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Investigation of Antiviral Potential of Food Carotenoids and Apocarotenoids against RNA-dependent RNA Polymerase of Hepatitis C Virus

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Abstract
Hepatitis C disease have been a global health threat and affects a significant portion of world population. Hepatitis C have also been a silent health threat for Turkiye, where there are around half million people infected with Hepatitis C Virus (HCV). Disease burden and mortality are expected to increase gradually in the next 20 years in Turkiye. Unavailability of enough data on the currently-available drugs in routine clinical practice, their side effects and interactions with other drugs, and their efficacies on the less common genotypes indicates the necessity of alternative treatment options. Natural products from herbal and medicinal plants can indeed provide an alternative as being drug-like dietary supplements. In particular, the carotenoids and apocarotenoids are underexplored in their antiviral potential, including anti-HCV activities. Therefore, we focused on the virtual screening of various carotenoids and apocarotenoids against the RNA-dependent RNA polymerase (RdRp) of HCV. Molecular docking experiments showed strong binding affinities of the ligands to both palm and thumb domains of RdRp of HCV. In fact, some of them such as neoxanthin, crocin, canthaxanthin and cryptoflavin bound quite strongly to both domains compared to native ligands and current antiviral drugs. MD simulation for neoxanthin-RdRp complex confirmed the stability of the ligand within the binding cavity of RdRp throughout 100 ns simulation. This clearly indicated the potential of carotenoids, specifically neoxanthin, as RdRp inhibitor in treating HCV. Thus, this study not only discovered anti-HCV drug candidates with the properties of easy-to-access and low cost, but also paved the way for the development of carotenoid or apocarotenoid based dietary supplement candidates for the prevention and treatment of HCV.

1. Introduction

Hepatitis C is a blood borne disease and affects around 3% of the world population. The cause of hepatitis C, Hepatitis C virus (HCV), has been a global threat for the public health. According to World Health Organization (WHO), around 3-4 million new cases of HCV infection has been annually reported, indicating an ongoing global public health issue [1], [2]. Additionally, HCV is one of the main causes of advanced liver diseases such as hepatocellular carcinoma, cirrhosis, and liver failure [2], [3]. The transmission of HCV is mostly due to direct percutaneous exposure to blood and becomes different based on the classifications of the countries. The infection rates in low and high endemic countries differ at around 20%. In developed regions of the world, the intravenous drug abuse is the most common way of transmission for HCV, while invasive procedures or unsterile medical procedures and instruments are the most widely-seen reasons for the transmission of HCV in poor regions [1], [4], [5].

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In addition, people who inject drugs, men who have sex with men, and prisoners were reported as high-risk populations [6]. Furthermore, there currently exist seven genotypes and numerous subtypes of the virus, which complicates targeting of treatment [7]. Globally, HCV genotypes 1 is the most common hepatitis C infection occupying of 44% of all cases, while genotypes 3 and 4 causes 25% and 15% of all hepatitis C cases, respectively [6].

Based on the data and information provided in the Turkiye Viral Hepatitis Prevention and Control Programme (2018-2023), in Turkiye, approximately 250,000-550,000 in the population over the age of 18 are infected with HCV and the vast majority of them is not aware that they are infected with HCV [8], [9]. In another study conducted in 2014, the number of HCV infected people in Turkiye was estimated to be around 514,000. However, according to the same study; 81,300 (16%) people was HCV-infected, the annual number of newly diagnosed patients was 5,500 (1.1%), and the annual number of patients receiving treatment was estimated to be 4,200 (0.8%). Based on the data from the Ministry of Health in Turkiye, anti-HCV positivity was 3.8% in hemodialysis patients, 1.7% in peritoneal dialysis patients, 1.96% in kidney transplant patients, and 7.6% in liver transplant patients. HCV was also reported to be the second most common cause of liver transplantation in Turkiye. It was also pointed out that if HCV is not treated and does not become under control, the disease burden and mortality are expected to increase gradually in the next 20 years [9]–[11].

The treatment of hepatitis C has been historically prolonged and leads to multiple several severe side effects [12]. In 1990s, pegylated interferon and ribavirin (PEG/RBV) combination therapy was the only treatment option. However, this antiviral agent did not have high efficacy in the eradication of HCV and led to common side effects [13]. Genotype 1 was particularly more resistant to interferon and this led to the lower success rate of the combination therapy for genotype 1 compared to other genotypes [14]. In 2011, first direct acting antiviral agents (DAA), boceprevir and telaprevir, were approved to treat the genotype 1 together with the combination therapy. DDA led to an increase in the rates of sustained viral response, yet greater toxicity, high cost, and less safety in patients with advanced diseases were observed for these drugs [15]. Since 2011, various antiviral drugs such as DNA and RNA polymerase inhibitors, RNA protease inhibitors, and RNA serine protease inhibitors have been approved for hepatitis C. These recent drugs had shorter therapies, less toxicity and did exclude the PEG/RBV therapy from the treatment process of hepatitis C patients [14], [15]. Nevertheless, the majority of the current scientific evidence for these drugs depends on expert opinion, case-control series, cohort studies. It was also reported that some of phase 2 and 3 clinical trials for these antiviral agents were conducted with a reduced number of patients and select groups. Unavailability of adequate amount of data on the use of these drugs in routine clinical practice, their side effects and interactions with other drugs, and their efficacies on the less common genotypes set forth the necessity of alternative treatment options/drugs [15]. The new candidates should be easy-to-access, effective for all genotypes, and low cost. Particularly, natural products from medicinal and herbal plants might provide an alternative option for HCV as being drug-like dietary supplements.

Carotenoids and their cleaved derivatives, apocarotenoids, are widely seen in nature with more 1200 identified members [16], [17]. They were isolated mainly from plant and algal species, yet some non-phototrophic fungi, bacteria and archaea also produce carotenoids and apocarotenoids [18], [19]. These colorful natural products help to protect photosynthetic organisms from the photo-oxidative damages. On the other hand, in non-photosynthetic organisms, they protect the cells from oxidative damages with their strong antioxidant properties [20]. Furthermore, carotenoids and apocarotenoids have various applications such as food colorants, food supplements and nutraceuticals for cosmetics industry, and pharmaceuticals. Particularly, their health-promoting properties render them as alternative option for the treatment and prevention of various diseases such as cardiovascular diseases, COVID-19, cervical and prostate cancers [21]–[23].
Even though the health benefits and biological activities of carotenoids and apocarotenoids have been explicitly reported in literature, their antiviral potential was underexplored. Particularly, their antiviral potential against HCV, for which there is a need for low cost, and easy-to-access drug and/or dietary supplement candidates, was not extensively studied. In literature, there are two studies in which bacterioruberin and pigment extract including carotenoids were tested for their antiviral potentials [24], [25]. The study with bacterioruberin was aimed to conduct *in vitro* antiviral tests for this carotenoid against RNA-dependent RNA polymerase (RdRp) of HCV and revealed that bacterioruberin is indeed a promising RdRp inhibitor [24]. Additionally, in literature, some of carotenoids including astaxanthin, lycopene and β-carotene were reported to have preventive and supportive of treatment in viral diseases, yet no direct antiviral potentials of these

natural compounds were studied [26]–[29]. Therefore, in this study, we evaluated the antiviral potentials of some of the food carotenoids and apocarotenoids against RdRp of HCV using molecular docking and molecular dynamics (MD) simulation. Our findings indicated that these food natural products often had low binding energy scores. In particular, neoxanthin had the strongest affinity to RdRp of HCV, showing the potential inhibitory effect against the receptor. The molecular docking results was further confirmed by MD simulation, during which neoxanthin was stable in the RdRp of HCV. Our results not only uncovered the potential of food carotenoids and apocarotenoids as antiviral agents, but also provide an initial data for the development of dietary supplementary candidates for the treatment and prevention of hepatitis C disease.

2. Material and Method

2.1. Ligand library and preparation

The ligand library was designed to be consist of carotenoids and apocarotenoids. In addition, the original substrates for the receptor protein structures as well as known antiviral agents were added to the library. Upon the creation of the ligand library, their 3D structures were downloaded from PubChem database in sdf format. For the compounds without 3D structures, their 2D structure files were downloaded and converted to 3D structures using Open Babel GUI software. Subsequently, the conversion of all the sdf files with 3D structures to pdb files were also conducted in Open Babel GUI software. In the last step of ligand preparation, AutoDock Tools software was used, in which aromatic carbon and rotatable bonds were detected, torsion number was automatically set, non-polar hydrogens and Gasteiger charges were added to all ligands. They all were saved as pdbqt files [30].

2.2. Receptor Preparation

The receptor protein was the Hepatitis C virus RNA-dependent RNA polymerase NS5B. Two different crystal structure files (PDB IDs: 2GIR and 2GIQ) were available for this protein with non-nucleoside polymerase inhibitors (NNI-2 and NNI-3). NNI-2 binds to the palm binding domain, while NNI-3 binds to the thumb 1 site of RdRp of HCV [31]. In this study, it was aimed to investigate the potential non-nucleoside inhibitors with strong binding affinities towards both domains. Both receptors were prepared using AutoDock Tools software, with which water molecules were deleted from the PDB files of the receptor proteins, and polar hydrogen atoms and Kollmans charges were added to them. Then, both were saved as pdbqt files to proceed molecular docking experiments [30].

2.3. Molecular docking experiments

In order for conducting a virtual screening for the molecules in the ligand library against RdRp of HCV, the grid boxes were determined for both target domains of RdRp of HCV. This was done using the positions of the NNI ligands interacting with the specific target domains in the receptor. The grid box coordinates for 2GIR was at x = −33.10, y = -12.39, and z = 34.80 with sizes of 33.54, 38.52, and 28.90 Å. For 2GIQ, it was at x = 14.14, y = -8.40, and z = -13.10 with sizes of 47.24, 41.07, and 34.82 Å. After determining the grid boxes, the prepared flexible ligands were docked against the fixed rigid receptor protein using AutoDock Vina in the Windows command line window upon the preparation of config files with necessary information (receptor, grid box coordinates, number of modes: 10, energy range: 8 and exhaustiveness: 16) [32]. Pymol was used for the visualization purposes.

2.4. Molecular dynamics simulation

Molecular docking experiments resulted in the indication of strong binding affinity of neoxanthin towards both target sites of RdRp of HCV. Although similar strong binding affinities were shortlisted for some other ligands, neoxanthin with the lowest binding energy score was selected for further validation of its stable binding to the receptor protein using MD simulation. The NPT ensemble MD simulation was performed using the 2GIQ-neoxanthin complex with the parameters at 300 K temperature for a duration of 100 ns. Since the drugs perform their actions in human body under constant temperature and pressure conditions. Therefore, NPT ensemble condition was enabled during the simulation process and kept the temperature and pressure constant while allowing the system volume to change during the simulation. Desmond module of Schrödinger was used to perform MD simulation under energy-minimized complex system and isosmotic environment was created to neutralize the existing charges by the addition of 0.15 M NaCl [30], [33].

3. Results and Discussion

3.1. Design of the ligand library

The ligand library was designed to include carotenoids and apocarotenoids, mostly found in the herbal and medicinal plants. The list of the all ligand molecules used in this study was tabulated in Table 1. Various food-derived carotenoids as well as some of the glycosylated derivatives were included in the library. For instance, β-carotene is very common in many foods such as dark orange and green fruits, and vegetables, such as mango, apricot, cantaloupe, carrots, red peppers, pumpkin, sweet potatoes, broccoli, and leafy greens. β-cryptoxanthin are commonly found in tropical fruits, such as mango, papaya and tangerine. Leafy greens, corn, and green vegetables such as broccoli, brussel sprouts, green beans, peas, and zucchini are the major sources for lutein. Tomato has lycopene as the dominant carotenoid, which can also be found in other fruits such as watermelon, pink grapefruit, and Japanese persimmons. Zeaxanthin is available in egg yolks, corn, corn meal, and leafy greens. Neoxanthin is found as a major carotenoid in all green leaves and other photosynthetic tissues and in many algae, and in some flowers [34]–[38]. On the other hand, glycosylated carotenoids such as zeaxanthin diglucoside, bacterioruberin diglucoside, and astaxanthin diglucoside are found in algae and bacteria including thermophilic, endophytic and psychrotrophic bacteria [17], [39]–[41]. The glycosylated derivatives were added to the ligand library to evaluate the effect of glycosyl group on the antiviral potential of carotenoids. The library also has some commonly known apocarotenoids from herbal plants such as saffron [42]–[44]. The carotenoid database, including the apocarotenoids, was also used in designing the ligand library as a helpful database [16]. We also added the native NNI molecules from the crystal structures of the RdRp of HCV and these pre-confirmed compounds allowed to compare our results with the known RdRp inhibitors [31]. Finally, some of the currently-used antiviral drug molecules such as favipiravir, remdesivir, sofusbavir, paxlovid and molnupiravir were also added for the comparison of the results with the current-used antiviral agents.

3.2. Molecular docking

To validate the molecular docking protocol, we firstly conducted molecular docking with the native ligands found in the crystal structure of RdRp of HCV. NNI-2 and NNI-3 were reported to bind palm and thumb

Table 1. List of compounds in the ligand library and their binding energy scores against Thumb 1 and Palm domains of RdRp of HCV. Binding energies are in kcal/mol.

Compounds	Thumb Domain	Palm Domain		Compounds	Thumb Domain	Palm Domain
Alpha-carotene	-9.0	-8.9		Zeaxanthin Diglucoside	-8.8	-9.3
Cucurbitaxanthin A	-8.7	-9.4		Astaxanthin Diglucoside	-9.1	-9.7
Cucurbitaxanthin B	-8.7	-9.0		Bacteroruberin Diglucoside	-8.7	-9.0
Beta-carotene	-9.3	-9.1		Bixin	-6.9	-7.2
Phytoene	-6.2	-7.6		Safranal	-5.5	-5.6
Luteoxathin	-9.2	-9.9		Picocrocin	-6.9	-8.0
Cryptoflavin	-9.8	10.3		Crocetin aldehyde	-6.9	-6.7
Canthaxanthin	-9.3	-9.2		Crocetin	-7.3	-7.3
Capsanthin	-8.9	-9.3		Crocin	-8.2	-9.7
Capsarubin	-8.3	-9.5		3-hydroxycyclocitral	-5.6	-5.5
Lycopene	-8.5	-8.1		Crocusatin H	-6.3	-6.4
Violaxanthin	-8.6	-9.1		Apo-12'-capsorbibal	-7.2	-8.1
Beta-cryptoxanthin	-8.9	-9.2		Apo-12'-zeaxanthinal	-7.8	-7.7
Fucoxanthin	-9.1	-9.4		Apo-12'-violaxanthal	-8.0	-8.0
Lutein	-9.1	-9.5		Native ligand (NN2)	-8.9	-8.9
Neoxanthin	-9.0	-10.4		Native ligand (NN3)	-8.2	-8.3
Phytofluene	-7.5	-6.9		Favipiravir	-5.2	-5.9
Latoxanthin	-9.0	-9.4		Molnupiravir	-6.9	-8.0
Zeaxanthin	-9.2	-9.2		Paxlovid	-7.9	-8.2
Astaxanthin	-9.2	-9.7		Remdesivir	-7.6	-8.2
Bacterioruberin	-9.1	-7.7		Sofusbavir	-7.9	-8.5

domains of RdRp of HCV, respectively [31]. The binding energy score of NNI-2 to the palm domain is -8.9 kcal/mole, while the one of NNI-3 to thumb domain is -8.3 kcal/mole. This indicated their strong binding affinities towards the corresponding domains of RdRp of HCV and validated the molecular docking protocols. Then, all the carotenoids and apocarotenoids, as well as some of the approved antiviral drugs were docked against both domains of RdRp. The binding energy scores of each ligand were tabulated in Table 1. Our docking experiments revealed that in general carotenoids and long-chain apocarotenoids bound strongly to both palm and thumb domains of the receptor protein. In specific, various carotenoids such as cryptoflavin, neoxanthin, astaxanthin, capsarubin, luteoxanthin had very low binding energies towards palm domain, whereas canthaxanthin, cryptoflavin, β-carotene, neoxanthin had strong affinities to the thumb domain of RdRp of HCV. Another important finding is that the glycosylated carotenoids did not change the binding affinities, except bacterioruberin diglucoside had an improved the binding affinity from -7.7 to -9.7 kcal/mole towards only palm domain. For the other glycosylated carotenoids, there is no significant change in the binding energy scores.

In addition, a glycosylated apocarotenoids, crocin, had lower binding energies to both domains of receptor protein compared to its non-glycosylated derivative, crocetin. This indicated that glycosylated carotenoids and apocarotenoids might provide better biological activities. Similar phenomena were recently reported for glycosylated flavonoids against the main protease of SARS-CoV-2 in an *in silico* study [45]. The glycosylated natural products were known to have a better solubility and pharmacological properties [46]. On the other hand, for the smaller apocarotenoids such as safranal, crocusatin H and 3-hydroxycyclocitral, the binding affinities to both domains were weak. Similar results were obtained for favipiravir as a quite small antiviral agent. The other currently-used antiviral agents has low binding energy scores for both domains of RdRp of HCV with an average score of around -8 kcal/mole (Table 1). Obviously, many carotenoids had indeed stronger binding affinities towards both palm and thumb domains as compared to these approved antiviral drugs, indicating the high antiviral potential of carotenoids and apocarotenoids.

3.3. Molecular dynamics simulation

Since the binding energy score of neoxanthin was the lowest among all (Table 1), the MD simulation was performed for the ligand-receptor complex with neoxanthin in order to confirm its stability within palm domain of RdRp of HCV. MD simulation was executed for 100 ns using the Schrodinger Desmond program. The Root Mean Square Deviation (RMSD) was measured to observe the average change in the backbone of receptor protein as well as structural shifts. As shown in Figure 1, RMSD profile for the receptor protein revealed that the protein was stable throughout the simulation with around 1.5-1.75 Å RMSD value, which was perfectly acceptable. Similarly, RMSD value for neoxanthin ligand was also within the acceptable range (2.4-3.2 Å), although there was a fluctuation for around 50 ns. However, it was stabilized at around 3.2 Å at the end of MD simulation. Despite this short fluctuation, ligand was adjusted to be stable within the binding pocket of the palm domain. RMSD trajectories indicated stable behaviors for both ligand and receptor molecules

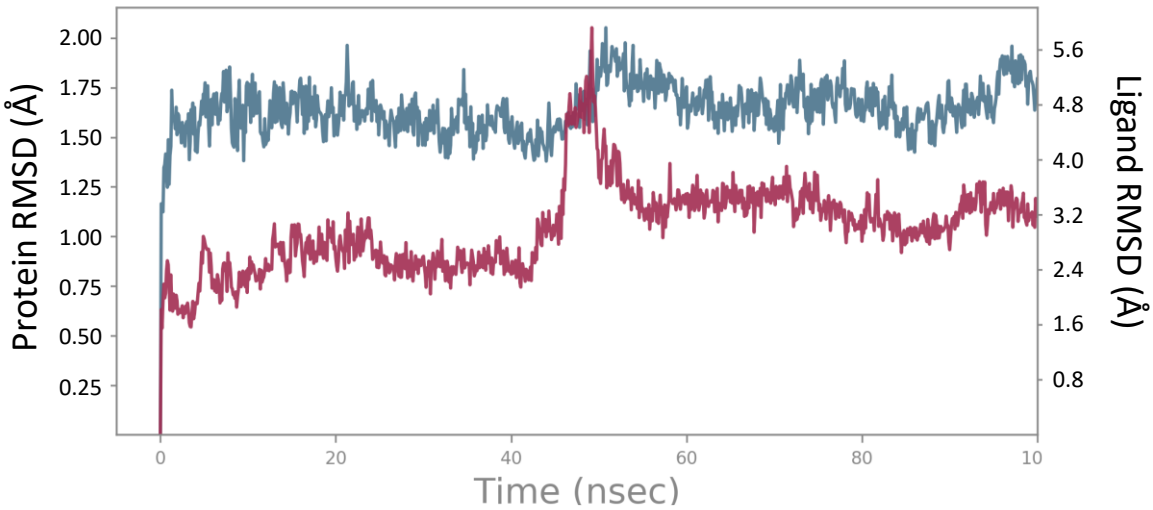

Figure 1. RMSD profiles of the backbone of RNA-dependent RNA polymerase of Hepatitis C virus (blue) and neoxanthin (pink) throughout 100 ns MD simulation.

within the complex with very little fluctuations (Figure 1).

Root Mean Square Fluctuation (RMSF) profile was also measured to characterize local changes in the protein chain and ligand atom positions. Figure 2 showed RMSF profiles for both protein and ligand. RMSF value observed for the majority of the residues of receptor protein was stable within the range of 0.6-2 Å. But there are some residues, particularly terminal residues were fluctuated up to around 3 Å (Figure 2A). We evaluated the RMSF values for the residues located in the binding pocket and found that they were not among the fluctuated residues. This confirmed the acceptable fluctuations within the receptor protein active site. RMSF value of neoxanthin showed that the majority of its atoms was stable within the range of 1-2 Å, except atoms numbered as 7, 8, 9, 41 and 44 had small fluctuations. All of these atoms are located in the ring with epoxy functional group (Figure 2B).

46.77%, which were conserved during MD simulation. The stability of the receptor-ligand complex during MD simulation was due to the various interactions such as hydrogen bonds, hydrophobic and ionic interactions, and water bridges. These interactions demonstrated in Figure 3. Neoxanthin had hydrophobic interactions with the following amino acid residues of RdRp of HCV: ALA15, ALA16, PHE193, TYR195, CYS366, TRP397, PRO404, VAL405, MET414, TYR415, ILE447 and TYR448, while the hydroxyl group of neoxanthin created water bridges with some of the residues of receptor protein (Figure 3). The intensities of all the interactions were measured throughout the simulation period and demonstrated as interaction fractions in Figure 4. Some of these interacting residues such as MET414, ILE447, TYR448, ASN316 and PHE193 were listed among the key residues interacting with native NNI-2 molecule. Particularly, the mutant RdRp of HCV with

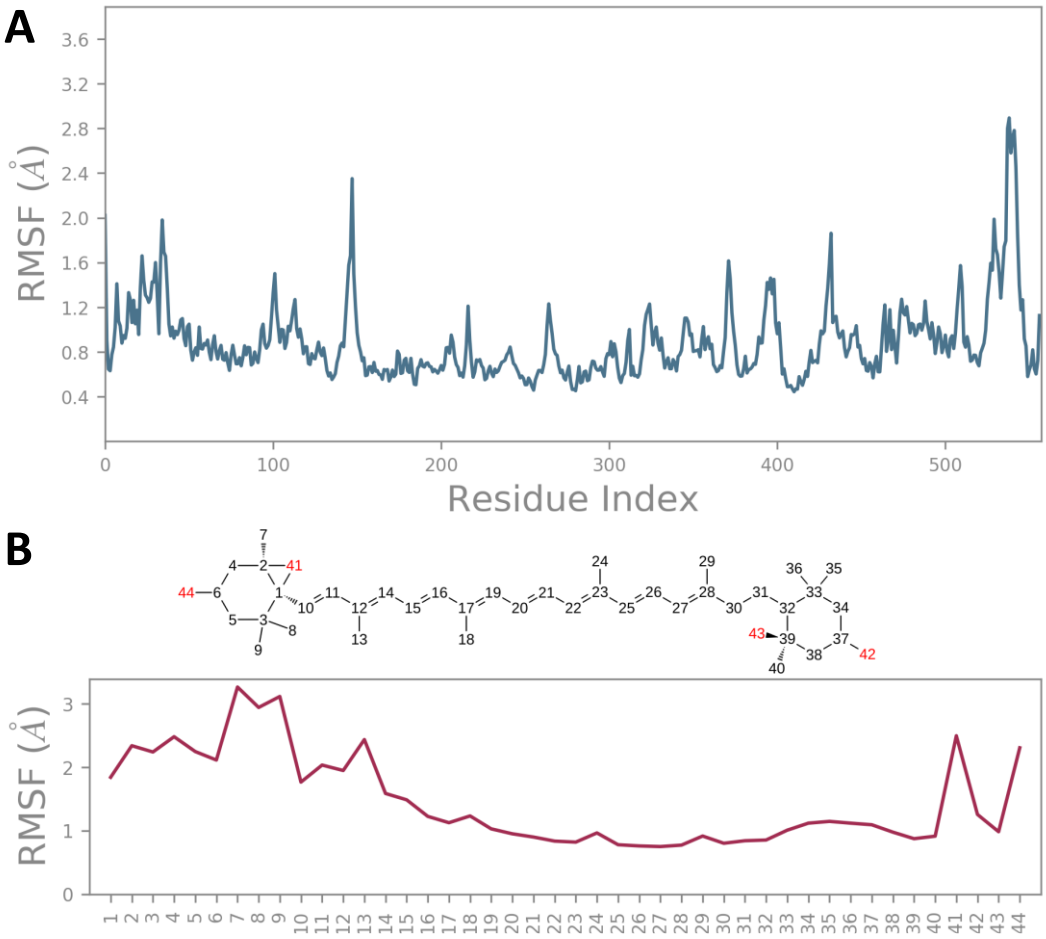

Figure 2. RMSF profiles of RNA-dependent RNA polymerase of Hepatitis C virus (A) and neoxanthin throughout 100 ns MD simulation and its structure (B).

Protein secondary structure elements (SSE) analysis revealed that receptor protein had α-helices (37.844%) and β-sheets (9.33%)) with a total SSE of

MET414LEU replacement was reported to lead to increased resistance to NNI molecules [31].

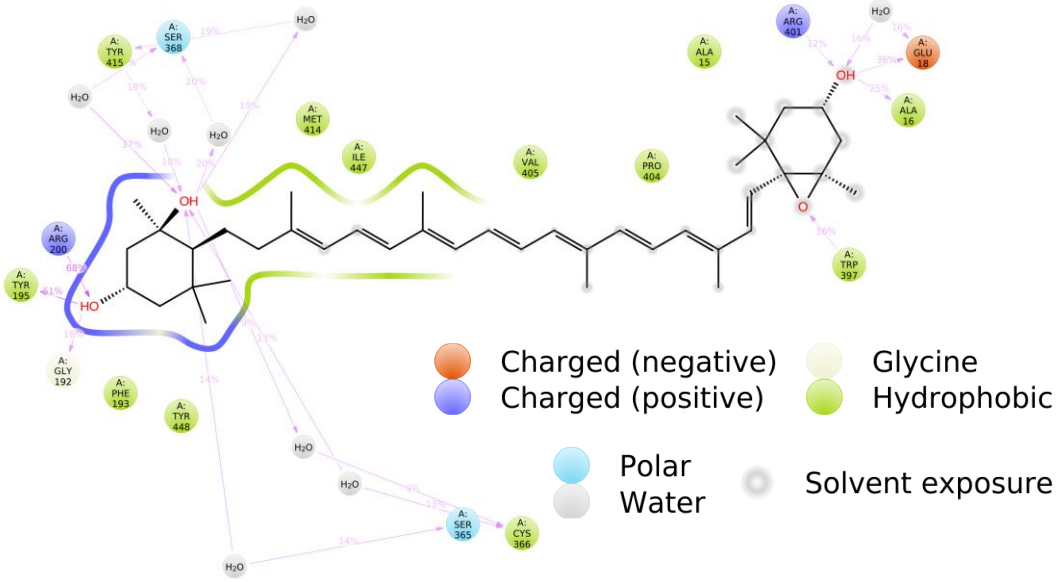

Figure 3. The protein–ligand contacts observed after MD simulation for RNA-dependent RNA polymerase of Hepatitis C virus complexed with neoxanthin.

3.4. Antiviral potential of hit molecules

A wide variety of health benefits and biological activities have been reported for carotenoids and apocarotenoids, yet their antiviral activities have not been extensively investigated. In addition, in literature, some of carotenoids and apocarotenoids such astaxanthin, lycopene, β-carotene and crocin were reported and suggested to have preventive and supportive of treatment in viral diseases [27], [28], [47]–[50]. Nevertheless, it is due to the antioxidant and anti-inflammatory properties of these molecules. In other words, their antiviral properties were not directly examined. In specific, their antiviral potential against HCV, for which there is a need for low cost, and easy-to-access drug and/or dietary supplement candidates, was not extensively studied. Thus, they attracted the attentions of researcher as a new research area exploring their antiviral capacity. In particular, COVID-19 pandemic speeded up this new research field to discover and develop new antiviral agents and/or dietary supplements for the treatment and prophylaxis of COVID-19. For example, in a recent paper, fucoxanthin and siphonaxanthin were tested against SARS-CoV-2 using *in silico* as well as *in vitro* techniques. Siphonaxanthin from *Codium fragile* exhibited a strong antiviral activity (IC50: 87.4 μM) against SARS-CoV-2. With its low acute toxicity and promising pharmacokinetic properties, siphonaxatnhin were suggested to be used in the treatment and prevention of COVID-19 through the inhibition of ACE2 binding site of spike protein of SARS-CoV-2 [51]. In another study, carotenoid-including microalgae extracts showed antiviral activity against Herpes Simplex Virus Type 1. Ethanol extract of *Haematococcus pluvialis* showed the inhibition of virus infection by around 85% [25]. Furthermore, the antiviral potential of bacterioruberin was investigated in an *in vitro* study against RdRp of HCV. This study revealed that bacterioruberin is a promising RdRp inhibitor for the treatment of Hepatitis C disease [24]. We observed the same results in this study for bacterioruberin, which showed quite strong binding affinities to both domains of RdRp, indicating its potential as RdRp inhibitor in the treatment of Hepatitis C disease.

Neoxanthin as one of the hit molecules is known to have photoprotective role as an antioxidant compound [52], [53]. However, to our knowledge, there is no study, even *in silico* studies, showing its antiviral potential. Another important hit molecule in this study is cryptoflavin. In a study, it was listed among the carotenoids of marine-origin extracts, which showed antibacterial activities against isolated from human specimens such as *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* [54]. However, no antiviral potential of this carotenoid was reported in literature. Furthermore, astaxanthin and canthaxanthin also had low binding energies and were reported to be used as an anti-aging agent and supplement in the treatment of Alzheimer's and Parkinson's disease, high cholesterol, strokes and cancer, respectively [55]. They were also tested

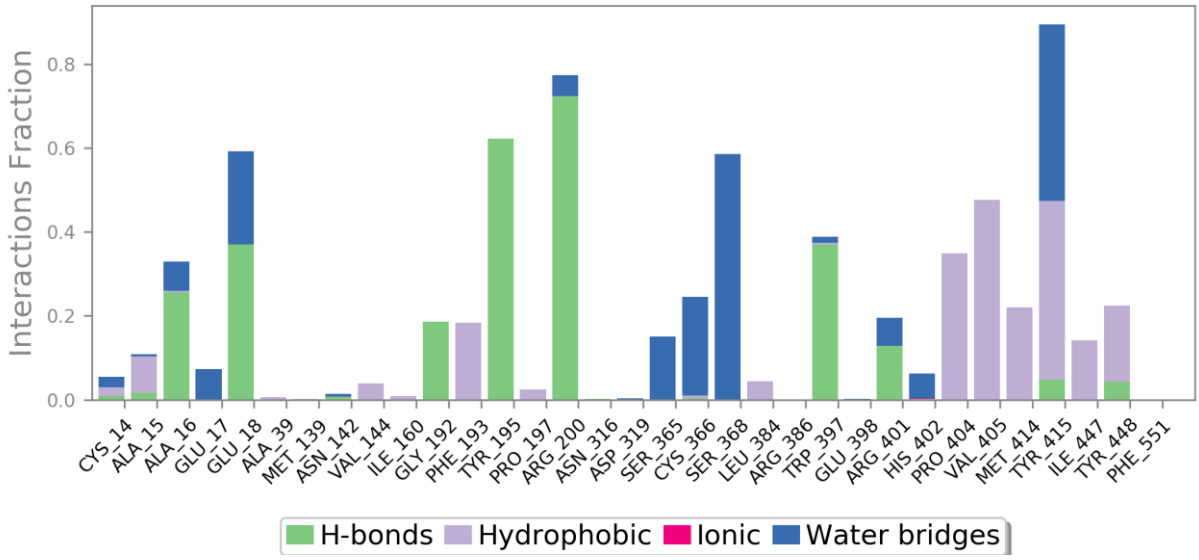

Figure 4. Interaction fractions for the protein–ligand complex observed during MD simulation for RNA-dependent RNA polymerase of Hepatitis C virus complexed with neoxanthin.

papain-like protease of SARS-CoV-2 and had the lowest binding energies (canthaxanthin, –9.4 kcal/mole and astaxanthin, –9.3 kcal/mole) [56]. In our study, such low binding energy scores were also found for these two important carotenoids as non-nucleoside RdRp inhibitors. The most promising apocarotenoids in this study was crocin, which is the primary pigment for the color of saffron herb. It was suggested to be considered as prophylactic agent during COVID-19 pandemic and some *in silico* studies exhibited its antiviral potential against SARS-CoV-2 [23], [50], [57]. For instance, crocin had strong binding affinities towards various drug targets of SARS-CoV-2, showing its multiple mechanisms of action, which is important for its effectiveness in the treatment of various mutants of SARS-CoV-2 [23]. This study pointed out the antiviral potential of crocin against HCV.

Considering the health benefits and various biological activities of carotenoids and apocarotenoids, as well as their potential for being dietary supplements, most of the molecules in this study, particularly the ones with low binding energies, can be used in the treatment and prevention of various viral diseases, including Hepatitis C, as antiviral agents and/or dietary supplements. In fact, further *in vitro* and *in vivo* studies are required to confirm the initial findings presented in this study.

4. Conclusion

Molecular docking screening of selected carotenoids and apocarotenoids revealed that the majority of them had relatively strong binding affinities to both palm and thumb domains of RdRp of HCV. In fact, some of them such as neoxanthin, crocin, canthaxanthin and cryptoflavin bound quite strongly to both domains. MD simulation for neoxanthin-RdRp complex confirmed that it was stable within the binding cavity of RdRp throughout 100 ns simulation process, indicating the inhibitory effect of neoxanthin on the drug target protein of HCV. Additionally, since carotenoids and apocarotenoids have been originally found in various plants and fruits, they can be considered as potential dietary supplement candidates. Thus, this study not only discovered antiviral drug candidates in treating Hepatitis C disease, but also opened the door for the development of carotenoid or apocarotenoid based dietary supplement candidates for the prevention and treatment of HCV. The availability of current literature with *in vitro* studies on bacterioruberin as anti-HCV candidate supported our findings that the compounds in this study with low binding energies can indeed become RdRp inhibitors for HCV. However, further studies are still essential to confirm the findings of our *in silico* study for the translation of this idea into clinic.

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Statement of Research and Publication Ethics

This study complies with research and publication ethics.

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