increase in temperature in each case, which revealed that the quenching mechanism followed the static quenching [32] in the Aloe-emodin : DNA complex formation. Gibbs free energy, enthalpy and entropy values for Aloe-emodin binding with four DNA oligomers are summarized in Table 6. It was found that the change in Gibbs free energy values is negative for all the complexes of Aloe-emodin : DNAs. The negative ΔG value revealed that the interaction of Aloe-emodin with DNA oligomers is spontaneous and exothermic in nature. Marked negative values of enthalpy change for Aloe-emodin : DNA complexes underlined the similarity with other reported intercalators [19]. The obtained values proved that Aloe-emodin interacting with decamers in intercalation mode. The enthalpy change also revealed that the energy for Aloe-emodin : DNA complexes was lower than the reactant entity (Aloe-emodin and DNA oligomer) due to the liberation of energy during the complex formation and is favourable for intercalation interaction of Aloe-emodin with DNA (1 to 4). The calculated values of entropy change (ΔS) also reported a negative value which was unfavourable. This underscored the complex formation through intercalation mode as reported by various studies [19]. It has been described that intercalation mode of interactions is an enthalpy driven while groove binding is supported by entropy values. Enthalpy and entropy values also suggested the role of the forces like H-bonding, van der Waals and non-bonded interactions in the complex formation of AE and DNA. It was assumed that the negative entropy values may be caused by the structural change of DNA upon AE : DNA binding [33-36].

On overall thermodynamic analysis of Aloe-emodin : DNAs formation, it was concluded that the complex formation is spontaneous, endergonic, feasible and energy driven process. It was also revealed that the complex formation was facilitated by non-bonding interactions like H-bonding and van der Waals.

**Table 6: Thermodynamic Parameters ΔH, ΔG and ΔS for Aloe-emodin with four DNA Oligomer (DNA-1 to DNA-4).**

| | ΔH kcal/mol | ΔG (kcal/mol) | | | | ΔS (kcal/mol) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Temperatures (°C) | | | | Temperatures (°C) | | | |
| | | 10 | 20 | 30 | 40 | 10 | 20 | 30 | 40 |
| AE:DNA-1 | - 21.3 | -7.5 | -7.6 | -6.4 | -6.5 | -0.1 | -0.1 | -0.1 | -0.8 |
| AE:DNA-2 | -15.6 | -6.8 | -6.5 | -6.4 | -6.0 | -0.8 | -0.8 | -0.7 | -0.7 |
| AE:DNA-3 | -23.1 | -7.5 | -7.4 | -6.3 | -6.4 | -0.12 | -0.12 | -0.1 | -1 |
| AE:DNA-4 | -23.7 | -7.2 | -7.5 | -6.0 | -6.2 | -0.12 | -0.12 | -0.1 | -0.1 |

### 3.4. Molecular Docking Studies

In the present study, the Molecular Docking was performed to analyze the binding site information and the comparative efficiency of Aloe-emodin with GC-rich and AT-rich DNA. In each docking, the 10 most complimentary structures were analyzed, in all the performed 20 runs. Docking technique can utilize the flexibility of drug molecule to understand the mode of binding by recognizing the active site on macromolecules. Since the proposed DNA oligomers utilized in the experimental studies lack intercalation cavity that could not easily generated through software, therefore these oligomers were not used for dock calculations. In order to generate the model for investigation of intercalation mechanism and sequence preference of Aloe-emodin, GC and AT-rich DNA duplexes with intercalation cavity were required. To solve this problem, two DNA PDBs were chosen: 4BZV [Complex structure of MLN 944 - d(TACGCGTA)$_2$] and 1G3X [Complex structure of 9-acridine-peptide drug – d(CGCGAATTCGCG)$_2$. The ligand compounds were removed by chimera software to generate the specific intercalation cavity [18, 20].

The Figure 6 indicated the intercalation of Aloe-emodin in GC cavity of selected DNA structure. The theoretical calculation from Docking studies exhibited an excellent similarity with experimental results. Theoretical results concluded that complexation was facilitated by H-bonds, van der Waals and electrostatic forces (Table 7) showing the similarity with experimental data obtained from thermodynamic studies. Complex with minimum binding energy was preferred for structure illucidation using DiscoveyStudio Visualizer. Figure 6.1 illustrates the stacking distance along with H-bond and bond length formed between atoms of the DNA base pair and drug molecule for complexation. Data obtained for stacking distances between AE and GC rich DNA has been summarized in Table 8.

**Table 7: H-Bonds Distance between Aloe-emodin and GC Rich DNA**

| H-bond between atoms | Bond length (Å) |
|---|---|
| AECH15:G3O3′ | 1.60 |
| AECH15:C4O4′ | 1.59 |

**Table 8: Stacking Distance between Aloe-emodin and GC Rich DNA**

| Atoms of Drug and DNA Base | Distance (Å) |
|---|---|
| AEO1:G3 | 2.08 |
| AEO1:C4 | 1.77 |
| AEO:G15 | 1.78 |

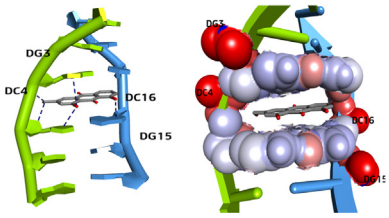


**Figure 6: Aloe-emodin Binding with DNA Duplex at 5′-Gp-C-3′ Intercalation Cavity, with Surface View of Aloe-emodin Sandwiched between GC Base.**

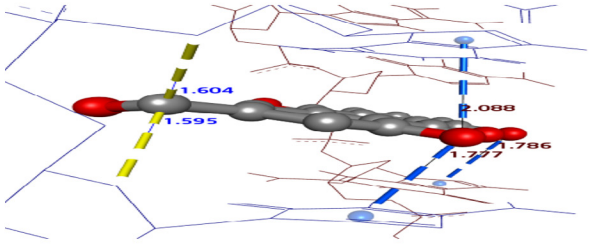


**Figure 6.1: H-bonds (shown in yellow color) Distance in Å and Stacking Distance between AE and Adjacent DNA Base Pair (shown in blue color)**

Aloe-Emodin also intercalates in the DNA

structure possess AT rich intercalation cavity (Figure 7). For complex formation, $\pi-\pi$ stacking interactions, van der Waals and electrostatic forces played a dominant role. The stacking distance along with H-bond (Figure 7.1) and bond length formed between atoms of the DNA base pair and drug molecule for complexation have been summarized in Table 9. The binding energy and binding constants values obtained from docking data has been given in Table 10.

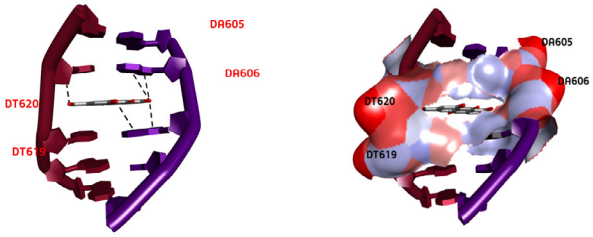


**Figure 7: Aloe-emodin Binding with DNA duplex at 5′-Ap-A-3′ Intercalation Cavity, with Surface View of Aloe-emodin Sandwiched between AA Base.**

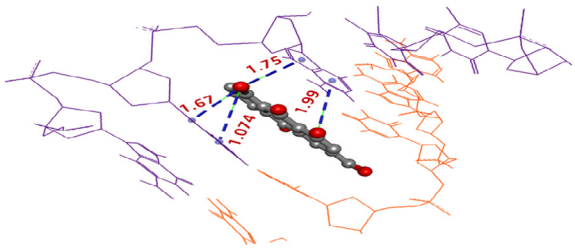


**Figure 7.1: Stacking Distance Between AE and Adjacent DNA Base Pair in Å (shown inroyal blue color)**

**Table 9: Stacking Distance between Aloe-emodin and AT Rich DNA**

| Atoms of Drug and DNA Base | Stacking Distances (Å) |
|---|---|
| AEO16:A606 | 1.99 |
| AEO17:A605 | 1.67 |
| AEO17:A605 | 1.07 |
| AEO17:A606 | 1.75 |

**Table 10: Binding Energy and Binding Constants from Molecular Docking Studies**

| Complex | Binding Energy (ΔGcal) (kcalmol$^{-1}$) | Binding Constants (ΔGcal) $M^{-1}$ |
|---|---|---|
| Aloe-emodin:DNA (GC) | -7.89 | $6.40 \times 10^5$ |
| Aloe-emodin:DNA (AT) | -7.28 | $2.26 \times 10^5$ |

## Conclusions

The anthraquinones are a class of compounds with established anticancer properties due to their promising binding properties. The genetic information carrier i.e. nucleic acid or DNA tends to be major target for small molecules and this interaction can help in cancer therapy by blocking those DNA sequences as a result of drug binding. Therefore, successful designing of small therapeutic substances needs to be investigated. However, it is important to search for more effective anticancer agents. In this regard, plant-derived herbal drugs have drawn the attention of researchers. Here, the interaction of Aloe-emodin with four custom designed oligonucleotide DNA sequences have been carried out to determine the sequence specificity. Using Spectroscopic and Molecular Docking studies, the structural and thermodynamic profiles of Aloe-emodin has been investigated. Both Spectroscopic and Molecular Docking experiments confirm the presence of intercalation binding mode via $\pi$-$\pi$ stacking of Aloe-emodin with DNA sequences mentioned. The hypochromic effect with bathochromic shifts have been observed in the peak maxima. Aloe-emodin has shown complementary binding with all DNA sequences. Aloe-emodin has shown strong preference towards GC rich DNA as compared to AT rich DNA sequences demonstrating its sequence specificity. The pH change has also caused a significant alteration in the complexation of Aloe-emodin with all DNA sequences as can be observed from change

in binding constant values. The quenching study using potassium iodide (KI) has also confirmed the presence of intercalation mode of binding and GC preference of aloe-emodin. Thermodynamic studies suggest that the complex formation is energy driven, spontaneous, feasible and endergonic process. Enthalpy and entropy change revealed that the non-bonding interaction like van der waals, H-bonding and electrostatic forces contribute in the binding of aloe-emodin with oligonucleotides. Molecular Docking studies of aloe-emodin with GC and AT specific DNA sequences are in good agreement with our experimental results which confirmed that the aloe-emodin intercalates in both the DNA structures but has preferential binding towards GC specific DNA structure. Molecular Docking studies also confirm the presence of non-bonding interactions like H-bonds, van der Waals and electrostatic forces in complex formation. Thus, these studies enhance our knowledge on the binding of small molecules to the defined DNA base pair sequences.

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**Conflicts of Interest**

The authors certify that they have no affiliations with or participation in any organization or entity with any financial interest.

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