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STUDIES ON INTERACTION OF NORBIXIN WITH DNA: MULTISPECTROSCOPIC AND *in silico* ANALYSIS

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Abstract:

The interaction of food colorant norbixin with Calf Thymus DNA (CTDNA) was investigated through UV-Visible spectroscopy, Fourier Transform Infra Red (FTIR), Circular Dichroism (CD), Nuclear Magnetic Resonance (NMR), DNA melting studies, electrophoretic analysis, histological staining technique and molecular docking studies. The results indicated that norbixin interacted with CTDNA by partial intercalation mode. The binding constant (K) of norbixin with CTDNA was calculated to be 5.08 x 10^5 Mol^{-1} L. FTIR and CD studies were coupled with H^1NMR spectra revealed that norbixin intercalates partially and binds to the groove's, phosphate group, deoxyribose sugar of DNA and also induces conformational transition of B-form to A-form DNA. Agarose gel electrophoretic and histological staining technique results further prove that, norbixin specifically binds to the DNA in the cell. Moreover, molecular docking studies on the specific binding of norbixin with CTDNA have exhibited lowest conformation energy score of -3.2. Therefore, this food colorant has the ability to interact with DNA and it could emerge as a promising class of natural DNA targeted therapeutic.

Key words: Norbixin, apocarotenoid, DNA, interaction, Spectroscopic, *in silico* analysis

1.Introduction:

In recent years, many studies were carried out on the binding interaction of small molecules or drug with DNA and has created a great significance in cancer research. DNA (Deoxyribonucleic acid) is a prime target for many chemotherapeutic agents, which has affected the replication, transcription, topoisomerase activity and also induced DNA cleavage, noncovalent cross-linking [1,2]. Understanding, the structural and functional interaction of drugs with DNA provides a further information on mutant genes, origin of diseases and also structural properties of the DNA [3]. Consequently, the interaction mechanism between the drugs and DNA occurs mainly through three noncovalent mode (i.e.,) by intercalation, groove binding and electrostatic interaction [4-7]. The specific binding of drug with DNA could induce a change in conformation and also thermodynamic stability in DNA [3]. Therefore, it is necessary to investigate the in-depth interaction mechanism between the drug molecules and DNA, which could lead to the design of novel DNA targeted drugs [8].

Norbixin is one of the major orange-red food colorant obtained from the seed of *Bixa orellana* (commonly known as annatto or lipstick tree) [9]. It is used as a natural coloring agent in dairy products, smoked fish, sausage, snack food, ice cream, cosmetics and body care product. The Acceptable Daily Intake (ADI) of norbixin is 0-0.065mg/kg body weight/day [10,11]. Kovary et al., (2001) reported that pro and antioxidant properties of norbixin prevented the plasmid DNA from oxidative damage induced by H_2O_2 [12]. Hagiwara et al., (2003) also reported that thirteen week feeding of annatto extract containing 91.6% norbixin at 0.1% dietary level has not exerted adverse effects in Sprague-Dawley rats [13]. Though there are various pharmacological reports available on norbixin, but the therapeutic ability of it still remains an elusive. Furthermore, these food colorants were consumed in our day to day life, without understanding the insight mechanism occurring in our human system. Hence, for the first time the interaction of food colorant norbixin with DNA was systematically investigated using various spectroscopic techniques such as UV-Visible spectrophotometer, FTIR, CD spectrum, NMR and further evidenced through electrophoretic analysis, histological staining technique, molecular docking studies. As

a result, the binding mode of norbixin with DNA implies that this food colorant can be used as a model for the development of DNA targeted therapeutics.

2.Materials and methods:

2.1.Materials

The sodium-calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis, MO). Ethidium bromide and Agarose was purchased from Himedia, India. The gel loading dye was purchased from Merck Bioscience, India. The analytical grade solvent was used.

2.2.Preparation of CTDNA stock solution

The CTDNA stock solution was prepared by dissolving CTDNA in Tris-EDTA buffer at 4°C for 24hour to obtain a homogeneous solution. The concentration of the CT-DNA solution was determined by UV absorbance at 260nm using a molar extinction coefficient ε_{260} =6600 M^{-1} cm^{-1} [8]. The absorbance at 260nm and 280nm was recorded in order to determine the purity of CTDNA solution. The A_{260}/A_{280} ratio was found to be 1.85 depicting that the DNA was sufficiently free from protein.

2.3.Preparation of norbixin

The bixin was isolated from the seed of *Bixa orellana* as per Kovery et al., (2001) protocol [12]. The purity of bixin was checked through UV-Vis spectrophotometer (Supplementary Fig.1) and compared with the published data [12]. The final yield of bixin is 0.5g, which was obtained from 50g of *Bixa orellana* seed. The norbixin was obtained through the saponification of bixin (i.e.,) by agitating with 5% NaOH solution (bixin: NaOH, 1:3 (mol/mol)) for 24hour at 37˚C. Water was evaporated to dryness at 45˚C and then crystallized norbixin was kept at -20˚C, until further use. The formation of norbixin was confirmed through UV-Visible spectrophotometer, Fourier Transform Infrared, High performance liquid chromatography (Supplementary Fig. 2-4).

2.4.UV-Visible Analysis

The various absorbance and wavelength of the different peaks during each experimental setup were determined through a wave scan range from 200 to 800nm as per the protocol of Rehman et al., 2014;

Bhakta and Siva, 2012 with certain modification [14, 8]. The UV–visible spectra were recorded on a Shimadzu UV-2401 PC UV–Vis spectrophotometer. The absorbance spectrum of norbixin was examined. The interaction of norbixin with CTDNA was studied at the constant concentration of norbixin (0.15µg/3ml) to be monitored for the spectral shift with the addition of various concentrations of CTDNA (10, 20, 30, 40 µg).

2.5.FTIR studies

Infrared spectra were recorded on a Magna 750 FT-IR spectrophotometer (DTGS detector, Ni- Chrome source and KBr beam splitter) with a total of 100 scans and a resolution of 4 cm^{-1}. Solution spectra were recorded after 1 h of incubation, using AgBr windows. Obtained spectra were analyzed using OMNIC software specific to the spectrophotometer. In the present investigation, the binding property of norbixin (5mg/ml) was examined with CTDNA (3mg/ml) through FTIR spectrum.

2.6.CD studies

Conformational changes were recorded on a Jasco J-715 spectropolarimeter, in a 3-cm rectangular cuvette associated with an optical path length at 25±0.2 °C. Temperature was kept in check using a Peltier type temperature control system. The interaction of norbixin with different concentrations of CTDNA (10, 20, 30, 40, 50 µg) were recorded for five times with a bandwidth 1.0n. and resolution of 0.5nm with a scan speed of 100nm/min.

2.7.NMR spectroscopy

The interaction of norbixin with CTDNA was determined through ^{1}H NMR spectral data obtained at 400MHz using Burker Ascend Model. The 10mg norbixin and 10mg CTDNA mixed thoroughly in D_2O (Deuterium oxide) before adding to the NMR tube. The chemical shift was reported in part per million (ppm) based on the interaction of norbixin with CTDNA and then it was compared with ^{1}H NMR spectrum of norbixin and DNA alone.

2.8.DNA melting studies

The Thermal denaturation studies were determined using Shimadzu UV-2401 PC UV–Vis spectrophotometer with a thermoelectric temperature controller. The absorbance at 260nm was observed for the CTDNA (20µg/ml) alone and CTDNA-norbixin (1:1) ratio at different temperature. The melting temperature region (T_m) was determined through the almost linear melting region (i.e.,) the start and end temperature of the melting process.

2.9.Histological Staining Procedures

Histological staining of onion tissue was performed as per Bhakta et al., (2013) method [15]. Fine sections of *Allium cepa* inner peel were dipped in isotonic solution (0.9% NaCl) for 10 min. The sections were stained with norbixin (10 mg/ml) and allowed to stand for 5 min. The excess stain was washed away with double distilled water and the stained section was observed under microscope adjusted at 10x magnifications.

2.10.Agarose Gel electrophoresis analysis

The reaction mixture containing a constant concentration of CTDNA (1µg) with the different concentrations of norbixin (10, 20, 30, 40, 50µg) was incubated at 37˚C for 30min. The 10µl of reaction mixture was added to 5µl of gel loading dye (bromophenol blue). The samples were subjected to 0.8% agarose gel prepared in TBE buffer (45mM Tris, 45mM boric acid and 1mM EDTA, pH 7.3) at a constant voltage of 100V for 1hr (until the bromophenol blue had passed through 50% of the gel) in TBE buffer. After electrophoresis, the gel was stained for 1hr by soaking it in a 0.5µg/ml ethidium bromide solution at room temperature. The stained gel was photographed using Image Quant 300 Gel Imaging System.

2.11.Docking Simulation of ligand with DNA

The target 3D structure of octamer (PDBID: 1DSC) was retrieved from the protein data bank (*www.rcsb.org*). The receptor DNA (1DSC) and the ligand norbixin was taken for *in silico* docking studies using Auto Dock4.0. For docking calculations, the co-crystallized ligands were identified and removed from the structure of 1DSC and the energy minimization was done for both DNA and ligand by using UCSF Chimera. The program UCSF (http://www.cgl.ucsf.edu/chimera) was added Gasteiger

charges (computed using ANTECHAMBER) and running 10,000 steps for 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 ligand were set to be rotatable. The docking program was done using the Lamarckian Genetic Algorithm (LGA) method [16]. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interaction of complex DNA with norbixin was analyzed using python molecular viewer software.

3.Results and discussion:

3.1.Absorption spectrum

The binding mechanism of the molecules with DNA was studied through the most convenient absorption spectroscopy technique [17]. Based on the interaction of drugs or molecules with DNA, the absorption spectra shows hyperchromism and hypochromism, that confirms the change in the duplex structure of the DNA. The hyperchromism with blue shift arises from the disruption of the secondary structure of DNA duplex and the hypochromism with red shift indicates the stabilization of DNA duplex through the intercalation or electrostatic binding of the molecule with the DNA [18, 19]. The absorption spectrum of norbixin displayed two maximum absorption at 480nm and 450nm wavelength (Supplementary Fig. 1). With the addition of CTDNA, the absorption peak at 480nm and 450nm decreased suddenly and then gradually increases at higher concentration of CTDNA without any wavelength shift (Fig 1). This kind of interaction with DNA indicates, the partial insertion of molecules with the DNA and also it can be a minor or major groove binding of a molecule with DNA as reported by Darabi et al., (2014) [19]. The overall affinity of the molecule towards the DNA mainly depends upon the structure of the molecule, especially on the geometric size, hydrogen bonding and hydrophobicity [20]. The absorption relationship between the norbixin and CTDNA is expressed by double reciprocal equation [21]

$$1/(A_0-A)=1/A_0+1/(K*A_0*C_{DNA}) \qquad \text{Equation (1)}$$

where A_0 and A is the absorbance of norbixin without and in the presence of CTDNA, respectively. K is the binding constant between the norbixin and CTDNA. C_{DNA} is the concentration of CTDNA. The dependence of $1/(A_0 - A)$ on the reciprocal value of the CTDNA concentration

$1/C_{DNA}$ is linear, with a slope equal to the value of $1/K A_0$. The value of $1/ A_0$ is fixed on the ordinate. The constant K is the quotient of an ordinate $1/ A_0$ and slope $1/K A_0$.

From Fig. 2, the double reciprocal plots of $1/ (A_0 - A)$ versus $1/C_{DNA}$ were linear. The binding constant of norbixin with CTDNA is $K=5.08x10^5$ Mol^{-1} L was calculated from Equation (1). Similarly, the binding constant value of ethidium bromide-CTDNA ($K=4.3x10^5$ Mol^{-1} L) is equal to the binding constant value of Norbixin-CTDNA [22]. The result from UV-Visible spectrum suggested that the partial intercalation and also groove binding was exhibited by norbixin towards the CTDNA.

3.2.FTIR spectroscopy

Infrared band at 1614 cm^{-1}, 1606 cm^{-1}, 1664 cm^{-1} and 1493 cm^{-1} shows the spectrum of CTDNA, which was assigned to the vibration stretches of guanine, adenine, thymine and cytosine nitrogenous base respectively [23]. The stretching vibration at 1664 cm^{-1} due to the thymine (C2=O2) band, which was shifted to 1658 cm^{-1} in norbixin-CTDNA adduct (Fig. 3). Moreover, the thymine oxygen group present in the minor groove of the DNA. The change in the band of the thymine oxygen group indicates the minor groove binding of norbixin to CTDNA [23]. The cytosine band at 1493 cm^{-1} exhibited a shift to 1479 cm^{-1} that shows the norbixin does not intercalate with guanine –cytosine base pair and also never binds to the major groove of CTDNA.

The spectrum observed for the CTDNA phosphate asymmetric and symmetric stretching of bands at 1224 $cm^{-1}$and 1086 cm^{-1} [23] was shifted to 1236 cm^{-1} and 1093 cm^{-1}, after the addition of CTDNA to norbixin (Fig. 3). The vibrational band of CTDNA backbone and deoxyribose sugar at 969 cm^{-1} and 896 cm^{-1} respectively [23] gets shifted to 964 cm^{-1} and 864 cm^{-1} respectively in norbixin-CTDNA complex. Ghosh et al., (2013) stated that the disappearance of certain characteristic peak indicates the probable interaction of dye-CTDNA adduct formation [24]. The disappearance of a characteristic vibration stretch of norbixin

at 3439 cm^{-1}, 3143 cm^{-1}, 2470 cm^{-1} shows the plausible interaction between dye and CTDNA (Fig. 3). The result of UV-Visible spectrophotometer is slightly accordance with FTIR spectroscopy, which shows that norbixin-CTDNA complex established by the minor groove binding and also with the phosphate group, deoxyribose sugar of the CTDNA.

3.3.CD analysis

The change in the conformation of DNA occurs through various binding modes of a molecule, which was detected by powerful sensitive CD technique. The CD spectrum of free DNA shows the positive band at 279nm, which was due to the base stacking and it is the main characteristics of right hand B-DNA [14]. Ivanov et al., (1973) reported that the interaction of small molecules with DNA could induce change in the positive band [25]. Groove binding and electrostatic interaction of small molecules shows less or no perturbation on the base stacking and helical band [26]. In our present study, the positive band at 279nm was increased with the addition of increasing concentration of CTDNA to norbixin (Fig. 4). Hegde et al., (2012) reported that increased positive band at 279nm occurs, due to the conformational transition of B-DNA to A-DNA [7]. The increase in CD band was associated with each base transition, which occurs due to the coulombic interaction and also close contact between hydrophobic base stacking of nucleotides and polymer [27]. The CD studies further confirm that norbixin induces conformation change upon the interaction with CTDNA.

3.4.NMR studies

The interaction of norbixin with CTDNA was indicated through the line widths and chemical shifts of the ^{1}H-NMR signal. Three distinct types of interaction mode were determined by ^{1}H-NMR signal. In Type 1 binding, intercalation of the molecules between the base pair of the DNA was shown as total line broadening in ^{1}H-NMR signal. For Type 2 binding, the line broadening and upfield chemical shift of the signal originates mainly for two reasons: the weak restrictions of molecular tumbling in the DNA-complex and also slow rate exchange between the various DNA binding sites and the unbound state. This type 2 binding mode indicates that the molecule may partially intercalate with DNA. If no line broadening

and up-field shift was observed in the ^{1}H-NMR signal that confirms the high binding affinity of the molecule towards the DNA. The Type 3 binding mode observed mainly due to the binding of the molecule with the groove's of DNA [28]. Our result shows that several new signals were formed based on the significant interaction between the CTDNA and norbixin. The comparative ^{1}H-NMR spectra of norbixin in the presence and absence of CTDNA were given in Fig. 5 (a-d). After the addition of CTDNA, ^{1}H-NMR signal of dicarboxylic acid of norbixin C-1 and C-25, C-15 at δ6.5 and δ5.9, gets shifted to δ6.7 and δ6 respectively, which indicate the line broadening and upfield shift. It confirms that the norbixin binds with CTDNA through Type 2 binding mode. The norbixin exhibited strong interaction with the CTDNA, which was indicated by no line broadening and upfield shift at δ3.9-3.4, δ5.7-5.1, δ2.1-1.1. The result from ^{1}H-NMR accordance with the absorption spectrum (i.e.,) norbixin binds to CTDNA through partial intercalation.

3.5.Melting curve studies

The melting curve of CTDNA in the presence and absence of norbixin was given in Fig. 6. At increasing temperature, the absorption spectrum of CTDNA at 260nm was raised due to the dissociation of double stranded CTDNA to single stranded CTDNA [29]. In DNA melting studies, the intercalation of the compound with the CTDNA indicated through increase in melting temperature (T_m) of 13-14°C and the non-intercalative binding mode shows no apparent change in the T_m value [30]. The melting temperature of the CTDNA alone is 64±0.2°C. After the addition of norbixin to CTDNA (1:1) ratio, the T_m value increases to 70±0.2°C. The small increase in T_m (6°C) indicates external binding and partial intercalation of the norbixin with CTDNA [30].

3.6.Electrophoretic studies

The electrophoretic pattern shows the binding of norbixin with CTDNA (Fig. 7). Vujcic et al., (2013) reported that the interaction of molecules could cause DNA damage, which can be accessed through agarose gel electrophoresis [18]. Ouyang et al., (2008) also reported that norbixin never induced DNA damage [31]. The binding of norbixin has quenched the fluorescence of ethidium bromide in the entire

sample, when compared to the CTDNA alone (control). The interference of norbixin in the intercalation mechanism of ethidium bromide with CTDNA was demostrated more in lane 3 and followed by lane 4, 5 as shown in Fig. 6. The result has suggested that norbixin quenched the binding affinity of the ethidium bromide towards the DNA.

3.7.Histological staining

The binding mode of the dye with bio-macromolecular DNA is studied through simple staining technique, which also reveals the cell nucleus morphology. The visualization and quantification of the cellular constituents can be determined only by histological staining of specific organelle in the cell [32]. Eventhough, there are many synthetic dyes were used for the nuclei staining, in which most of them are considered to be harmful to the environment and also carcinogenic. In order to replace such synthetic dyes, natural dyes are non toxic and environmental friendly [33]. Fig. 8 indicated the specific binding of norbixin with the nucleus. The norbixin is a basic dye with pH=12 [34]. Furthermore, Michaelis (1947) reported that Paul Ehrlics's research has shown that basic dye binds to the highly acidic nature of the DNA in the cell [35]. Finally, the histological staining of *Allium cepa* by basic norbixin dye implies that the preferential interaction of norbixin dye with the cell nuclei.

3.8.Low conformational energy of norbixin

The binding pattern was analyzed to predict the small molecule how it binds to the receptors of known 3D structure. The flexible docking was performed for the ligand norbixin with target CTDNA (Fig. 9). The investigation of CTDNA interaction with norbixin was performed to find the conformational energy of norbixin through Autodock server. The negative and lowest value of ΔG bind specifies strong favorable bonds between CTDNA and the novel ligand. Hence, norbixin is subjected for its validation of binding energy and ligand efficacy. In our study the norbixin shows negative value (-3.2) which represent valid and reasonable potential binding modes of the inhibitors. The docking results illustrations that Van der Waals (VDW) force in association with the hydrogen bond and dissolved energies predicted from norbixin. If the VDW energies are positive, it is a sign that the ligand is not fitting well into the active site

[8, 24]. However, the result of the study shows that VDW force energies are considerably negative for norbixin which fitting well into the DNA molecule. Each Autodock run shows ten conformations which are present in rank order based on the scores generated by the docking server. Hence, the first conformation is the best conformation and having lowest binding energy. Therefore norbixin shows lowest conformation energy -3.2, thus we hope that the norbixin can be used for anti-therapeutic agent with the desired biological activity.

4.Conclusion:

In our present study, the binding affinity of apocarotenoid norbixin with CTDNA was examined through various techniques, in which each technique reveals the interaction mechanism of norbixin with CTDNA. The partial intercalation and groove binding of norbixin with CTDNA was shown through absorption spectrum, DNA melting and NMR studies. Further, the FTIR result demonstrated the in-depth binding mechanism of norbixin with CTDNA (i.e.,) it interacts with the minor groove and also with phosphate group, deoxyribose sugar of CTDNA. The norbixin has induced conformational transition of B- form to A- form DNA, which was indicated by the increased positive band in the CD spectrum. The simple visualization techniques such as gel electrophoresis and histological staining have recommended that norbixin have the ability to interact with the DNA. In addition, the molecular docking studies have proven that norbixin interacts with CTDNA through lowest conformation energy score of -3.2. Finally, our result has shown that the norbixin binds with CTDNA through partial intercalation, groove binding, phosphate group and deoxyribose sugar. So, the utilization of this food colorant significantly leads to the discovery of novel DNA-targeted therapeutics.

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Reference:

[1] M. Yang, J. Jin, G. Xu, F. Cui, H. Luo, Chemical Physics 428 (2014) 1–5.

[2] G.M. Bleckburn, M.N. Gait, Nucleic Acids in Chemistry and Biology, IRL Press, New York, 1990, 297–332.

[3] S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar, A.M. Khalid, Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 205-217.

[4] P. Zhu, G. Zhang, Y. Ma, Y. Zhang, H. Miao, Y. Wu, Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy 112 (2013) 7-14.

[5] S. Zhang, X. Sun, Z. Jing, F. Qu, Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy 82 (2011) 213– 216.

[6] G. Zhang, X. Hu, J. Pan, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 78 (2011) 687-94.

[7] A.H. Hegde, S.N. Prashanth, J. Seethramappa, Journal of Pharmaceutical and Biomedical Analysis 63 (2012) 40– 46.

[8] D. Bhakta, R. Siva, Applied Biochemistry and Biotechnology 167 (2012) 885-896.

[9] R. Siva, F.P. Doss, K. Kundu, V.S.V. Satyanarayana, V. Kumar, Industrial Crops and Products 32 (2010) 48-53.

[10] W. Auttachoat, D.R. Germolec, M.J. Smith, K.L.Jr. White, T.L. Guo, Food and Chemical Toxicology 49 (2011) 2638–2644.

[11] H. Noppe, S. Abuin Martinez, K. Verheydden, J. Van Loco, R. Companyo Beltran, H.F. De Brabander, Food additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk assessment. 26 (2009) 17-24.

[12] K. Kovary, T.S. Louvain, M.C. Costa e Silva, F. Albano, B.B. Pires, G.A. Laranja, C.L. Lage, I. Felzenszwalb, British Journal of Nutrition 85 (2001) 431-40.

[13] A. Hagiwara, N. Imai, Y. Doi, K. Nabae, T. Hirota, H. Yoshino, M. Kawabe, Y. Tsushima, H. Aoki, K. Yasuhara, T. Koda, M. Nakamura, T. Shirai, Cancer Letters 199 (2003) 9-17.

[14] S.U. Rehman, Z. Yaseen, M.A. Husain, T. Sarwar, H.M. Ishqi, M. Tabish, Plos one 9 (2014) e93913.

[15] D. Bhakta, S. Lulu, G. Jayaraman, S. Babu, R. Siva, Interdisciplinary Sciences Computational Life Science 5 (2013) 53-59.

[16] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Journal of Computational Chemistry 19 (1998) 1639–1662.

[17] J. Zhu, L. Chen, Y. Dong, J. Li, X. Liu, Spectrochimica Acta Part A, molecular and biomolecular spectroscopy 124 (2014) 78-83.

[18] M.T. Vujcic, S. Tufegdzic, I. Novakovic, D. Djikanovic, M.J. Gasic, D. Sladic, International Journal of Biological Macromolecules 62C (2013) 405– 410.

[19] F. Darabi, H. Hadadzadeh, M. Ebrahimi, T. Khayamian, H.A. Rudbari, Inorganica Chimica Acta 409 (2014) 379–389.

[20] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton, Journal of the American Chemical Society 111 (1989) 3051-3058.

[21] S. Zhang, X. Sun, F. Qu, R. Kong, Spectrochimica Acta Part A, molecular and biomolecular spectroscopy 112 (2013) 78-83.

[22] J.B. LePecq, C. Paoletti, Journal of molecular biology 27 (1967) 87-106.

[23] D.K. Jangir, S. Kundu, R. Mehrotra, Plos one 8 (2013) e69933.

[24] P.Ghosh, G.P. Devi, R. Priya, A. Amrita, A. Sivaramakrishna, S. Babu, R. Siva, Applied Biochemistry and Biotechnology 170 (2013) 1127-1137.

[25] V.I. Ivanov, L.E. Minchenkova, A.K. Schyolkina, A.I. Poletayev, Biopolymers 12 (1973) 89-110.

[26] G. Zhang, X. Hu, P. Fu, Journal of Photochemistry and photobiology B: Biology 108 (2012) 53-61.

[27] N. Shahabadia, N. Fatahi, M. Mahdavi, Z.K. Nejad, M. Pourfoulad, Spectrochimica Acta Part A-molecular and biomolecular spectroscopy 83 (2011) 420- 424.

[28] W. Zhong, J.S. Yu, Y. Liang, K. Fan, L. Lai, Spectrochimica Acta Part A, molecular and biomolecular spectroscopy 60 (2004) 2985-2992.

[29] F. Gao, H. Chao, F. Zhou, Y.X. Yuan, B. Peng, L.N. Ji, Journal of Inorganic biochemistry 100 (2006) 1487-1494.

[30] S. Roy, R. Sadhukhan, U. Ghosh, T.K. Das, Spectrochimica Acta Part A- molecular and biomolecular spectroscopy (2015) doi:10.1016/j.saa.2015.01.041 (in press)

[31] D. Ouyang, R. Zhang, L. Yi, Z. Xi, Food and chemical toxicology 46 (2008) 2802-2807.

[32] M. Alfert, I.I. Geschwind, Proceedings of the National Academy of Sciences 39 (1953) 991-999.

[33] R. Siva, Current Science 92 (2007) 916-925.

[34] P.H. Todd, Norbixin adducts with water-soluble or water-dispersible proteins or branched-chain or cyclic polysaccharides. Google patents (1991) US5053240 A.

[35] L. Michaelis, Cold spring harbor symposia on quantitative biology 12 (1947) 131-142.

Figure legend:

Fig. 1: Interaction of norbixin with CTDNA was investigated using UV-Visible spectrum.

Fig. 2: The double reciprocal plot obtained from the equation 1 giving the binding constant value of norbixin-CTDNA adduct, $K=4.3x10^5 Mol^{-1}$ L.

Fig. 3: FTIR spectrum of (a) CTDNA with norbixin (b) Norbixin

Fig. 4: CD spectrum of CTDNA (10-50µg) in the absence and presence of constant a concentration of norbixin.

Fig. 5: H^1 NMR studies (a) CTDNA alone (b) Norbixin alone (c) CTDNA-Norbixin adducts (d) chemical structure of norbixin.

Fig. 6: DNA melting curve of CTDNA (20µg/ml) in the presence and the absence of norbixin (20µg/ml).

Fig. 7: Electrophoretic analysis of the interaction of CTDNA with norbixin (1,2,3,4,5 µg) (lane 2-6) respectively; lane 1: Control CTDNA (without norbixin).

Fig. 8: Histological staining technique (a) Tissue section of *Allium cepa* peel without staining of norbixin (blue arrow denotes nucleus) under 10x magnification; Histological staining of *Allium cepa* peel with norbixin (black arrow denotes stained nucleus) under 10x magnification.

Fig. 9: Molecular docking of norbixin with DNA (a) 3D structure of norbixin; (b) 1DSC DNA; (c)The docked structure of norbixin with 1DSC DNA . The binding energy of norbixin-CTDNA adducts is -3.2.

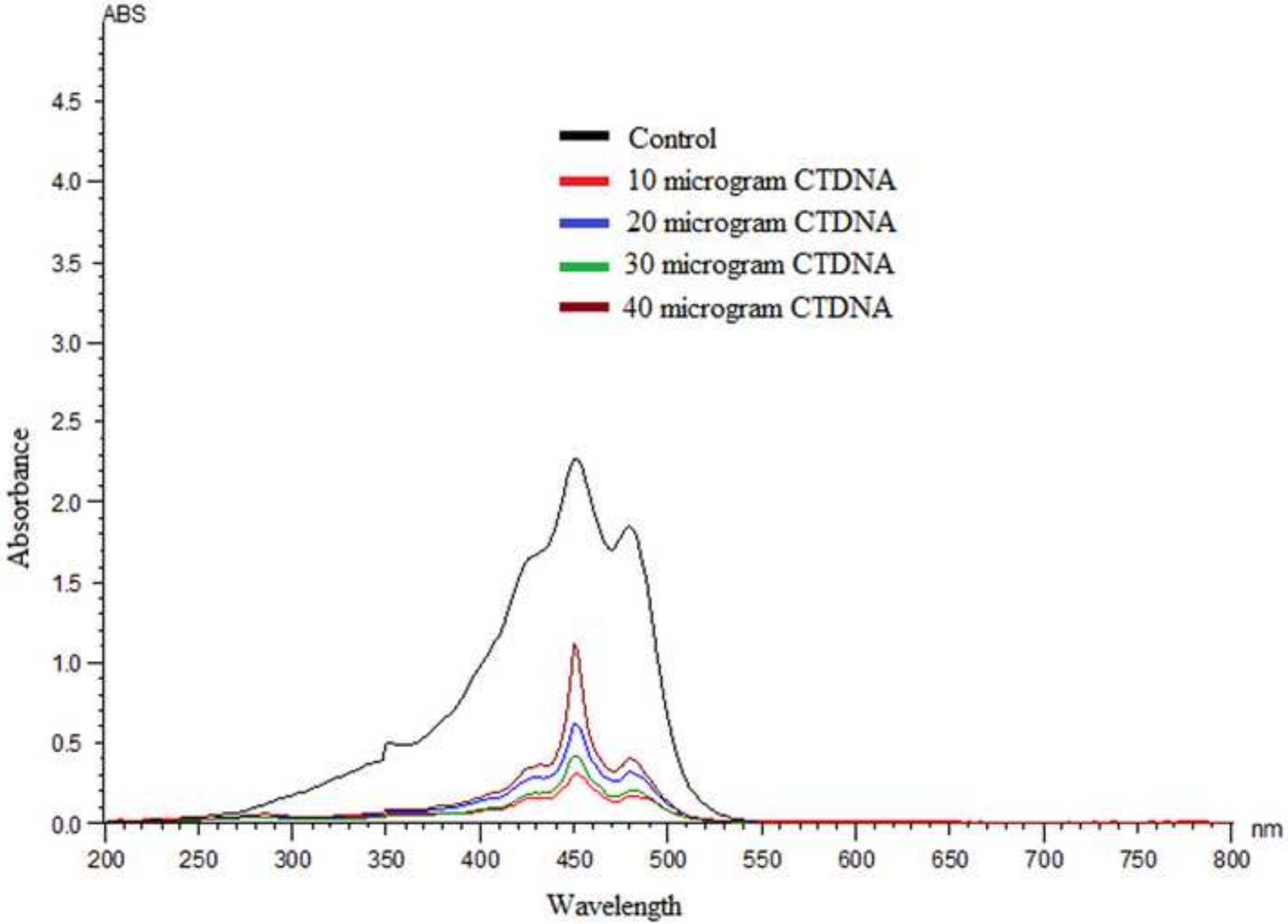
Supplementary Figure

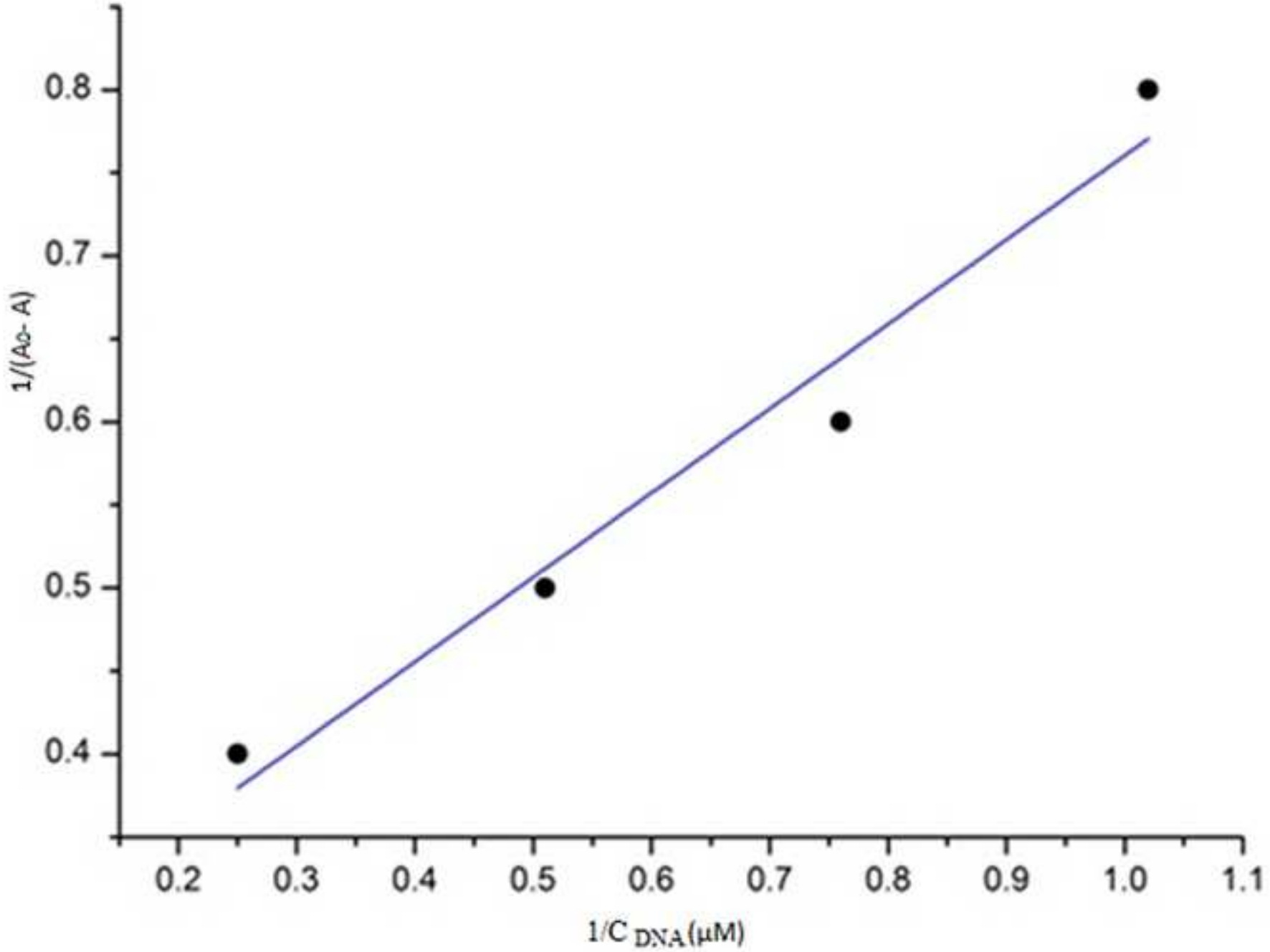
Supplementary Fig.1: UV-Visible absorption spectra of bixin isolated from *Bixa orellana* seed with absorption wavelength of 502nm and 470nm in chloroform solvent.

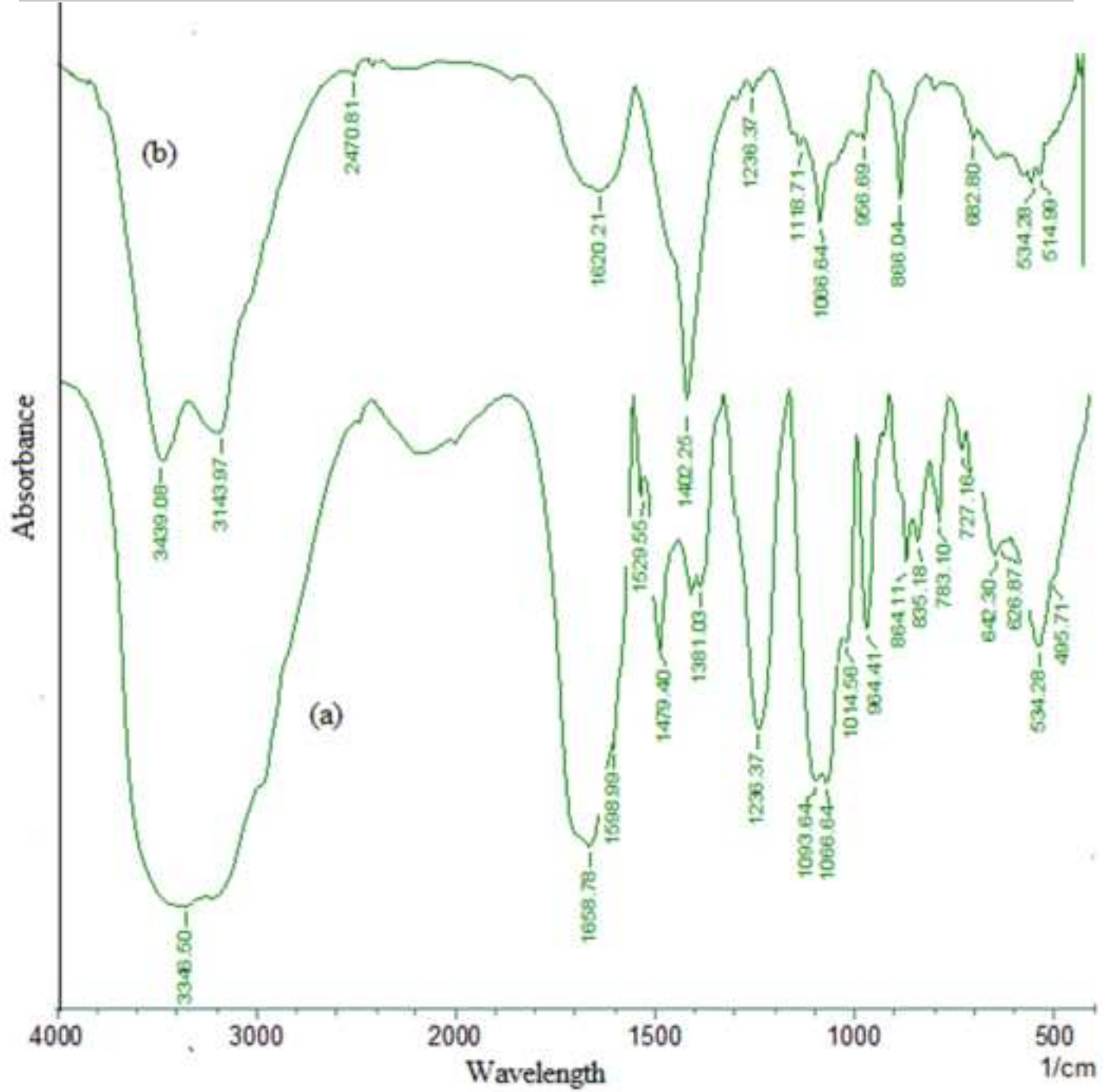
Supplementary Fig.2: UV-Visible absorption spectra of norbixin obtained through saponification of bixin. The absorption wavelength of norbixin is 480nm and 450nm in water.

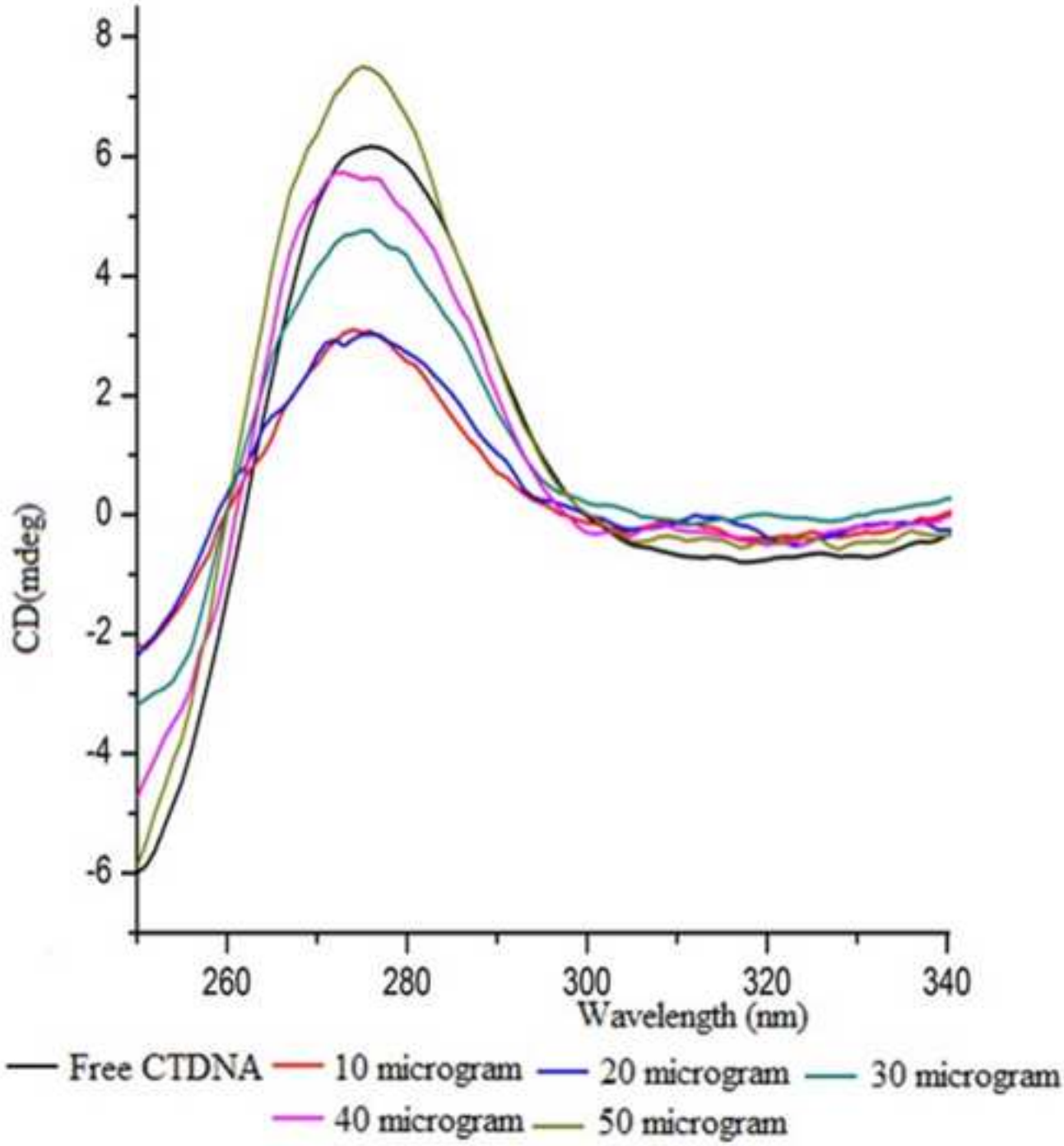
Supplementary Fig.3: FTIR spectra of norbixin.

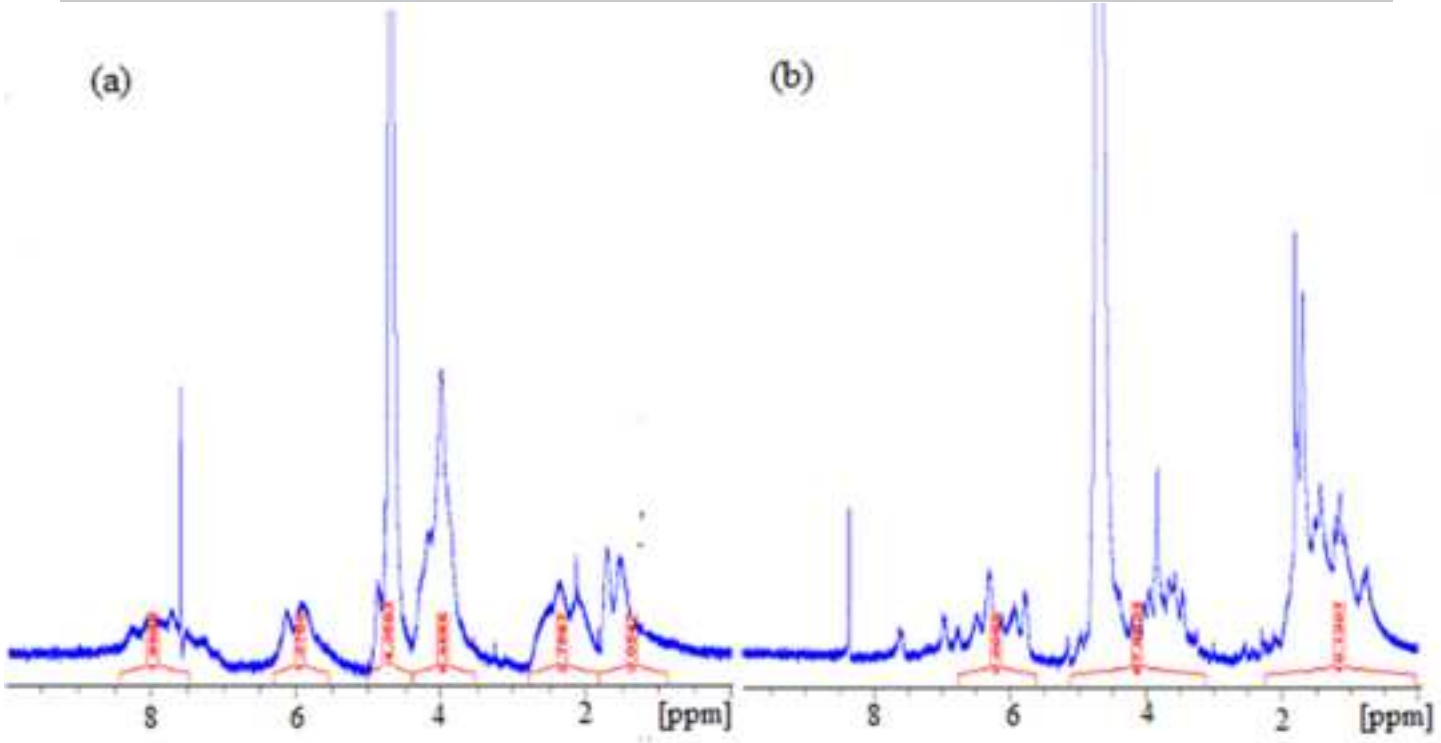
Supplementary Fig.4: HPLC chromatogram of norbixin was detected at particular wavelength of 450nm; moblie phase- acetonitrile: water: glacial acetic acid (70:29:1); flow rate: 1ml/min and the retention time of norbixin is 9.89 min.

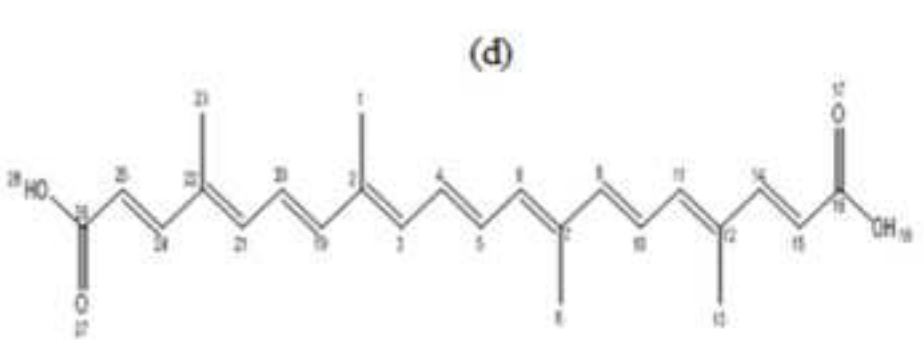


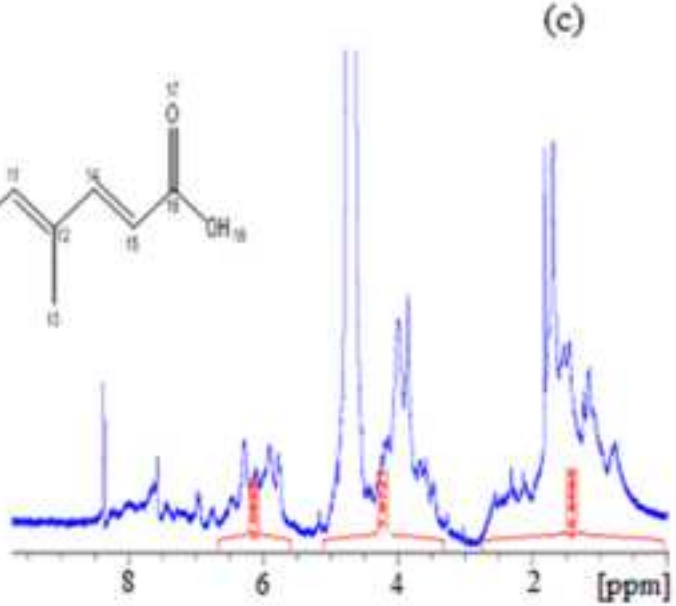


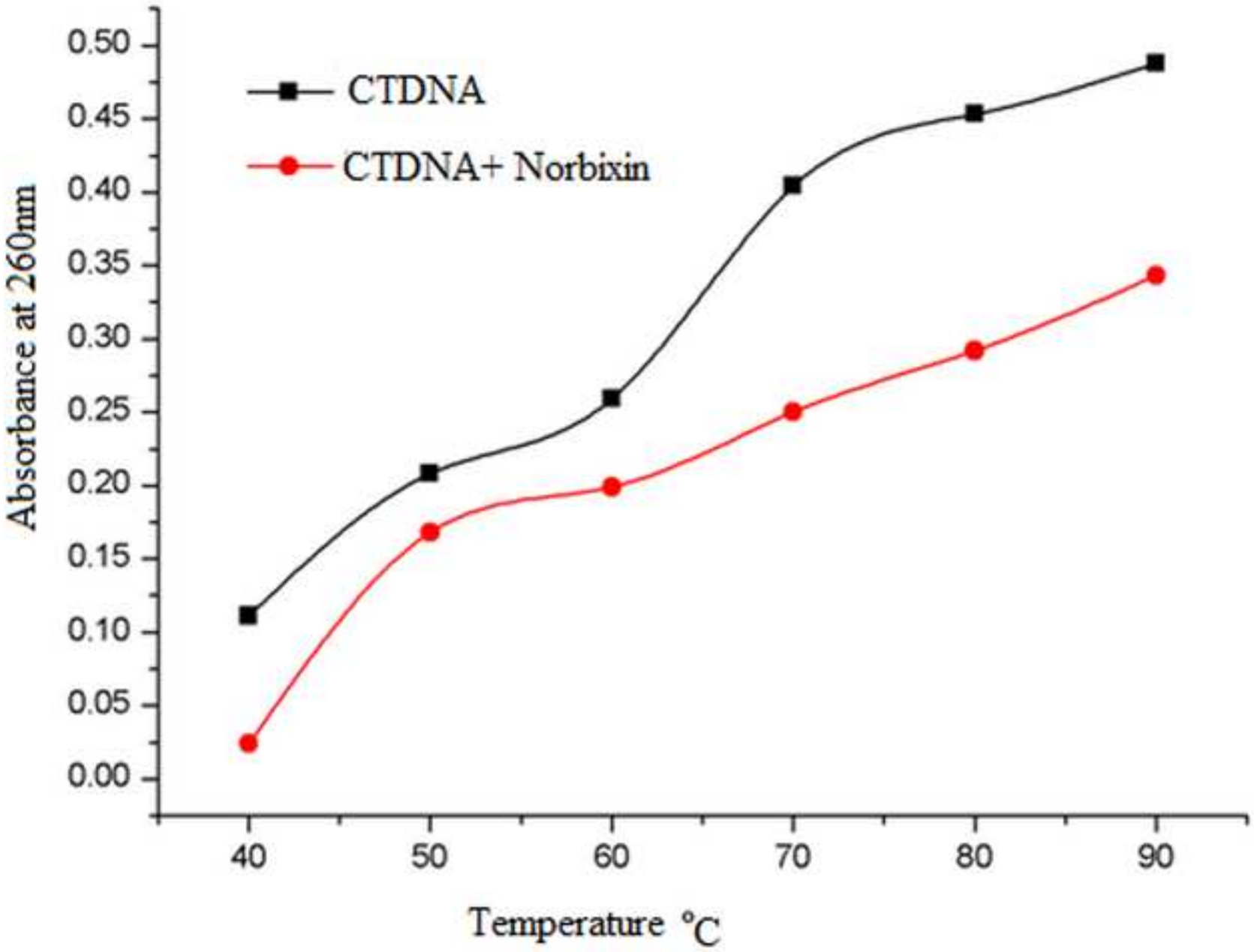


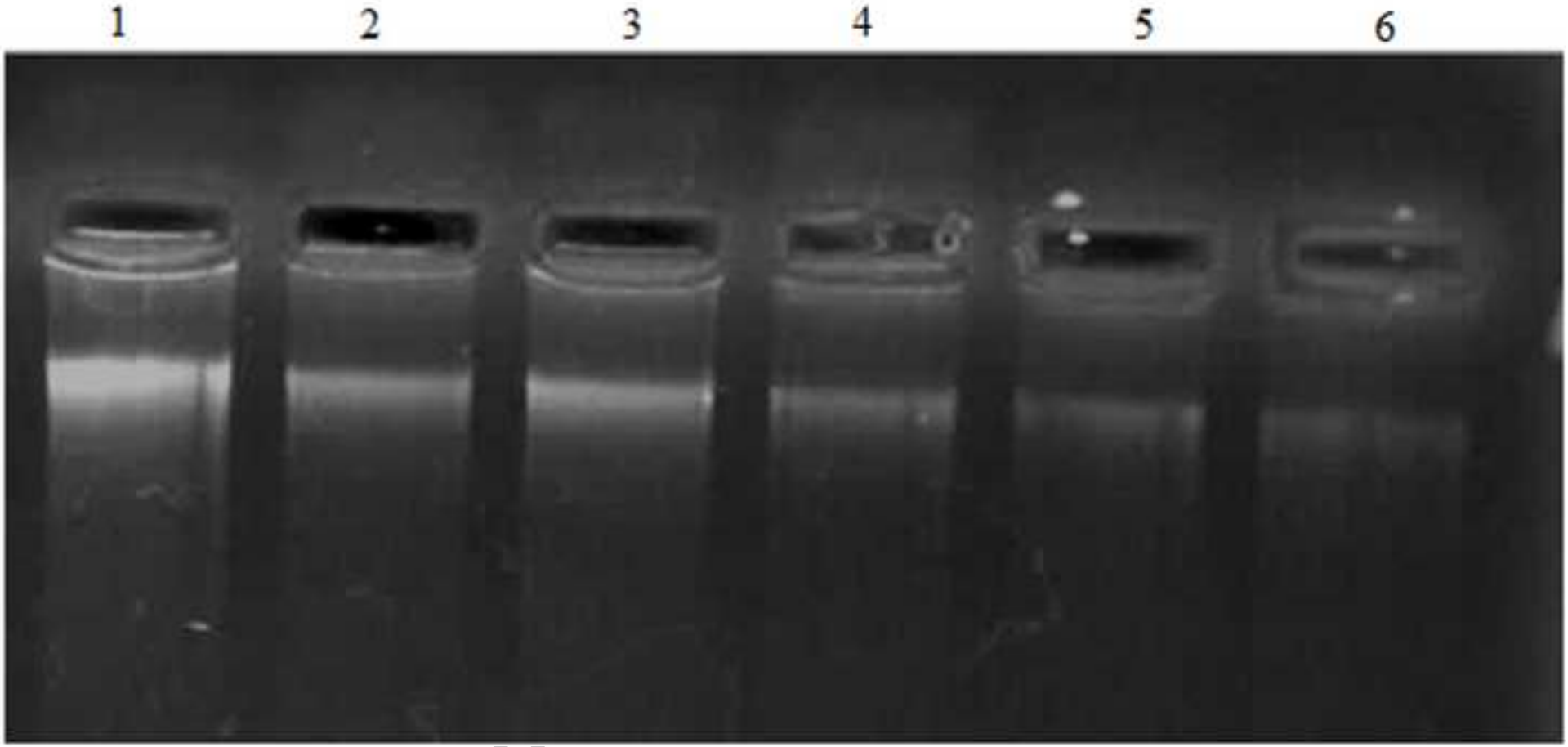

8
6
4
2
0
-2
-4
-6
CD(mdeg)
260
280
300
320
340
Wavelength (nm)
Free CTDNA
10 microgram
20 microgram
30 microgram
40 microgram
50 microgram

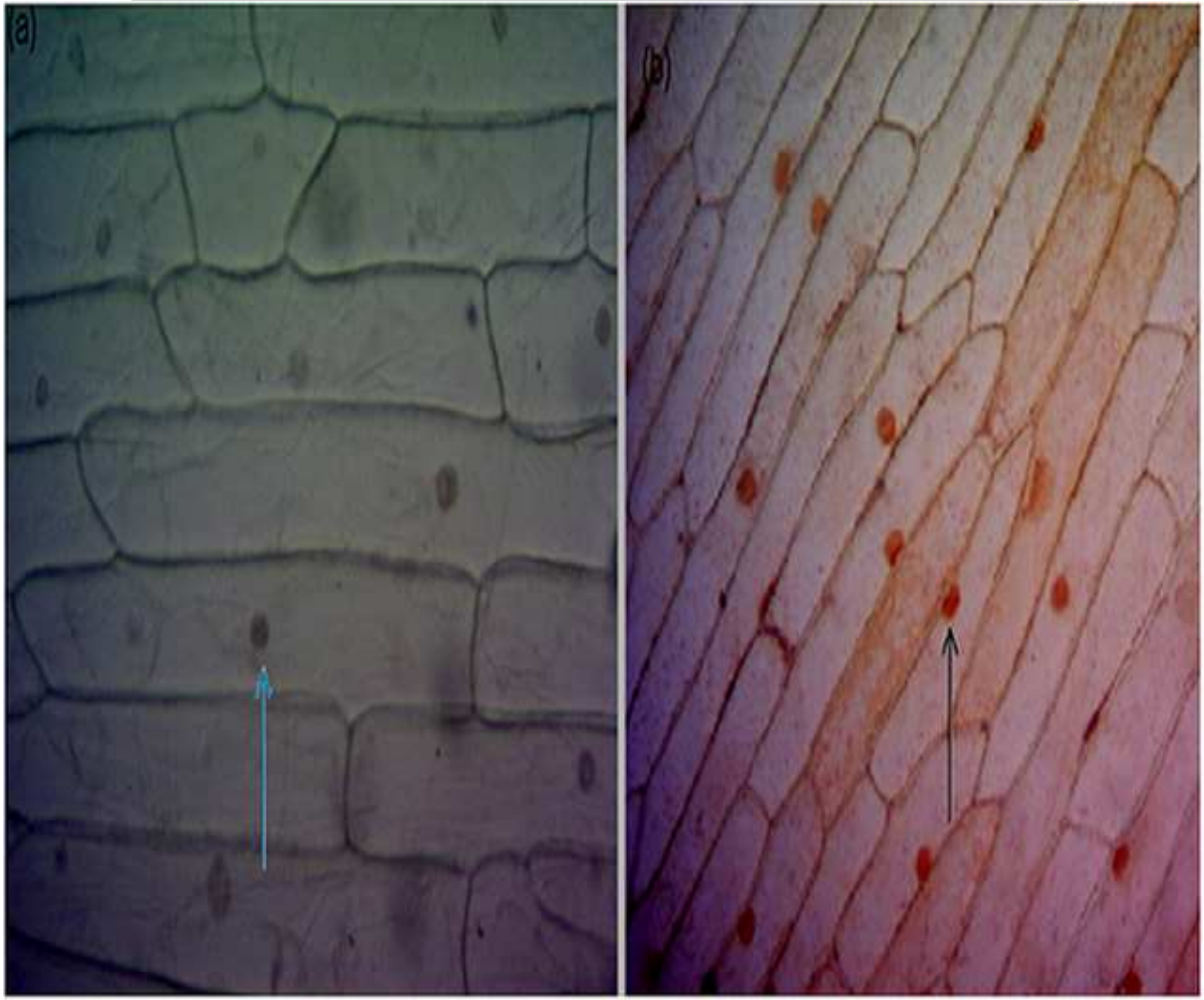


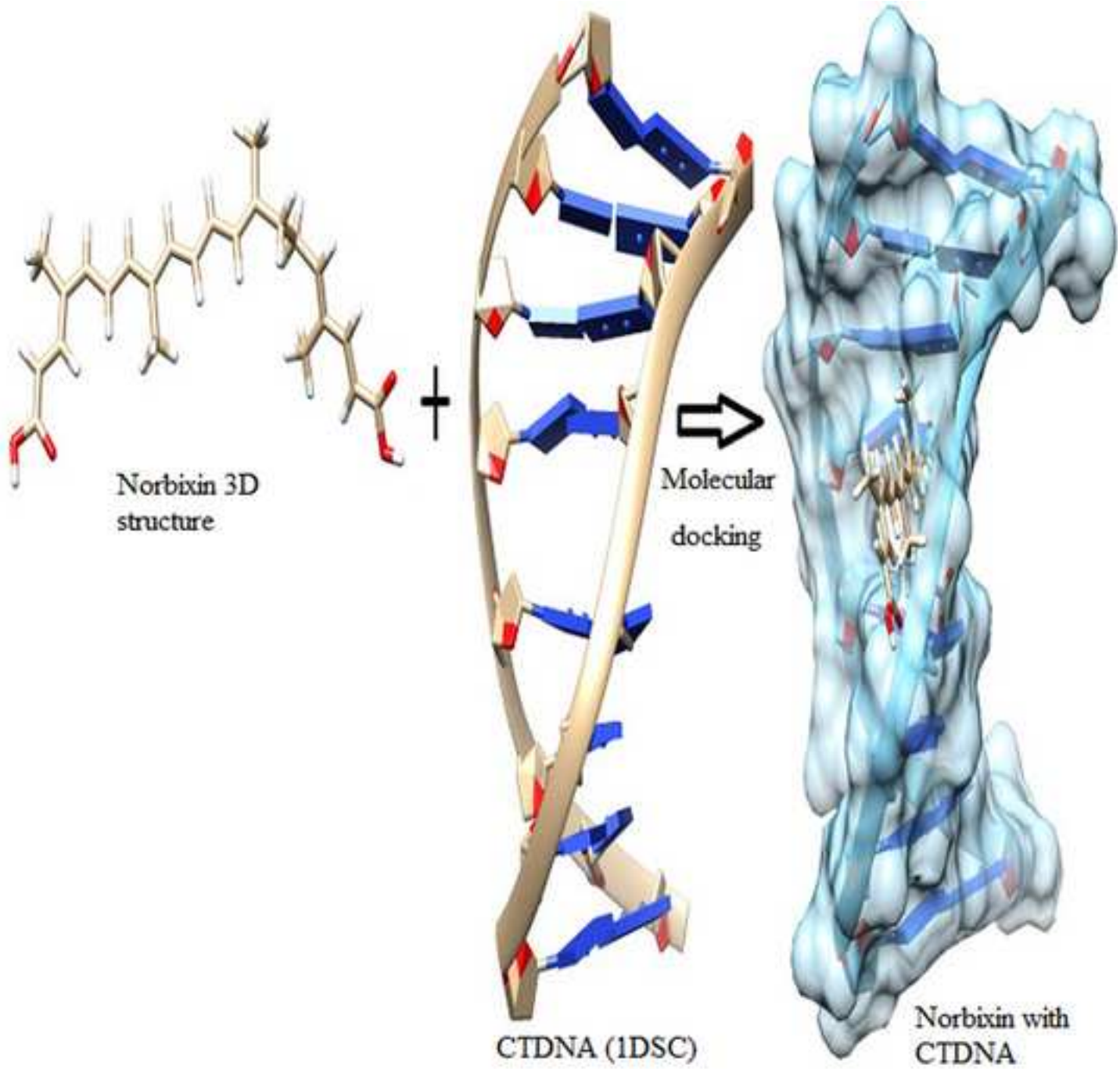


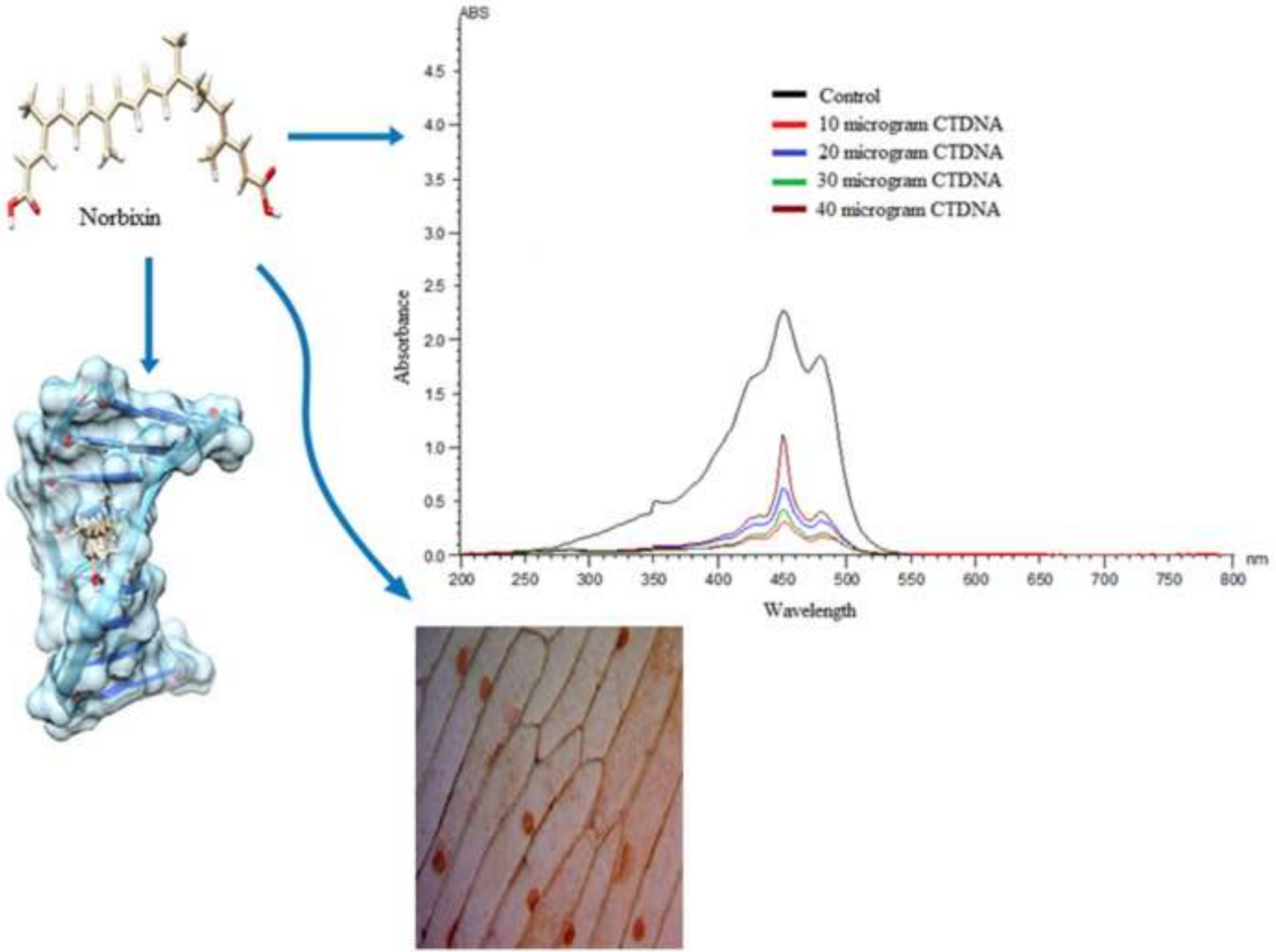












Highlights:

- The interaction between food colorant norbixin with CTDNA
- Partial intercalation and groove binding of norbixin with CTDNA
- Lowest conformation energy of norbixin.