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Investigating binding potential of carotenoid pathway bioactive molecules for ACE2 receptor of SARS-CoV-2: Possibility of a saffron based remedy for novel coronavirus!

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ABSTRACT

Purpose: Given the rising number of novel coronavirus named SARS-CoV-2 (COVID-19) cases, the purpose of the present study was to explore saffron bioactive compounds against COVID-19 since saffron is used in fever, bronchitis, cold, respiratory disorders and is recognized for its anti-inflammatory, antioxidant and immunomodulatory effects. **Research method:** COVID-19 engages the host cell surface receptor angiotensin-converting enzyme 2 (ACE2) through its spike protein receptor-binding domain (RBD). The idea was to check atomistic interaction of these bioactive molecules with ACE2 for obstructing its interaction with RBD, in order to screen and assess the likelihood of these molecules for drug development. Based on ligands' molecular weight, we chose smaller bioactive molecules (picrocrocin, safranal, lutein) for interaction with cell ACE2 of the host. **Findings:** Flexible molecular docking followed by atomic level interaction study indicated that lutein and picrocrocin form various interactions with different amino acid residues of ACE2**.** In depth analysis revealed that these interactions with the majority of the residues of ACE2 could be crucial for RBD binding and, therefore, can disrupt the interaction between RBD and ACE2. The study provides a hit for further analysis using *in vitro*, animal models and clinical studies. **Limitations:** In this study dynamic approaches such as molecular dynamics and semiemperical quantum mechanical (SQM) methods have not been used. **Originality/Value:** By preventing the interaction of RBD with ACE2, lutein and picrocrocin may prove helpful in the development of therapeutics for COVID-19 management.

INTRODUCTION

Humanity has faced many pandemics like Black Plague, Smallpox, Tuberculosis, Malaria, Spanish Flu, and plant-based remedies have been and shall continue to be used to treat these diseases (Garcia, 2020). The respiratory pandemic COVID-19 (Coronavirus disease 2019) is the third epidemic of zoonotic origin to occur in the present century. COVID-19 was declared as a pandemic by WHO on 12th March 2020 and its causative agent was named as SARS-CoV-2 (severe acute respiratory syndrome corona virus 2) (Jiang et al., 2020). Based on information shared on World Health Organization (WHO) and Centres for Disease Control and Prevention, USA (CDC, USA) portals, people suffering from SARS-CoV-2 may possess fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, the new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhoea, and some patients may need hospitalization (Husaini et al., 2021). The incubation period of COVID-19 ranges from 1 to 14 days, with an average of 5-6 days in most patients. In the absence of effective allopathic drugs and until the free and mass availability of effective and affordable vaccines, WHO guidelines on social distancing, wearing masks, frequent hand washing, etc. are an important management strategy. There are promising results on vaccine development; however, their free access, affordability, and efficacy against the newer variants of SARS-CoV are causes of concern.

Many research groups have turned to repurpose other drugs due to the long time-frames that usually imply finding a good candidate (Garcia, 2020). Antimalarials like chloroquine, hydroxychloroquine were tested against COVID19 (Colson et al., 2020; Mitjà et al., 2020; Singh et al., 2020). Other plant-based antimalarials, artemisinin and an elixir based on *Artemisia annua* extract, “covid-organics” was distributed as a cure against COVID19 in many African countries (Garcia, 2020). Carotenoids and the compounds generated from the oxidative cleavage of their double bonds (apocarotenoids) play a protective function as scavengers of oxygen radicals and enhance the immune system's function. COVID-19 has been shown to engage the host cell ACE2 through its spike protein receptor-binding domain (RBD) (Adedeji et al., 2013; Prabakaran et al., 2006). The spike protein (S) of SARS-coronavirus (SARS-CoV) interacts with its cellular receptor, Angiotensin Converting Enzyme 2 (ACE2) via Receptor Binding Motif present in receptor-binding domain (RBD). Saffron is a high value low volume spice that grows throughout Mediterranean Europe and Western Asia between 10° west and 80° east longitudes and 30 to 50° north latitudes, and has countless biological properties like anticancer, antimutagenic, and antioxidant (Husaini, 2014). We recently showed that it could be useful in the management of covid related abnormalities, including stress, depression, anxiety, panic, sleep disturbances, and post-traumatic stress disorder associated with long-term mental health management during the pandemic (Husaini et al., 2021). Saffron bioactive compounds are generated through apo-carotenoid pathway, which is a branch of the carotenoid biosynthesis pathway. These pathways produce medicinally important compounds like β-carotene, lycopene, lutein, crocin, picrocrocin and safranal. We wanted to check the atomic scale interaction of 3 bioactive molecules of the carotenoid and apo-carotenoid pathway with ACE2 to block its interaction with RBD. We used flexible mode molecular docking approach to screen these bioactive molecules for interaction with ACE2 and found some candidates for further analysis using in vitro, animal model, and clinical studies for drug development.

Till 31st May, nearly 169.60 million people were infected worldwide, out of which about 3.53 million lost their lives (WHO, 2021). It does not differentiate between nations on the basis of ethinicity or economic wellbeing as the maximum number of deaths

(approx.32916501) were reported in the USA, and the second maximum (approx. 27894800) were in India, irrespective of the contrasting development indices between these countries (WHO, 2021). Most people infected with coronavirus experience mild to moderate respiratory illness and recover without any special treatment. However, older people and those with underlying medical problems are likely to develop severe illness and may even progress to organ failure (Nikhat & Fazil, 2020). Poor diet quality has been associated with depressed immune function and increased susceptibility to infection (Brahmbhatt, 2020). There are many preclinical immune modulator based treatments currently in development like AT-100 (recombinant human surfactant protein D, rhSP-D reduces inflammation and modulates lung immune response), BPI-002 (small molecule as a potent T cell co-stimulator), 7HP-349 (small-molecule integrin activator as an oral adjuvant), Brilacidin (defensin mimetic candidate), PRTX-007 (oral small molecules which activate toll-like receptor 7) (Florindo et al., 2020).

SARS-CoV-2 is internalized by the cell via membrane fusion or endocytosis. The best-known mechanism of SARS-CoV-2 cellular infection is mediated by the cell surface receptor angiotensin-converting enzyme 2 (ACE2) (Hoffmann et al., 2020; Malik et al., 2020; Petitprez et al., 2020; Zhou et al., 2020). The SARS-CoV-2 spike binds to ACE2 via its receptor-binding domain (RBD). RNA is released, and viral proteins are synthesized through translation. A replication complex is formed using these proteins to create additional RNA, which then assemble with the viral proteins into new viruses and are released (Florindo et al., 2020). As this ACE2 receptor is predominantly present in the human epithelia of lung and small intestine, the SARS-CoV-2 is more likely to infect the respiratory and gastrointestinal tracts (Chen et al., 2020; Hoffmann et al., 2020; Malik et al., 2020; Zhou et al., 2020). The presence of ACE2 receptor on the endothelium, glial cells, and neurons may have a role to play in the partial or altered sense of smell too, as this may help SARS-CoV-2 entry into the brain through cerebral circulation (Xia & Lazartigues, 2008). Therefore, one of the viable strategies could be to check the interaction of carotenoid pathway bioactive molecules with ACE2 in order to hinder the interaction of the virus with it, thereby blocking its entry in the host cells. Herein we focus on the binding energy estimation of some vital bioactive compounds of carotenoid and apocarotenoid pathways (Lutein, Picrocrocin, and Safranal) for their binding affinity with ACE2 receptor.

MATERIALS AND METHODS

We retrieved the crystal structure of angiotensin-converting enzyme 2 or ACE2 (PDB ID: 1R42) from Protein Data Bank (PDB; Towler et al., 2004). The redundant items from 1R42 were removed using UCSF Chimera version 1.11.2 as per the widely followed protocol (Pettersen et al., 2004; Shankaran et al., 2017). Keeping in view that ACE2 is zinc metalloenzyme the heteroatom zinc was kept intact during the protein preparation (Ganai, 2021; Vickers et al., 2002). The crystal structure was further examined for missing residues using the Maestro (free version), and no residues were found to be missing (Mir et al., 2020). Following this, the amino acid residues of Angiotensin-converting enzyme 2 crystal structure were renumbered as per UniProt numbering using the structure editing option of the UCSF Chimera to avoid confusion among non-bioinformaticians (Pettersen et al., 2004).

We retrieved the coordinates of lutein, safranal, and picrocrocin from PubChem bearing PubChem CID: 5281243, 61041, and 130796, respectively (Kim et al., 2018). All the three ligands were converted into acceptable docking format through UCSF Chimera software (Pettersen et al., 2004).

We used GalaxyDockWEB for molecular docking. The prepared PDB file of receptor and the prepared ligand files were given as an input. From a given protein and ligand structure, this protein-ligand docking program (GalaxyDock) generates receptor-ligand complexes. During docking simulation, GalaxyDock treats receptor as rigid and ligand as flexible (Shin et al., 2013). The residues of ACE2 that have been proven to interact with receptor binding domain (RBD) of spike protein of SARS-CoV-2 were specified prior to docking to direct the ligands towards these residues. GalaxyDock generates 50 models for each receptor-ligand input (Shin et al., 2013). These models are ranked by their calculated binding affinity values, which are based on GalaxyDock BP2 score (Baek et al., 2017).

We used two separate methods for generating possible interactions between these ligands and ACE2. Protein–ligand interaction profiler (PLIP), which detects non-covalent interactions between ligand and protein in docked state was used (Ganai, 2021). The docked complex was used as input, and the interaction was finally generated by selecting the ligand molecule (Salentin et al., 2015). Further, the protein-ligand interactions were also generated by another program known as LIGPLOT. Hydrogen bonds and hydrophobic interactions between ligand and protein are generated by this program (Wallace et al., 1995).

RESULTS

Among the bioactive molecules, lutein and picrocrocin showed better binding affinity compared to safranal. While lutein-ACE2 showed a binding affinity value of -17.391, picrocrocin-ACE2, and safranal-ACE2 manifested these values as -15.837 and -12.235, respectively (Fig. 1 a).

Although the GalaxyDock generates 50 models for each protein and ligand input, only the top model based on a more negative value of binding affinity has been selected in each case. A recent study has shown that SARS-CoV-2 RBD interacts with ACE2 through hydrogen bonds and salt bridges. Aspartate 30 of ACE2 forms 2 salt bridges and 1 hydrogen bond with RBD of defined virus (Lan et al., 2020). Further, among the contact residues Lys 31, His 34, Asp 38, Glu 35, 37, Gln 42, Tyr 41, 83, Lys 353, Gly 354, and Arg 393 are prominent (Lan et al., 2020). Docking followed by interaction profile study indicated that lutein forms hydrogen bonding interaction with Asp 30, Lys 31, Asp 38 and His 34 (Fig. 1 b). LIGPLOT data of lutein-ACE2 revealed hydrophobic interactions with Lys 31, Glu 35, Asp 38, Asp 30, His 34, Glu 37, Arg 393, Gly 354, and Lys 353 (Fig. 1 c).

Thus it is evident that lutein forms interactions with the majority of the residues of ACE2 crucial for RBD binding. This suggests that lutein may prove as a potential molecule for COVID-19 by preventing the interaction of RBD with ACE2. Picrocrocin also targeted one residue having significance in RBD-ACE2 interaction (ARG 393). Picrocrocin also targeted certain residues lying adjacent or in the vicinity of RBD-ACE2 interacting residues. For instance, through hydrophobic interaction, picrocrocin interacts with Phe 40, the residue close to RBD-ACE2 interacting residues such as Phe 41, Asp 38, and Gln 42. Moreover, picrocrocin also targets Asp 350, the residue close to Lys 353 (involved in RBD-ACE2 binding) (Fig. 1 d). Thus picrocrocin interacts with multiple residues, but these residues except (Arg 393) are not directly involved in the interaction between RBD-ACE2.

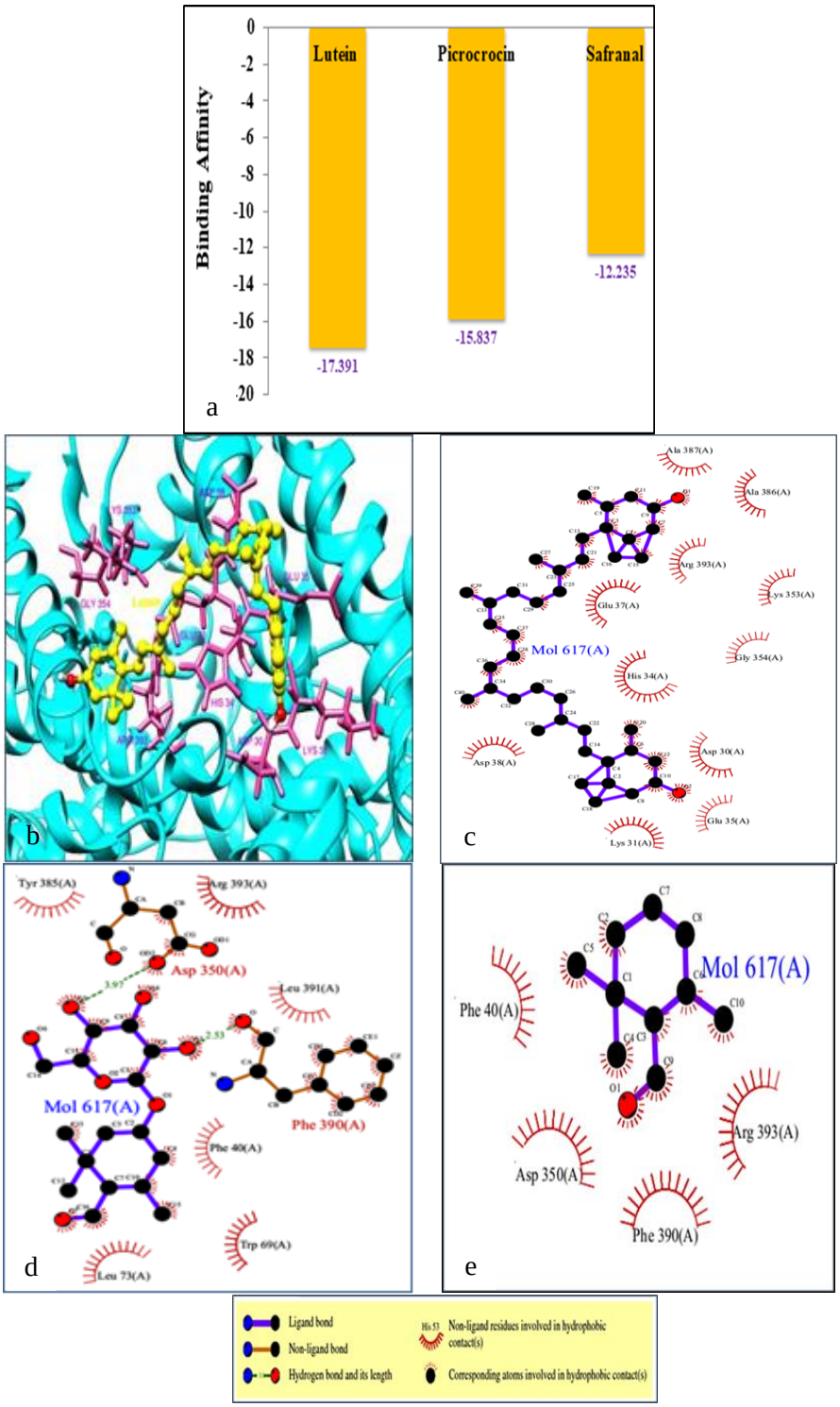

Fig. 1. Ligand -receptor interaction profile. a) Binding affinity of bioactive molecules lutein, picrocrocin and safranal against ACE2; b-c) Lutein (in yellow) in docked state with ACE2 (in cyan) and the contact residues: b) indicates the rendered image of defined docked complex wherein ligand lutein is shown in yellow colour and the residues of ACE2 which have been proven to be interacting are coloured as hot pink; c) specifies the residues of ACE2 making contact with lutein, wherein 11 residues of ACE2 form hydrophobic contacts with lutein; d) Interaction profile of ACE2-picrocrocin docked complex: Picrocrocin shows hydrogen bonding interaction with Asp 350 and Phe 390, and shows hydrophobic interactions with Tyr 385, Arg 393, Leu 73, Trp 69, Leu 391 and Phe 40; e) Interaction profile of safranal in complex state with ACE2: Safranal shows hydrophobic contacts with Arg 393, Phe 390, Phe 40 and Asp 350. For details kindly refer key provided at the bottom of the figure in yellow background.

However, certain ACE2 residues involved in RBD interaction are spaced close to residues displaying interactions with picrocrocin. Thus there is a possibility that picrocrocin may interfere RBD-ACE2 binding by inducing changes in the ACE2 structure. The binding affinity of safranal, from the docking study, proved to be relatively lesser (Fig. 1 e). Lutein and picrocrocin molecules may help disrupt the interaction between RBD and ACE2 and are potential molecules for further studies.

DISCUSSION

Saffron (*Crocus sativus* L.) is the dried orange-red trifid stigma of a perennial bulbous plant *Crocus sativus* L., a triploid male-sterile plant flowering in autumn, one of the costliest culinary spice of the world (Kafi et al., 2018). The introduction and popularization of organic saffron kitchen gardens have been advocated for health-promoting benefits and boosting the immunity of children and household members (Husaini & Wani, 2020). Both traditional and experimental evidence suggests the possible therapeutic effect of saffron and its constituents on respiratory disorders, which can be beneficial in the current pandemic (Husaini et al., 2021). Ancient Iranian physician Avicenna has stated that saffron oil can facilitate breath and strengthen the respiratory organs (Boskabady & Aslani, 2006; Boskabady et al., 2019; Mokhtari-Zaer et al., 2015). Saffron has been used in traditional medicine for the treatment of fever, bronchitis, cold, pertussis, asthma, and improvement of respiratory function. It is power-packed with B vitamins, vitamin C, carotenoids, and phytochemicals, which are believed to boost the immune response (Husaini et al., 2021). Bioactive constituents of saffron can affect both cellular and humoral immunity functions (Zeinali et al., 2019). (Bukhari et al., 2015) evaluated the antioxidant potential of saffron in normal human bronchial epithelial cells (NHBE) and the anti-inflammatory potential of safranal in a murine model of asthma. In bronchial epithelial cells, safranal significantly reduced oxidative stress via iNOS reduction and prevented apoptosis in these cells. This safranal mediated iNOS inhibition attenuated asthmatic features in the murine model of allergic asthma.

Umifenovir is considered for COVID-19 treatment in combination with protease inhibitors (Baig et al., 2020; Kadam & Wilson, 2017). It has been shown to inhibit SARS entry into target cells by disturbing ACE2 protein interaction under in vitro, further inhibiting fusion of viral envelope (Boriskin et al., 2008), also being effective in vivo against H1N1 (Liu et al., 2013), and influenza A viruses (Shi et al., 2007). Hydroxychloroquine sulfate and chloroquine phosphate are anti-malaria drugs shown to inhibit the terminal glycosylation of ACE2 in vitro against SARS-CoV (Vincent et al., 2005). This indicates that it can block SARS-CoV-2 infection by preventing the fusion of the virus with the cell membrane. Furthermore, hydroxychloroquine sulfate has been shown to affect immune cell activation and against lupus erythematosus (Alunno et al., 2019), which indicates its role in host immune response modulation during SARS-CoV-2 infection (Bao et al., 2020; Devaux et al., 2020; Hu et al., 2020).

Based on ligands' molecular weight, we chose smaller bioactive molecules (picrocrocin, safranal and lutein) for screening against ACE2. Small molecule docking with surface receptor angiotensin-converting enzyme 2 (ACE2) as of the therapeutic target has shown promising results for lutein and picrocrocin. We opine that these are good candidates for further analysis, using *in vitro*, animal model, and other studies and hypothesize that saffron may prove useful in development of therapeutics for COVID-19 or as an adjuvant to such drugs. The present study supports our views expressed at length in a recent review on the role of saffron constituents for the treatment of cardiovascular, central nervous system, and

respiratory disorders as well as their possible role in the overall management of COVID-19 (Husaini et al., 2021). These bioactive molecules could be useful in the symptomatic treatment of fever, bronchitis, cold, asthma, alveolar hypoxia, atherosclerosis, etc., as well as neuropsychiatric problems like depression, anxiety, panic, sleep disturbances and post-traumatic stress disorder associated with the long-term mental health management during the pandemic.

CONCLUSION

The pandemic began in Wuhan, China, in December 2019, took the world by storm and has played havoc in the health and economic sectors. The rise in the global demand for effective, affordable vaccines, therapeutics, and antiviral drugs caused a shift towards exploring options available in traditional medicines, especially immune-boosting ethno-botanically significant natural products. Saffron is used in fever, bronchitis, cold, respiratory disorders and is recognized for its anti-inflammatory, antioxidant and immunomodulatory effects. Carotenoid and apo-carotenoid biosynthesis pathways are good sources of compounds like β-carotene, lycopene, lutein, crocin, picrocrocin and safranal. In the present study, the docking interaction of lutein and picrocrocin with ACE2 for blocking its interaction with RBD shows the likelihood of their role in drug development against COVID-19 and as potential candidates for further analysis using in vitro, animal model, and clinical studies on drug development. The purpose of the present study is not to present these bioactive compounds as a solution to COVID-19 but only to explore their potential as an adjuvant to future drug formulations against SARS-CoV-2. The study could particularly be of interest to the drug development industry.

Abbreviations

COVID-19 (Coronavirus disease 2019); WHO (World Health Organisation); SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2); Centres for Disease Control and Prevention (CDC); ACE2 (angiotensin-converting enzyme 2); RBD (receptor-binding domain); PDB (Protein Data Bank)

Conflict of interests

There is no conflict of interest to declare

Authors' contributions

AMH conceived, designed, and wrote the article; SAG did atomistic analysis, created figures, and wrote the methodology.

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