Therapeutic Effect of Sargassum Tenerrimum Derived Compounds Against SARS-CoV-2 Using Molecular Docking

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Abstract- *Corona virus has desolated the world and is the biggest of pandemics in history, without any vaccination or treatment the mortality and morbidity rates are extremely high. The present study was undertaken to identify the potent drug from marine algae Sargassum tenerrimum derived compounds Fucoxanthin and Phlorotannin-Eckol against SARS-CoV-2 and their application as antiviral agent and an effective drug like compound against SARS-CoV-2 using in-silico tools. The 3-dimensional structures of target proteins were retrieved from Protein Data Bank and 3D Structure of drug molecules were retrieved from PubChem database. The molecular docking studies were performed on AutoDock Vina on four different proteins associated with COVID-19. The docking result reveals that Sargassum tenerrimum derived fucoxanthin and phlorotannin were found to be more effective against the target proteins as confirmed through their docking score and binding affinity and can be used as a potent drug against corona virus. Molecular Docking analysis reveals that phlorotannin shows good binding affinity at the active site of target proteins when compared to fucoxanthin and can be used as a potential SARS-CoV-2 inhibitor.*

Keywords- COVID-19, Sargassum tenerrimum, Fucoxanthin, Phlorotannin, Molecular Docking

I. INTRODUCTION

Corona Virus

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infections associated respiratory disease – COVID-19 (2019-nCoV) has spread rapidly around the globe. The outbreak of COVID-19 is posing major challenges to the worldwide health systems. It is also believed to have far-reaching consequences on the global economy if the spread of the virus is not effectively controlled [1, 2]. All over the globe scientists and physicians are focusing their attention on evaluating the usefulness of already existing drugs for preventing entry, replication and multiplication of the virus in the host system [3]. Efforts are also underway that focus on identifying new drug molecules for the management of COVID-19 pandemic. There are two approaches in novel drug development for this virus based respiratory syndrome. One approach focuses on developing drug molecules that target the viral proteins and viral RNA. The other approach focuses on drugs that target the host cell proteins which facilitate viral entry, viral replication and those involved in viral multiplication.

Gordon have clearly stated that identification of host dependence factors mediating virus infection may provide key insights into effective molecular targets for developing broadly acting antiviral therapeutics against SARS -COV-2 and other deadly corona virus strains [4]. In this host protein target-based drug discovery approach, scientists often caution that host-directed drugs are more likely to do harm than therapies that target the virus directly. The reason cited by them are while hitting a host target there is a possibility of hitting a host function which would lead to increased safety risk. This argument is counteracted by the view that drugs that target the host proteins are a good choice as the virus is less likely to develop resistance as the host proteins are encoded in the human genome and not that of the virus. It is important to note here that developing antiviral for HIV, influenza and other diseases have led to the understanding that resistance is a major problem in developing antiviral [5]. Thus, targeting a human protein that's central in corona virus infections in general could lead to development of broader drug entities that could treat similar viral infections. Zhumla in their review Corona virus-drug discovery and therapeutic options have listed the Virus-based anti-CoV treatment options and Host-based anti-CoV treatment options. It is interesting to note that ACE2 receptor, membrane bound serine protease TMPRSS2 and Cathepsins B and L that was believed to be involved in viral entry into the lung airway epithelial cell adapted by SARS CoV 1 is also implicated as the viral entry route in

SARS CoV2 infection [6]. Hoffmann has demonstrated that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. They have also demonstrated that TMPRSS2 inhibitor approved for clinical use blocked entry and might constitute a treatment option for COVID -19 [7].

Literature searches on in-silico approach based drug identification for SARS CoV 2 resulted in many publications till date from the start of the pandemic in December, 2019. Review by Murgueitio discusses the application of virtual screening techniques for antiviral drug discovery [8]. Zhou using powerful network-based methodologies has suggested rapid identification of candidate re-purposable drugs and potential drug combinations targeting 2019-nCoV/SARS-CoV-2 [9]. Choudhary using in silico structure based virtual screening approaches and drug repurposing concept have identified inhibitors for SARS-CoV-2 cell entry. They had screened ligand from ligand libraries and known drugs for their ability to inhibit ACE2 receptor [10]. These studies highlight the importance of in silicon studies for rapid drug development for a pandemic like COVID 19. Studies reported after COVID 19 crisis, using in silico approaches by and large have focused on the inhibitory potential of known drugs, phyto-constituents and small molecules from ligand libraries on viral based proteins. Enmozhi have evaluated the potential of Andrographolide from Andrographis paniculata to inhibit the main protease of SARS -COV-2 (Mpro) through in silico studies such as molecular docking, target analysis, toxicity prediction and ADME prediction [11]. Rattis have shown that curcumin exhibited better potential inhibition against COVID-19 main protease active site and ACE2 [12].

Sargassum tenerrimum

The marine algal species are a good source of nutraceuticals compounds. Among marine organisms, marine algae are rich source of structurally diverse bioactive compounds with various biological activities. The diverse classes of seaweeds, edible brown seaweed is considered to be the most nutritious and possesses a range of compounds with biological properties including anti-oxidant, anti-diabetic, anti-cancer, anti-coagulant, and anti-bacterial, anti-proliferative. Seaweeds are excellent sources of bioactive compounds such as polyphenols, carotenoids and polysaccharides. These bioactive compounds can be applied in the functional food, pharmaceuticals and cosmetic products as they bring health benefits to consumers [13].

Fucoxanthin

Fucoxanthin is one of the most abundant carotenoids, especially in brown seaweeds and contributes about 10% of the total estimated production of carotenoids in nature. They are colourful pigments synthesized in plants, seaweeds and other photosynthetic organisms as well in some non-photosynthetic bacteria and are involved in photosynthesis, hormonal synthesis, photoprotection and photomorphogenesis. Fucoxanthin has a unique structure, where it contains an unusual allenic bond and a 5,6-monoepoxide in its molecule. However, different brown seaweed strains produce different compositions and profile of fucoxanthin. Thus, it is suspected that the pigments from *Sargassum tenerrimum* are rich in fucoxanthin. Fucoxanthin has anti-cancer, anti-oxidant, anti-viral and anti-obesity properties. Fucoxanthin is also a radical scavenger, whereby previous studies reported strong DPPH radical scavenging activity exhibited by fucoxanthin from various sources has been reported [14].

Phlorotannin

Brown seaweeds accumulate a variety of phloroglucinol based polyphenols (Phlorotannin), which are formed from the polymerization of phloroglucinol (1,3,5-trihydroxybenzene) monomer units resulting in compounds with different molecular weight (from 126KDa to 650KDa). Based on the monomer's linkage, phlorotannin can be classified into four sub classes: fuhalols and phlorethols (ether linkage), fucols (phenyl linkage), fucophlorethols (ether and phenyl linkage), and eckols and carmalols (dibenzodioxin linkage). Phlorotannin is present in many marine organisms, especially in brown sea weed, where its concentration is highly variable depending on the species and the geographic area. There are several biological activities have been attributed to the phlorotannin, such as anti-oxidant, anti-bacterial, anti-inflammatory and anti-allergic, contributing for the reputation of brown seaweed as a source of healthy food [15].

Docking studies help to analyze the interaction between the drug compound and target protein. Generally, *in-silico* approach is cost effective and additionally save time and energy. This current study was aimed to evaluate the therapeutic effect of Fucoxanthin and Phlorotannin bioactive constituents present abundantly in marine brown algae *Sargassum tenerrimum* against SARS- CoV-2 proteins ACE2, TMPRSS2, Cathepsins B and L using molecular docking approach and also to evaluate drug likeliness properties of the potent compound.

II. METHODOLOGY

The crystallographic structures of Angiotensin converting enzyme ACE2, TMPRSS2, Cathepsin B and Cathepsin L were retrieved from RCSB PDB (PDB ID: 1R4L, 7MEQ, 2IPP and 1ICF). The downloaded protein structures were prepared by using BIOVIA Discovery studio. All the hetatoms and water molecules were removed. Then, hydrogen bond structures were optimized by adding atoms in missing loops or side chains. Finally, the processed proteins were saved in ".pdb" format. The structures of Fucoxanthin and Phlorotannin ligands were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in ".sdf" format (Pubchem ID: 5281239 and 145937). Then by using PyMol files were converted from ".sdf" to ".pdb" format. AutoDock Vina, freely available software was used to perform molecular docking. The processed proteins were inserted in AutoDock vina tab in ".pdb" format. Then the ligand molecules were loaded in ".pdb" format. Now both target proteins and ligands were saved in ".pdbqt" format. The docking region was selected by forming a grid box region. Finally, command prompt was given and AutoDock Vina was performed and the results were analyzed. The structure of protein-ligand complex was made using the tool PyMol 2.4 (https://pymol.org/2/). The target protein and ligand molecule were imported in ".pdbqt" format into the docking workspace. Once the protein and ligand was imported the complex was constructed and saved in ".pdb" format. Using BIOVIA Discovery studio tool protein-ligand complex was visualized. The protein-ligand complex was loaded in ".pdb" format on the graphical window and the charges were added. The 2-dimensional and 3-dimensional structures of protein and ligand interaction were visualized and the hydrogen bonds interaction and bond distances were analyzed. Then the drug likeliness properties were analyzed by retrieving the "SMILES" from PubChem and uploading the "SMILES" in pkCSM analysis online tool.

III. RESULTS AND DISCUSSION

Docking scores of fucoxanthin and phlorotannin with target proteins

The molecular docking was performed for proteins ACE2, TMPRSS2, Cathepsin B and Cathepsin L against *Sargassum tenerrimum* derived compounds fucoxanthin and phlorotannin. The result outcomes from the docking of fucoxanthin and phlorotannin with SARS-CoV-2 target protein were shown in the table 1. The proteins of target were docked on the binding pocket with *Sargassum tenerrimum* derived fucoxanthin and phlorotannin.

Table 1.Docking scores of fucoxanthin and phlorotannin with target proteins

Protein	Binding Affinity (Kcal/mol)	
	Fucoxanthin	Phlorotannin
ACE2	-10.8	-9.5
TMPRSS2	-7.5	-8.1
Capathecin B	-8.1	-8.2
Capathecin L	-8.5	-9.5

Based on the minimal energy and negative value, the best docking score was selected. According to the docking result it was analyzed that fucoxanthin showed less binding affinity with proteins TMPRSS2, Cathepsin B and Cathepsin L compared to phlorotannin and phlorotannin showed less binding affinity with protein ACE2 compared to binding score of fucoxanthin. Both fucoxanthin and phlorotannin shows best docking score and strong interaction at the active sites towards target proteins. This result reveals that phlorotannin shows good binding affinity with maximum number of target proteins.

Visualization of protein-ligand interaction

The complex structure of protein-ligand interaction was first built using PyMol tool. Then, the constructed complex structure was imported into BIOVIA Discovery studio and protein-ligand interactions were analyzed. The 3D and 2D interaction of fucoxanthin and phlorotannin with the SARS-CoV-2 based proteins ACE2, TMPRSS2, Capathecin B and Capathecin L were shown in figure 1 and 2. Fucoxanthin with ACE2 formed two (2) conventional hydrogen bond interactions, H-Donor with amino acid Lys363 at bond distance 2.42343 Å and H-Acceptor with Asp269 at bond distance of 1.95285 Å. Fucoxanthin with ACE2 also formed four (4) Pi-Sigma hydrophobic interaction with amino acids His374, Phe274 and Trp349 (2) with bond distances 3.77459 Å, 3.90606 Å, 3.93472 Å and 3.5547 Å and also formed six (6) Pi-Alkyl hydrophobic interaction with amino acid residues His345, His378, Phe504, His505, Tyr510 and Tyr515 with 4.74019 Å, 4.07283 Å, 4.88397 Å, 4.50706 Å, 4.28289 Å and 5.10813 Å bond distances.

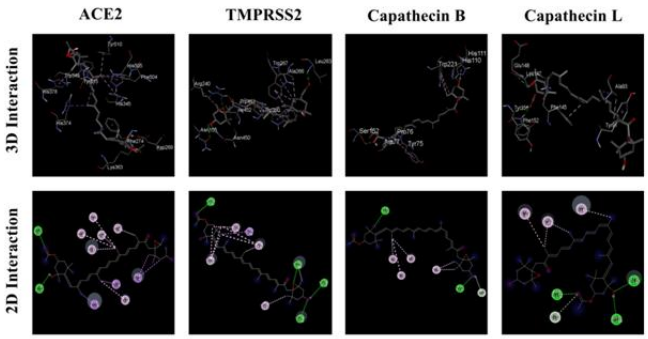

Figure.1 - 3D and 2D Interaction of Fucoxanthin with target proteins

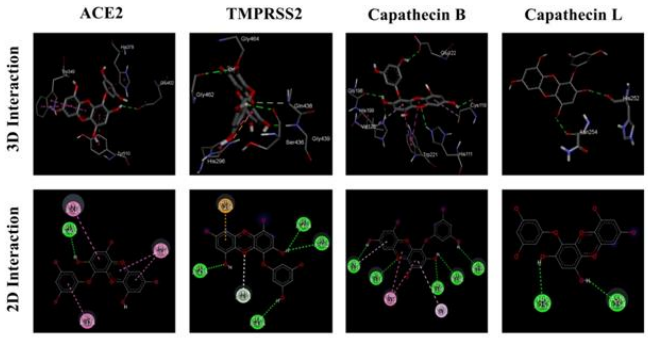

Figure.2 - 3D and 2D Interaction of Phlorotannin with target proteins

Fucoxanthin with TMPRSS2 formed four (4) conventional hydrogen bond interactions with Arg240, Leu263 and Asn450 as H-Donors and Asn155 as H-Acceptor with bond distances 2.15655 Å, 2.23533 Å, 2.65671 Å and 2.60795 Å respectively. Also, TMPRSS2 with fucoxanthin formed one (1) Pi-Sigma hydrophobic interaction with Trp380 at 3.90514 Å and nine (9) Pi-Alkyl hydrophobic interaction with Ala266, Trp267 (3), Trp453 (3), Ile452 and Arg240 with 4.40741 Å, 5.22772 Å, 5.42915 Å, 5.06571 Å, 5.29996 Å, 5.04979 Å, 5.45613 Å, 5.22772 Å and 4.55735 Å bond distances. Fucoxanthin with Capathecin B formed two (2) conventional hydrogen bond interactions with His110 and Ser152 with bond distances 2.10209 Å and 2.45056 Å and one (1) Pi-Donor hydrogen bond with His111 with 2.77883 bond distances. Furthermore, fucoxanthin with Capathecin B formed five (5) hydrophobic interactions as two (2) Alkyl form interactions with Ala77 and Pro76 at bond distances 4.26028 Å and 4.21059 Å and three (3) Pi-Alkyl form interactions with Trp221 (2) and Tyr75 at 5.00711 Å, 4.73429 Å and 5.25868 Å. Fucoxanthin with Capathecin L formed three (3) conventional hydrogen bond interactions with Lys147 and Phe152 as H-Donor at 1.9882 Å and 2.47359 Å and with Glu148 as H-Acceptor at 2.36335 Å. Other bonds include two (2) Alkyl hydrophobic interactions and two (2) Pi-Alkyl hydrophobic interactions with Ala93 (2), Tyr91 and Phe145 at bond distances 4.44279 Å, 3.60466 Å, 4.5807 Å and 3.94763 Å respectively. Phlorotannin with ACE2 formed one (1) H-Acceptor conventional hydrogen bond interaction with Glu402 at 2.86273 Å and four (4) Pi-Pi stacked hydrophobic bond interactions with Trp349 (2), His378 and Tyr510 at 4.54284 Å, 4.15123 Å, 4.30091 Å and 4.97525 Å bond distances. Phlorotannin with TMPRSS2 formed one (1) carbon hydrogen bond interaction with Gln438 at 3.56139 Å and four (4) conventional hydrogen bond with Gly439 (H-Donor) at 1.78946 Å and three (3) H-Acceptors with Ser436, Gly462 and Gly464 at bond distances 2.27186 Å, 2.36138 Å and 2.40117 Å respectively. Also phlorotannin with TMPRSS2 formed Pi-Cation electrostatic interaction and Pi-Pi T shaped hydrophobic interaction with His296 at 3.81896 Å and 4.78518 Å bond distances. Phlorotannin with Capathecin B formed two (2) H-Donor conventional hydrogen bond interaction with His111 and His199 at 2.54887 Å and 2.52843 Å distances and three (3) H-Acceptor conventional hydrogen bond interaction with Glu122, Gly198 and Cys119 with bond distances 2.47973 Å, 2.5937 Å and 2.31948 Å respectively. Additional interactions were two (2) Pi-Pi T shaped and two (2) Pi-Alkyl hydrophobic interactions with Trp221 (2), Val176 and Cys119 with distances 5.45286 Å, 5.24796 Å, 5.38291 Å and 5.08261 Å. Phlorotannin with Capathecin L formed two (2) H-Acceptor conventional hydrogen bond interactions with amino acid residues His252 and Asn254 with 2.39298 Å and 2.3514 Å bond distances. Based on the protein-ligand interaction results, phlorotannin shows more number of hydrogen bond interactions when compared to fucoxanthin. Previous studies have reported that the number of hydrogen bond interaction and the bond distances play a major role in influencing the ligand and protein interaction [16, 17]. The interaction of phlorotannin with target protein shows good binding affinity when compared to fucoxanthin. Therefore, phlorotannin was further analysed for drug likeliness property.

Drug likeliness properties analysis

By using pkCSM online server drug likeliness property analysis was done. Phlorotannin was analyzed for its drug property. The drug molecule was analyzed for Lipinski's Rule of Five and the results were tabulated in table 2. Lipinski's rule states that the hydrogen bond acceptor should be less than ten (<10), hydrogen bond donor should be less than five (<5), the molecular weight of the drug should be less than 500 Dalton, partition co-efficient (LogP) should be less than five (<5) and not more than one rule can be violated.

Table 2.Drug likeliness properties of Phlorotannin

Description	Value
Molecular Weight (Da)	372.3
Partition Co-efficient	2.5
H-Bond Acceptors	9
H-Bond Donors	6

The bioavailability and absorption of drug molecule was affected based on some physiochemical properties like molecular weight. The molecular weight of phlorotannin was 372.3 Da. The number of hydrogen bond acceptors and donors was nine (9) and six (6) and partition co-efficient was 2.5. According to Lipinski's rule of five the number of hydrogen bond donor should be less than 5 and for our drug phlorotannin the hydrogen bond donor was 6. Therefore, our drug molecule has violated one Lipinski's rule. But, violated of one Lipinski's rule was accepted. This could be easily interpreted that based on drug likeliness analysis phlorotannin can be a potent drug for the treatment of SAR-CoV-2.

IV. CONCLUSION

In the present *in-silico* study, we have effectively elucidated the therapeutic effect of *Sargassum tenerrimum* derived fucoxanthin and phlorotannin against SAR-CoV-2 associated proteins. Among the two compounds phlorotannin shows good binding with the target proteins when compared to fucoxanthin. Also, phlorotannin satisfies Lipinski's rule of five with one violation. Phlorotannin may act as a potent drug for the treatment of COVID-19 by inhibiting SAR-CoV-2 associated proteins. This study may open new avenue for the discovery of novel inhibitors targeting the SAR-CoV-2 proteins for the treatment of COVID-19.

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