Physicochemical, antioxidant properties of carotenoids and its optoelectronic and interaction studies with chlorophyll pigments

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The physicochemical and antioxidant properties of seven carotenoids: antheraxanthin, β-carotene, neoxanthin, peridinin, violaxanthin, xanthophyll and zeaxanthin were studied by theoretical means. Then the Optoelectronic properties and interaction of chlorophyll-carotenoid complexes are analysed by TDDFT and IGMPLOT. Global reactivity descriptors for carotenoids and chlorophyll (Chl*) are calculated via conceptual density functional theory (CDFT). The higher HOMO–LUMO (HL) gap indicated structural stability of carotenoid, chlorophyll and chlorophyll-carotenoid complexes. The chemical hardness for carotenoids and Chlorophyll is found to be lower in the solvent medium than in the gas phase. Results showed that carotenoids can be used as good reactive nucleophile due to lower µ and ω. As proton affinities (PAs) are much lower than the bond dissociation enthalpies (BDEs), it is anticipated that direct antioxidant activity in these carotenoids is mainly due to the sequential proton loss electron transfer (SPLET) mechanism with dominant solvent effects. Also lower PAs of carotenoid suggest that antioxidant activity by the SPLET mechanism should be a result of a balance between proclivities to transfer protons. Reaction rate constant with Transition-State Theory (TST) were estimated for carotenoid-Chlorophyll complexes in gas phase. Time dependent Density Functional Theory (TDDFT) showed that all the chlorophyll (Chl*, Chlβ)–carotenoid complexes show absorption wavelength in the visible region. The lower S1–T1 adiabatic energy gap indicated ISC transition from S1 to T1 state.

Chlorophyll and carotenoid pigments can be used as important optical molecular probes to observe different phases of plant performances and its development. Both Chlorophyll and carotenoid are biosynthesized in chloroplast. The synthesized plant carotenoids accumulate exclusively in plastids, and most importantly, chloroplast and chromoplast. Carotene and xanthophylls, both the oxygenated derivatives of carotenes are health promoters and have ability to quench singlet oxygen and scavenge toxic free radicals preventing or reducing damage to living cells. Carotenoids are also used to prolong the shelf life of pharmaceuticals as they have the ability to scavenge free radicals. Carotenoids react chemically with the free radicals and the system of conjugated double bonds is directly destroyed. Due to their long conjugated chains, carotenoids are highly reactive. The action of free radicals and other reactive oxygen species (ROS) partly causes diseases such as cancers, cerebral thrombosis and infarction. The small amount of adsorbed oxygen in the lungs is used to make harmful ROS, as hydrogen peroxide (H2O2) and the superoxide radical anion (O2·−), which when reacted with transition metals (Fe, Cu) in the human body, produce very reactive ROS [·OH] radicals, which causes harm to cells in the human body. Seven hundred characterized natural carotenoids are synthesized by plants and microorganisms that confer the yellow, orange and red colors. Twenty carotenoids have been detected in the human blood stream and tissues. Carotenoids are also used as nutritional supplements in food and pharmaceutical industries and in cosmetics due to their bright colors, nutrition and absorption of UV light. The antioxidant and prooxidant effects of carotenoids depends on various factors such as the concentration of carotenoids, molecular structure, action sites, oxygen pressure, interaction with other dietary antioxidants, and the methods used to induce oxidative stress.

The function and properties of chlorophyll and carotenoid reside in their chemical structure. Chlorophylls are cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring, while carotenoids are C40-tetraterpenoid skeleton which are classified in two groups as carotenes and xanthophylls. Plant chlorins (chlα and chlβ) have absorption bands around the blue and red spectral region in organic solvents. The structure of carotenoid is characterized by a linear chain of conjugated π-electron double bonds, while in oxygenic organisms, carotenoid
usually contain ring structures at each end, and most carotenoids contain oxygen atoms, usually as part of hydroxyl or epoxide groups. The absorption maxima is affected by the length of chromophore, the position of the end double bond in the chain or ring and the taking out of conjugation of one double bond in the ring or eliminating it through epoxidation. Carotenoids generally have three-peak absorption spectrum with well-defined maxima and minima though they show different optical characteristics in various solvents which depend on the polarizability of the solvent. The introduction of a carbonyl group in conjugation with the polyene system produces a bathochromic shift and the loss of fine structure, while β-carotene, cryptoxanthin and zeaxanthin all produces identical absorption spectrums as the influence of other substituents group are negligible. The biosynthesis and accumulation of carotenoids in dark-grown etiolated seedling is essential for the assembly of membrane structure and the development of chloroplast when seedlings emerge into the light. For photosynthetic systems, carotenoid is the associated pigment which collects light energy in the spectral region and transfers this energy to chl pigment as chl does not absorb this energy. Also in photoprotection, the role of carotenoid is to quench the triplet state of chl before it reacts with oxygen to form singlet oxygen species (ROS). Carotenoid regulates energy transfer in the light-harvesting antenna through xanthophyll cycle, to avoid over-excitation of the photosynthetic system by safely dissipating excess energy. So understanding the relationship between structure and photophysical properties of these pigments can provide insights into a better study of how photosynthesis works at the molecular level in chloroplast.

In this work, the physicochemical and antioxidant properties of seven carotenoids: antheraxanthin (a), β-carotene (b), neoxanthin (n), peridinin (p), violaxanthin (v), xanthophyll (x) and zeaxanthin (z) are studied by DFT method. Then the optoelectronic properties and interaction of these seven carotenoids were studied with chlorophyll Chla, Chlb. These fourteen complexes are Chla-antheraxanthin (Chlαa), Chla-β-carotene (Chlαb), Chla-neoxanthin (Chlαn), Chla-peridinin (Chlαp), Chla-violaxanthin (Chlαv), Chlα-xanthophyll (Chlαx), Chlα-zeaxanthin (Chlαz), Chlb-antheraxanthin (Chlβa), Chlb-β-carotene (Chlβb), Chlb-neoxanthin (Chlβn), Chlb-peridinin (Chlβp), Chlb-violaxanthin (Chlβv), Chlβ-xanthophyll (Chlβx), Chlβ-zeaxanthin (Chlβz) respectively. C-DFT (Conceptual Density functional Theory) has been used to calculate the global chemical descriptors of the carotenoids. The absorption properties for carotenoid, chlorophyll and chlorophyll-carotenoid have been studied by TDDFT method in water.

### Theoretical methods

First, a conformational analysis was performed by random rotations of the freely rotating bonds in the carotenoid complexes and chlorophylls in the range of 0° to 360°, generating 50 random structures for each system in this manner. Then the carotenoids are optimized by three different methods CAM-B3LYP** and M062x** and M062x/Lanl2dz:6-31G** and Wb97XD24/6-31G** and M062x/Lanl2dz:6-31G** by G16 software programme in gas phase. As Magnesium is present in Chlorophyll, Chla and Chlb are optimized with CAM-B3LYP/Lanl2dz:6-31G** by G16 software programme and M062x/Lanl2dz:6-31G** and Wb97XD/Lanl2dz:6-31G** methods with G16 software programme in gas phase. Finally the lowest minima structure by Wb97XD/Lanl2dz:6-31G** method was selected to study the physicochemical studies of carotenoid and chlorophyll in gas and solvent (water) medium by integral equation formalism-polarized continuum model (IEF-PCM) according to the SMD solvation model. The pictorial visualization of Electrostatic potential (ESP) of carotenoids by Gaussianview is given in Supplementary Fig. 1. The optimized structures of carotenoid and chlorophyll are given in Fig. 1.

The energy values by three different basis sets are given in Supplementary Table 1. Vibrational frequency analysis has been carried out for all complexes and no negative frequency is found. Now with these lowest minima structures of carotenoid and chlorophyll, various sites have been created and all structures are optimized with Wb97XD/Lanl2dz:6-31G** method in gas and water. Again the lowest minima structure has been selected for estimation of reaction rate (gas phase) calculations. Vibrational frequency analysis has been carried out to check the stability of complexes and no negative frequency has been found. See Fig. 2a and b for Chla-carotenoid and chlb-carotenoid complexes respectively. These optimized structures and IGMPLOT figures are visualized by Gaussianview programme. The same optimized geometries have been used to generate the intermolecular and intramolecular interactions with IGMPLOT.

Proton transfer reactions are of great importance in biomolecular processes of living organisms. In a gas phase environment, proton affinity (PA), is used which is defined as the negative of the enthalpy change at standard condition [i.e. temperature (298 K) and pressure]. Computation ab initio approaches can provide reliable values for proton affinities, which is important since it is difficult to determine experimentally. Chemical reactivity parameters have been used to predict the quantitative reactivity of the molecular system. The formulas used to calculate the proton affinities and global energy descriptors are given in Supplementary Information.

Antioxidants play very important role in the inhibition of the oxidative damage of the biomolecules. So quantitative analysis of the antioxidant activity of carotenoids has been obtained by the structure determination, energetics and global reactivity descriptors of carotenoid in gaseous and solvent (water) medium by DFT.

Transitivity code has been used to calculate the reaction rate constant with Transition-State Theory (TST) for reactions in gaseous phase. The graph Log K versus 1000/K were plotted for the fourteen carotenoid-chlorophyll complexes for the default temperature range 273.15 K to 4000.00 K. In this window, it is possible to estimate reaction rate constant with TST and several one-dimensional tunneling corrections, thermodynamic (AE, ΔH and ΔG) and kinetic (Eo, d, v, α, and a from ST model) properties of the reaction β = 1/kBT, where kB is the Boltzmann’s constant. The transition state calculations for carotenoid, chlorophyll and carotenoid-chlorophyll complexes have been carried out by G09 programme.

IGM (independent gradient model) represents a non-interacting system and the true ED gradient (\(\nabla V_o\)). It can be seen as a measure of electron sharing brought by ED contragradience. This model provides an automatic workflow that extracts the signature of interactions between selected groups of atoms. Also noncovalent
interaction (NCI) approach provides a visual understanding of the interactions present in chemical systems with both intermolecular and intramolecular interactions and to see the bond-by-bond picture that can be obtained from a wave function. In this way the specific interactions along reaction paths can easily monitor and the signature of inter and intra molecular interactions can be extracted. IGMPLOT is used to assess the role of non-covalent intramolecular interactions (intramolecular π–π stacking or hydrogen-bonding between two part of a single molecule along a reaction path).

TDDFT calculations for the complexes have been carried out on the ground state stable geometries with wB97x-D/Lanl2dz:6-31G** in solvent (water). The absorption wavelength, oscillatory strength and transitions for carotenoids, chlorophyll and chlorophyll-carotenoid interactions are given in Table 3 and Table 4 respectively. Transitions (%) are calculated by GaussSum32 software.

Results
The reactivity of seven carotenoids is studied by DFT-based global reactivity descriptors using Chemical reactivity theory (CRT). C-DFT33–37 used global reactivity parameters to predict structural and electronic properties of reactants and products which occur due to the chemical transformations during the reactions. It also gives a better microscopic insight to the whole interaction insight processes. To characterize the antioxidant property of carotenoids, it is important to analyse the global reactivity descriptors in detail. Chemical hardness measure the resistance to charge transfer, while the electronegativity measure the tendency to attract electrons in a chemical bond. The maximum electron flow between a donor and an acceptor is governed by the decomposition of binding energy between the atoms and it is determined by the factor electrophilicity index. Carotenoids act as

Figure 1. Optimized structures of carotenoids (wB97x-D/6-31G**) and Chl a and Chl b (wB97x-D/Lanl2dz:6-31G**) method by DFT method.
Figure 2. (a) Optimized Structures of Chylla-carotenoid complexes with wB97xD/Lanl2dz:6-31G** basis sets in solvent (water) by DFT method. (b) Optimized Structures of Chyllb-carotenoid complexes with wB97xD/Lanl2dz:6-31G** basis sets in solvent (water) by DFT method.
Scientists have indicated that antioxidant activity by the SPLET mechanism is a result of a balance between proclivity to transfer to another theoretical studies carried out for neutral ROS, i.e. ·OH and ·OOH, the average decrease in the vertical IP values is the antioxidant capacity of the compound. In our studied complexes, proton affinities (PAs) are much lower than the bond dissociation enthalpies (BDEs), which indicate that direct antioxidant activity in these complexes is mainly due to the sequential proton loss electron transfer (SPLET) mechanism. Lower PA of all the complexes is the antioxidant capacity of the compound. In our studied complexes, proton affinities (PAs) are much lower than the bond dissociation enthalpies (BDEs), which indicate that direct antioxidant activity in these complexes is mainly due to the sequential proton loss electron transfer (SPLET) mechanism.

Both carotenoids and Chlorophyll have higher HL gap which indicated structural stability. Chemical hardness of carotenoids is higher in gas state. It was also observed that the HL gap for carotenoids and chlorophyll in solvent is more than the HL gap of these complexes in gaseous state. Our Global chemical reactivity parameters (IP and EA), energy levels and energy gap (HOMO-LUMO) results are consistent with the previously calculated results for β-Carotene, Neoxanthin, Violaxanthin and Zeaxanthin in the gaseous phase, and methanol (solvent) using B3LYP/6-31+G(d,p) method. The results show narrow HL energy gap which benefits energy transfer process for the carotenoid. The experimental value of the band gap is in the range of (1.87–1.92) eV for Chla and (1.90–1.92) eV for Chlb. The HL gap for Chla is 1.883 in gas phase, while the HL gap for Chlb is 1.90 eV in aqueous medium. (See Fig. 2a). For Chla and Chlb, the ionization potentials are determined to be 4.19 eV and 4.45 eV in the PBE/DZP level; however, it is obtained 4.96 and 5.22 by using the Octopus code, as well as 4.79 and 5.17 by applying the B3LYP/6-31G* hybrid functionals, The dipole moment of Chla (4.79 D) is larger than that of Chlb (4.45 D) for Chla and Chlb, the ionization potentials are determined to be 4.19 eV and 4.45 eV in the PBE/DZP level; however, it is obtained 4.96 and 5.22 by using the Octopus code, as well as 4.79 and 5.17 by applying the B3LYP/6-31G* hybrid functionals, The dipole moment of Chla (4.79 D) is larger than that of Chlb (4.36 D). Carotenoids are well-known antioxidants and they have the ability to quench singlet oxygen and scavenging toxic free radicals preventing or reducing damage to living cells. In our studies, the complexes have lower µ and ω which predicted that these carotenoids are good reactive nucleophile, also indicating their antioxidant behaviour. So we calculated proton affinities (PAs) and bond dissociation enthalpies (BDEs) for the studied carotenoids. BDE (numerical parameter) is related to Hydrogen atom transfer (HAT) mechanism and characterizes the stability of the corresponding hydroxyl group. The lower BDE value indicates that the stability of the corresponding O–H bond is lower and the corresponding O–H bond can be easily broken. The antioxidant capacity of the compound. In our studied complexes, proton affinities (PAs) are much lower than the bond dissociation enthalpies (BDEs), which indicate that direct antioxidant activity in these complexes is mainly due to the sequential proton loss electron transfer (SPLET) mechanism. Lower PA of all the complexes indicated that antioxidant activity by the SPLET mechanism is a result of a balance between proclivity to transfer protons and the reaction kinetics of the conjugated base in the sequential electron transfer mechanism. See Table 2. The electron transfer between the antioxidant and the radical can be determined from the IP and EA. A lower IP means a higher probability of losing an electron. It is clear from the trends observed in IP values that all these carotenoid molecules are capable of electronic charge transfer to the neutral ROS. This trend is compared to another theoretical studies carried out for neutral ROS, i.e. OH and OOH, the average decrease in the vertical IP in the aqueous medium is 3.334 and 3.330, respectively. The EA also has crucial influence on the electron transfer between the antioxidant and the radical: a higher EA means a higher probability of gaining an electron.

Table 1. Calculated IP, EA, Global reactivity descriptors—electronegativity (χ), global hardness (η), global softness (S) and electrophilicity index (ω) in (eV) for studied carotenoid and Chlorophyll complexes with wB97x/D/6-31G** and wB97xD/Lanl2dz-31G** method respectively by DFT. *OH and *OOH parameters are taken from Ref.41.

| Complexes            | IP    | EA    | Electronegativity (χ) | Hardness (η) | Softness (S) | Electrophilicity Index (ω) |
|----------------------|-------|-------|-----------------------|--------------|--------------|---------------------------|
| Antheraxanthin       | 5.88  | 0.918 | 3.40                  | 2.481        | 0.2015       | 2.33                      |
| (Water)              | 4.77  | 2.182 | 3.48                  | 1.290        | 0.3864       | 4.67                      |
| β-carotene           | 5.79  | 0.862 | 3.33                  | 2.464        | 0.2029       | 2.24                      |
| (Water)              | 4.73  | 2.164 | 3.45                  | 1.280        | 0.3897       | 4.63                      |
| Neoxanthin           | 5.84  | 0.849 | 3.35                  | 2.496        | 0.2004       | 2.24                      |
| (Water)              | 4.76  | 2.149 | 3.45                  | 1.310        | 0.3830       | 4.57                      |
| Peridinin            | 6.16  | 1.205 | 3.68                  | 2.478        | 0.2018       | 2.74                      |
| (Water)              | 4.99  | 2.670 | 3.83                  | 1.160        | 0.4310       | 6.32                      |
| Violaxanthin         | 5.92  | 0.910 | 3.41                  | 2.505        | 0.1996       | 2.33                      |
| (Water)              | 4.53  | 2.184 | 3.36                  | 1.170        | 0.4263       | 4.80                      |
| Xanthophyll          | 5.83  | 0.863 | 3.35                  | 2.484        | 0.2013       | 2.25                      |
| (Water)              | 4.75  | 2.160 | 3.46                  | 1.300        | 0.3861       | 4.61                      |
| Zeaxanthin           | 5.78  | 0.854 | 3.32                  | 2.463        | 0.2030       | 2.23                      |
| (Water)              | 4.74  | 2.170 | 3.46                  | 1.290        | 0.3891       | 4.64                      |
| *OH (gas)            | 16.32 | 1.77  | 9.05                  | 7.279        | 0.069        | 5.65                      |
| (Water)              | 12.79 | 5.21  | 9.01                  | 3.792        | 0.132        | 10.71                     |
| *OOH (gas)           | 12.63 | 0.55  | 6.59                  | 6.041        | 0.083        | 3.602                     |
| (Water)              | 9.489 | 5.716 | 6.60                  | 2.887        | 0.173        | 7.543                     |
| Chlorophyll (Chl) α (gas) | 5.96  | 1.395 | 3.68                  | 2.283        | 0.2191       | 2.96                      |
| Chlorophyll (Chl) β (gas) | 7.26  | 0.665 | 3.96                  | 3.298        | 0.1516       | 2.38                      |
Strong intermolecular and intramolecular interaction has been observed among these complexes, with blue color isosurface plots between carotenoids and chlorophylls by IGMPLOT. The covalent chemical bond is intrinsically, SPLETT represents the most favorable process in the gas phase. By comparison, it is found that in the gas phase, the calculated PAs of carotenoids are significantly lower than the BDEs, and hence thermodynamically, the free radical scavenging process can be determined by the BDEs and PAs. Consequently, the higher electrophilicity index predicts greater propensity of the ROS to attract electrons from a generic donor molecule. In our studied complexes the electrophilicity index is lower than the electrophilicity index of ROS·OH and ·OOH, which indicates the high propensity of the ROS to attract electrons from these carotenoid molecules. The calculated redox potential is for the carotenoids higher than the experimental values. The electron donating ability of carotenoid is related with the HOMO energies also. The molecules with higher HOMO orbital energy have stronger electron donating abilities, which is strongly correlated to the IP values also. Thus, the thermodynamically preferred reaction pathway involved in the free radical scavenging process can be determined by the BDEs and PAs. By comparison, it is found that in the gas phase, the calculated PAs of carotenoids are significantly lower than the BDEs, and hence thermodynamically, SPLBT represents the most favorable process in the gas phase.

Table 2. Calculated HOMO–LUMO Gap (eV) (gas, water), Proton affinity (PA), bond dissociation energies (BDE) and redox potential (meV) for studied carotenoid complexes and chlorophyll with wB97xD/6-31G**, wB97xd/Lanl2dz:6-31G** method respectively.

| Complexes          | HL gap (gas) | HL gap (water) | PA   | BDE        | Redox potential |
|--------------------|--------------|----------------|------|------------|-----------------|
| Antheraxanthin     | 4.68         | 4.68           | 1.275| 1.555      | 591.04          |
| β-carotene         | 4.65         | 4.64           | 1.278| 1.529      | 561.87          |
| Neoxanthin         | 4.72         | 4.72           | 1.276| 1.565      | 560.82          |
| Peridinin          | 4.61         | 4.55           | 1.264| 1.428      | 574.32          |
| Violaxanthin       | 4.72         | 4.72           | 1.272| 1.563      | 577.21          |
| Xanthophyll        | 4.69         | 4.68           | 1.277| 1.546      | 576.44          |
| Zeaxanthin         | 4.65         | 4.64           | 1.279| 1.548      | 699.85          |
| Chlorophyll (Chl) a| 4.17         | 4.18           | 1.272| 2.169      | –               |
| Chlorophyll (Chl) b| 4.39         | 4.50           | 1.105| 1.962      | –               |

Strong intermolecular and intramolecular interaction has been observed among these complexes, with blue color isosurface plots between carotenoids and chlorophylls by IGMPLOT. The covalent chemical bond is intimately linked to the electron sharing between atoms. See Fig. 3a and b.

Transitivity code has been applied to estimate the reaction rate constant with Transition-State Theory (TST) for reactions in gaseous phase. Good correlation has been observed for all fourteen complexes in gas phase. See Fig. 4a and b. The biological activities and the spectral properties are influenced by the variation in the structures of the complexes. The biological activity in plants is the energy transfer, photoprotection and quenching of damaging singlet oxygen bioactivities in human and other organisms. A wide variety of computational investigations have been used for computing excitation energies and absorption profiles, ranging from time-dependent density functional theory (TD-DFT) and DFT/multireference configuration interaction (MRCI) to various wave function methods such as SAC-CI, CC2, and ADC(2). The calculated position of the first peak is about 1.85 (1.91) eV for Chl a (Chl b), which agrees well with existing experimental data. In another study the absorption energies of the Qy Band of Chl a and Chl b in acetone, diethyl ether, or ethanol were in the range (500–700) nm with CAM-B3LYP/e–31G* basis sets, while experimental results show (672 nm) and (652 nm) for Chl a and Chl b in ethanol. DLPNO–STEOM–CCSD calculations predict two excited states in the Q region, at 1.75 and 2.24 eV for chla in gas phase, while the absorption peaks of chla were observed in the range (600–700) nm range. Our TDDFT calculations show that the absorption wavelengths of the carotenoids are in visible region (432–452) nm, while chla and chlb has (356, 380) nm wavelength (water) respectively. See Table 3. The oscillatory strength of carotenoids are > 2, which is justified by the other theoretical results. The wavelengths for β-Carotene, Neoxanthin, Violaxanthin and Zeaxanthin in the gaseous phase and methanol (solvent) using TDDFT with the CAM-B3LYP/e–31+G(d,p) are (293–303) nm, which is slightly lower than our TDDFT results (430–452) nm measured in aqueous solvent. In one of the studies, the calculated TDDFT Excited States Energies (eV) were (2.20–3.65) eV using SVWN, B3LYP, and CAM-B3LYP Functionals for peridinin using Quantum Monte Carlo and Many Body Green's Function Theory. The Chla-carotenoid and Chlb-carotenoid complexes have wavelength (432–476) nm in visible region. Higher (89–90)% HOMO→LUMO transitions was observed for carotenoids, while chlorophyll has (68, 73)% transitions for HOMO→LUMO + 1. The % transitions are higher for carotenoid-chlorophyll complexes also. See Table 4. chlaz diade has absorption wavelength (340 nm) using CAM-B3LYP/6–31+G(d,p) method, while chl ap has two absorption wavelengths peaks in (440–480) nm range in aqueous state with MM/QM method.

The first excited triplet state transitions were calculated with wB97xD/6-31G** basis sets for Chl(a,b) and carotenoids and wB97xd/Lanl2dz:6-31G** basis sets were used for the Chlorophyll–carotenoid interactions. The wavelength for chlorophyll and carotenoid lies in (588–750) nm range (See Table 3) while the first triplet wavelength for chl-car complexes lies in (432–555) nm range. See Table 5. The results are consistent with the previous experimental results for ppLHCSR1-Vio (488 nm) and ppLHCSR1-Zea (491 nm) at pH 7. When pH is lowered to 5, the carotenoid bands slightly red-shifts to 489 nm (ppLHCSR1-Vio) and 492 nm (ppLHCSR1-Zea).
**Figure 3.** (a) Intermolecular and Intramolecular interactions of Chlα-carotenoid complexes by IGMPLOT. The visualized images have been taken from Gaussview programme. (b) Intermolecular and Intramolecular interactions of Chlβ-carotenoid complexes by IGMPLOT. The visualized images have been taken from Gaussview programme.
In another studies on Chl (a, b), the triplet energy is 0.8 eV lower than that of the S1 state. The S₁–T₁ adiabatic energy gap is lower for the studied complexes which is an implication on the ISC transition from S₁ to T₁ state. According to the energy gap law, for electronic states with similar geometries, the smaller the energy gap, the larger is the ISC rate. So there will have a faster ISC rate for triplet formation in chl-car complexes. Certainly, carotenoids play major role in the photosynthetic apparatus for quenching of the Chl triplet state through the triplet–triplet energy transfer (TTET) mechanism. The direct population of the carotenoid triplet state by intersystem crossing (ISC) is a low probability event, due to a very short lifetime of the excited state of these molecules, that is dominated by internal conversion. Energy transfer from the 'Chl, which is populated with a yield of ~0.6 in the absence of other quenching mechanisms, is efficient in photosynthetic systems because of the short average inter-pigment distances and because the 'Car lays at an energy level which is below that of 'Chl. Also it is well known that 'Chl is an efficient sensitizer of singlet oxygen (¹O₂), which is an highly reactive species and plays major role in photo-oxidative stress.

**Conclusions**

The chemical reactivity, antioxidant properties of carotenoids and chlorophyll has been studied by DFT. Results indicated high stability and lower reactivity for carotenoids and chlorophyll complexes. Lower proton affinities (PAs) than the bond dissociation enthalpies (BDEs) indicate that the direct antioxidant activity in these carotenoids is due to the SPLET mechanism with dominant solvent effects. Higher HL gap was observed for carotenoid
Figure 4. (a) Reaction rate constant graph for Chylla-carotenoid complexes (ln(k)) versus 1000/T(K). (b) Reaction rate constant graph for Chyllb-carotenoid complexes (ln(k)) versus 1000/T(K).
in solvent (water) as compared to the HL gap in gas phase. Stabilized intermolecular and intramolecular interaction has been visualized between chlorophyll-carotenoid interactions. A good coorelation for reaction rate constant with temperature by Transition-State Theory (TST) was estimated for carotenoid-chlorophyll complexes in gas phase. TDDFT results showed that all the carotenoid-chlorophyll complexes have absorption spectra in visible region. Further the lower $S_1$–$T_1$ energy gap implicated ISC transition from singlet to triplet state.
Data availability

The optimized structure (CIF) coordinates of all fourteen complexes are given in Supplementary Information.

Received: 13 April 2021; Accepted: 28 July 2021
Published online: 15 September 2021

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Table 3. Absorption wavelength (nm) (Singlet states and Triplet states), Oscillatory Strength (f) and transitions for studied carotenoids (wB97xD/6-31G**) and Chlorophyll (wB97xD/Lanl2dz:6-31G**) by TDDFT in solvent (water) medium.

| Complexes   | Singlet states | Triplet states |
|-------------|----------------|----------------|
|             | Wavelength (nm) | f   | Transitions | Wavelength (nm) | f   | Transitions |
| Antheraxanthin | 436.35         | 4.1365 | H→L (89%)  | 605.24         | 1.8395 | H→L (69%) |
| β-carotene   | 440.51         | 4.1761 | H→L (89%)  | 604.95         | 2.7927 | H→L + 1 (38%) |
| Neoxanthin   | 432.08         | 4.2227 | H→L+1 (90%) | 597.40         | 2.3260 | H→L+2 (34%) |
| Peridinin    | 452.09         | 2.8365 | H→L (90%)  | 642.31         | 2.2258 | H→L+3 (33%) |
| Violaxanthin | 431.63         | 4.1115 | H→L (90%)  | 588.16         | 1.6288 | H→L (23%) |
| Xanthophyll  | 435.99         | 4.0844 | H→L+1 (89%) | 596.28         | 2.4379 | H→L+2 (35%) |
| Zeaxanthin   | 440.49         | 4.1694 | H→L (89%)  | 605.72         | 3.5519 | H→L+3 (48%) |
| Chlorophyll (Chl) a | 356.57       | 1.1354 | H→L+1 (73%) | 730.65         | 0.0003 | H→L+2 (68%) |
| Chlorophyll (Chl) b | 380.98       | 1.4282 | H→L+1 (68%) | 675.85         | 0.0098 | H→L+3 (68%) |

Table 4. Absorption wavelength (nm) (Singlet States), Oscillatory strength and transitions for Chla-carotenoid and Chlb-carotenoid complexes with wB97xD/Lanl2dz:6-31G** method by TDDFT in solvent (water) medium.

| Complexes | λabs | f   | Transitions | Complexes | λabs | f   | Transitions |
|-----------|------|-----|-------------|-----------|------|-----|-------------|
| Chlaa     | 440.47 | 4.3868 | H→L+1 (89%) | Chlba     | 440.82 | 3.8688 | H→L+2 (89%) |
| Chlab     | 444.36 | 3.4976 | H→L+1 (89%) | Chlb     | 447.34 | 4.2932 | H→L+2 (87%) |
| Chlan     | 433.25 | 4.4038 | H→L+1 (89%) | Chln     | 554.23 | 4.7344 | H→L+2 (91%) |
| Chlap     | 475.92 | 2.5918 | H→L+1 (89%) | Chlp     | 476.46 | 2.7656 | H→L+1 (90%) |
| Chlav     | 432.85 | 4.2830 | H→L+1 (89%) | Chlw     | 432.91 | 4.1286 | H→L+2 (91%) |
| Chlax     | 444.21 | 2.8298 | H→L (89%)  | Chlax     | 442.24 | 4.0839 | H→L+2 (88%) |
| Chlaz     | 445.63 | 4.3302 | H→L+1 (89%) | Chlaz     | 444.76 | 4.4578 | H→L+2 (89%) |

Table 5. Absorption wavelength (nm) (Triplet states), Oscillatory strength and transitions for Chla-carotenoid and Chlb-carotenoid complexes with wB97xD/Lanl2dz:6-31G** method by TDDFT in solvent (water) medium.

| Complexes | λabs | f   | Transitions | Complexes | λabs | f   | Transitions |
|-----------|------|-----|-------------|-----------|------|-----|-------------|
| Chlaa     | 793.87 | 0.00030 | H→L (42%) | Chlba     | 715.88 | 0.00000 | H→L (37%) |
| Chlab     | 798.17 | 0.03020 | H→L (49%) | Chlb     | 730.86 | 0.00000 | H→L (39%) |
| Chlan     | 683.07 | 0.0486 | H→L+3 (46%) | Chln     | 758.54 | 0.00000 | H→L+1 (41%) |
| Chlap     | 795.63 | 0.00048 | H→L+2 (92%) | Chlp     | 743.34 | 0.00210 | H→L+1 (44%) |
| Chlav     | 775.77 | 0.00050 | H→L+2 (72%) | Chlw     | 742.37 | 0.00050 | H→L+3 (42%) |
| Chlax     | 791.11 | 0.00000 | H→L (98%) | Chlx     | 734.85 | 0.00048 | H→L+1 (40%) |
| Chlaz     | 795.09 | 0.00040 | H→L+3 (41%) | Chlhz    | 791.77 | 0.00035 | H→L+2 (54%) |
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Acknowledgements
RS acknowledges the financial assistance by DST WOSA Project (SR/WOS-A/CS-69/2018). RS is thankful to her Mentor Dr. Shrish Tiwari, Bioinformatics, CSIR-Centre for Cellular and Molecular Biology, and Dr. G. Narahari Sastry, Director, CSIR-NEIST for the technical support. The author also acknowledges Prof. G.M. Gurbanov, Azerbaijan Medical University, Baku, Azerbaijan, for sharing the initial idea to work on carotenoids.

Author contributions
This manuscript has been solely written by the corresponding author.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-97747-w.

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