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Study of Naturally-derived Biomolecules as Therapeutics against SARS-CoV-2 Viral Spike Protein

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PK, UC and MY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SP, ZM and SM helped in managing the literature data and writing the author MS. Authors SAK and MS managed the graphical presentation for the study. All authors read and approved the final manuscript.

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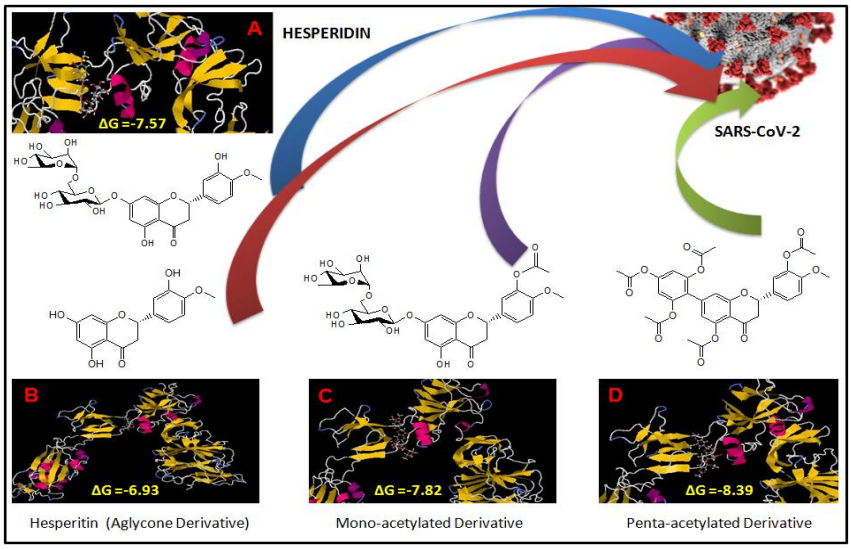
ABSTRACT

The SARS-CoV2 virus, the causative agent for COVID-19 disease has to lead to more than 3.1 million deaths and crossed 146 million infections worldwide so far. Although vaccines development and emergency authorization has been approved by several governments, there has been great concern about its side effects for the long term and its effectiveness against new mutated strains. A resurgence of COVID-19 or related disease can be catastrophic. There is an urgent need to look for effective antiviral agents for many coronavirus strains with minimum side-effects, and maximum efficacy globally. Several, naturally-derived biomolecules have proved their excellent effect on several infectious diseases in a multi-mode fashion by targeting several pathways as well as increasing efficacy with high safety profile. Integrate computational prediction design was used in the study to examine the pharmacology of bioactive compounds of natural origin against SARS-CoV2 spike protein. Keeping these facts we have computationally examined 16 naturally occurring compounds using to evaluate their effectiveness against the SARS-CoV2 virus using the molecular docking technique. Hesperidin derivatives are known to ameliorate diabetes, co-morbidity for coronavirus, as well as help in preventing post coronavirus complications. We found the binding free energy of Hesperidin with spike protein to be -7.57 kcal/mol, the aglycone derivative to be -6.93 kcal/mol, hesperidin monoacetyl derivative to be -7.82 kcal/mol, and hesperidin pentaacetyl derivative to be -8.39 kcal/mol. Our findings revealed that acetylated derivatives of hesperidin showed significant improved remarked binding affinity while aglycone derivative hesperetin showed a decrease in binding affinity. Our studies give a new direction where natural bioactive compounds and their derivatives can be modulated and used after clinical trials to effectively inhibit coronavirus infection as well as diabetes simultaneously with a high safety profile.

Graphical Abstract



Keywords: Active biomolecules; hesperidin; SARS-CoV2; molecular docking.

ABBREVIATIONS

ACE : Angiotensin-Converting Enzyme.
I-TASSER : Iterative Threading Assembly Refinement
mTOR : Mechanistic Target of Rapamycin
SARS-CoV2 : Severe Acute Respiratory Syndrome Coronavirus 2
SEC : Size-Exclusion Chromatography
SPR : Surface Plasmon Resonance

1. INTRODUCTION

SARS-CoV-2 belongs to the Coronaviruses family of *Coronaviridiae*, order of *Nidovirales* originated in Wuhan, Hubei Province of Republic

of China in December 2019 [1] and recognized as crown-spikes bearing, single-strand RNA viruses possessing a helical nucleocapsid [2], major known to cause acute and chronic respiratory, enteric, and central nervous system diseases in animals and humans [3]. So far, the globally infected population crossed 146 million with 3.1 million deaths [4]. In India, till now infected population reached 16.9 million with 0.19 million deaths, and additionally, the second COVID wave is appeared with a very high spreading rate [4] and the situation has caused tremendous damage to human life and the economy. Initiation of the infection takes place when the virus enters the human body where viral spike protein associates with the ACE2 protein [5] and switches on the downstream pathways like mTOR pathways [6]. Due to the high infectivity and severity of COVID-19 infections, many pharmaceutical companies worldwide took a leading role and invested in vaccine and medicine development. Many governments have been approved emergency authorization use of vaccines. The fundamental drawback is that we do not know for sure yet how long these vaccines protect us [7]. They may not be effective in the long run as viruses are mutating and they may not be effective for the new variants [8,9]. Recently, few countries have made vaccinations to combat COVID-19, but unfortunately, their effectiveness, as well as side-effects, are common such as injected site, tiredness, headache, muscle pain [10-12], questioned by scientists so far. As of April 2021, at least eleven different vaccines across three platforms have been rolled out in many countries. Vulnerable populations in all countries are the highest priority for vaccination [13]. Also, providing vaccine to a large population is a cumbersome task and manufacturing of the vaccine may take an unusually long time [14,15].

The emergences of structural genomics have paved strong considerations to develop novel drugs targets. In the present era, computer-based approaches have been utilized to build new possible lead compounds to combat several infectious diseases. In this regard, structure-based drug design is considered one of the most innovative and powerful approaches in drug design and development. Recent advances in computational methods for lead discovery include various commercially available software for de novo drug design, iterative design, selectivity discrimination, and estimation of ligand-binding affinities [16]. Furthermore, molecular dynamics simulation of multiple copies of molecular building blocks found capabilities to assess the molecular dynamics as well as inhibiting affinity towards targeted biological activities [17]. The multiscale biomolecular simulations are useful in drug design and development. Generally, the molecular dynamic (MD) simulation approach can be utilized to identify the drug binding sites on the target protein as several simple tools [18]. Most of the approved antiviral drugs are somehow directly or indirectly associated with side effects, which eventually raise the need for the development of antivirals based on natural phytochemicals [19]. Some of the viral diseases can be cured by approved antiviral drugs, but others still do not have any vaccines or drugs available. The natural compounds have a very high safety and compatibility profile having excellent biological properties with diversified applications [20]. Several studies revealed that plant-derived biomolecules are associated with protective proficiency [18-21]. Ghildiyal et al. described exhaustively that the development of antivirals is shifting towards plant-derived products as they are less toxic and has less chance to develop resistance [22]. The epidemic of viral diseases is a global concern, mandating an urgent need for the most promising antivirals. In addition, natural compounds can act in a multi-mechanistic manner by targeting several different pathways involved in viral regulation [23]. Post coronavirus complications and long term COVID-19 effects like onset of diabetes in otherwise normal patients [24] also need to be considered to completely cure coronavirus and related diseases. Herein we have performed molecular docking study by using Swissdock and Autodock Viena to examine the efficacy of 16 natural compounds and the derivatives in combating this unusual viral problem.

2. METHODS AND MATERIALS

Ligands were prepared by downloading the structures from PubChem/PubMed database and drawn using ChemDraw ultra pro 12.0.2 software. The I-TASSER software was used to assess intercalation through computational model spike protein for COVID-19. In the case of swiss-dock, the computationally modelled spike protein and .mol2 format of ligands were imported on the Swiss-dock server platform to predict binding site alteration and binding affinity values. While for Autodock-Vina, the pdbqt files of both proteins and ligands were made using MGL-Tools and by taking binding side prediction from Swiss-dock, grid box was prepared. Once

the grid box was set, ligand's and protein's pdbqt files were submitted for autodock study for further evaluation.

3. RESULTS AND DISCUSSION

Active compound that is suitable for constructing the model will be selected in the pharmacophore recognition process. Then, conformation analysis is used to find the binding conformation of the molecule, and to determine the pharmacophore. In recent years, with the development of compound databases and computer technology, the virtual screening of databases using the pharmacophore model has been widely used and has become one of the important systems for the identification and discovery of lead drugs [25]. Additionally, the identification of new probable drug can be carried out using the determination of pharmacophores with two approaches, a) if the target structure is available, the possible pharmacophore structure can be inferred by analyzing the action mode of receptor and drug molecule; and b) when the structure of the target is unknown or the action mechanism is still unclear, a series of compounds will be studied for pharmacophores, and information on some groups that play a key role in the activity of compound [26]. Traditionally, identifying active compounds from natural products rely on the experimental evaluation of natural products in a set of biological assays available. The broader coverage of chemical space of natural compounds as compared to synthetic molecules gives an advantage to the former to identify novel structural classes. For a long time, around 80% of drugs found their sources directly in natural products or compounds inspired by natural sources. Natural products have a rich history in drugs with therapeutic importance [27]. It has been reported that, since 1994, 50% of the approved drugs have been rooted based on natural phytoconstituents [28]. Despite the fact that this approach has given rise to the successful identification of lead compounds and approved drugs discussed above, it is anticipated that combining computational approaches with experimental-based natural product research will enhance the success rate. In this regard, the integration of *in silico* studies with herbal medicines has been gained popularity globally [29] using the synergy between well-established drug discovery approaches such as virtual screening and combinatorial chemistry.

The binding affinities are reported in Table 1, whereas Table 2 demonstrates the binding affinity of the derivatives of hesperidin. The binding energy (ΔG) values range from -6.72 kcal/mol for Myricetin to -8.27 kcal/mol for silibinin in bioactive natural compounds. The representative Autodock result for Hesperidin and spike protein association is shown in Fig. 1. The MD simulations study was done using the GROMACS platform. The simulations were run for the nanosecond timescale. The simulations were done to examine the stability of the protein complexes. Fig. 2 shows the binding affinity of sixteen targeted bioactive natural compounds with the different biomolecules as ligands. The MD simulation results for the protein-ligand complex with hesperidin and its derivatives are shown in Fig. 3.

Hesperidin is selected further for generating derivatives and docking studies. A considerable good binding affinity was observed for a few of the targeted natural compounds against the COVID-19 spike protein. Acetylation, easy to handle and cope with the better yield in synthesis, is an organic esterification reaction with the introduction of acetyl functional group into hydroxyl substitution [30]. The acetyl moiety is an active site of a variety of organic compounds as well as drugs including acetylcysteine, acetyl-CoA and acetaminophen, acetylsalicylic acid respectively [31]. So, we further analyzed the effectiveness of the acetylated hesperidin.

Table 1. Binding affinity of selected bioactive natural compounds with different ligands

SN	Ligands	ΔG (Kcal/mol)	SN	Ligands	ΔG (Kcal/mol)
1.	1,9-Dimethyl-8-azaxanthin	-6.73	9.	Myricetin	-6.72
2	Alpha Copaene	-6.78	10.	Oridonin	-7.66
3.	beta-Farnesene	-6.83	11.	Rubijervine acetate	-7.46
4.	Excavatolide M	-7.31	12.	Schisantherin A	-8.01
5.	Geniposide	-7.20	13.	Silibinin	-8.27
6.	Hesperidin	-7.57	14.	Silymarin	-7.52
7.	Isolinoleic acid	-7.58	15.	Spirosolane	-7.70
8.	Methyl rosmarinate	-7.70	16.	Withanolide D	-8.05

Table 2. Binding affinity of Hesperidin and its derivatives

S. No.	Ligands	ΔG (Kcal/mol)
1	Hesperidin	-7.57
2.	Mono-acetylated Hesperidin derivative	-7.82
3.	Penta-acetylated Hesperidin derivative	-8.39
4.	Hesperetin	-6.93

Fig. 1. Representative auto dock Vina results for Hesperidin-Spike protein interaction

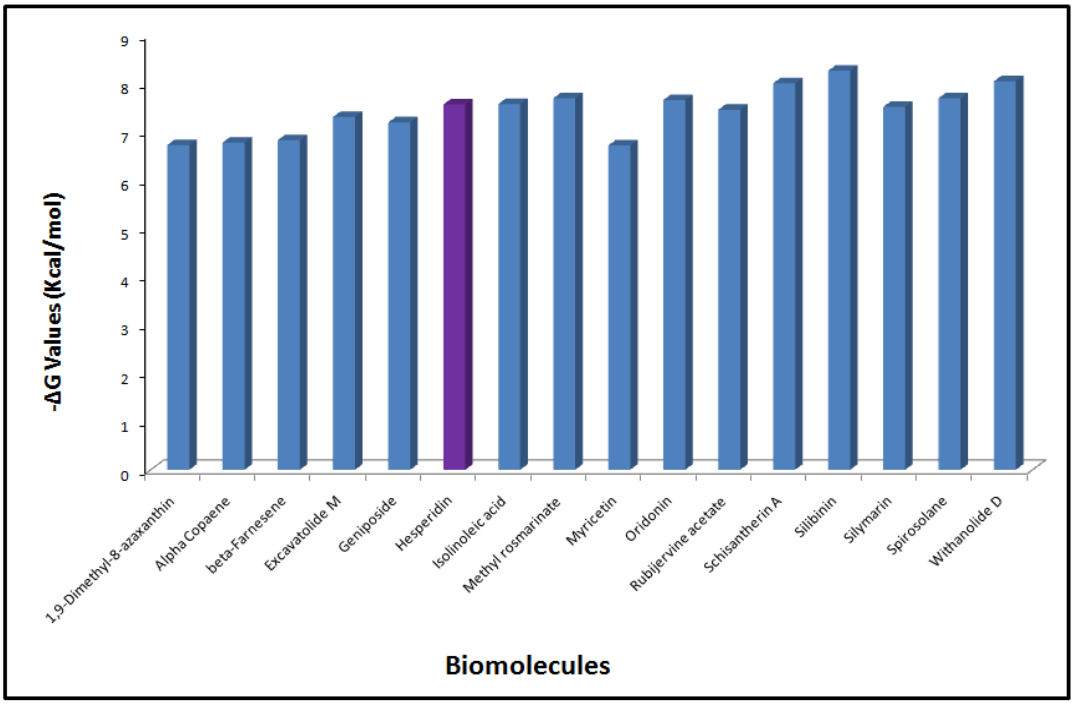


Fig. 2. The ligand-protein interaction results for 16 natural compounds (Hespiridin was selected for further study)

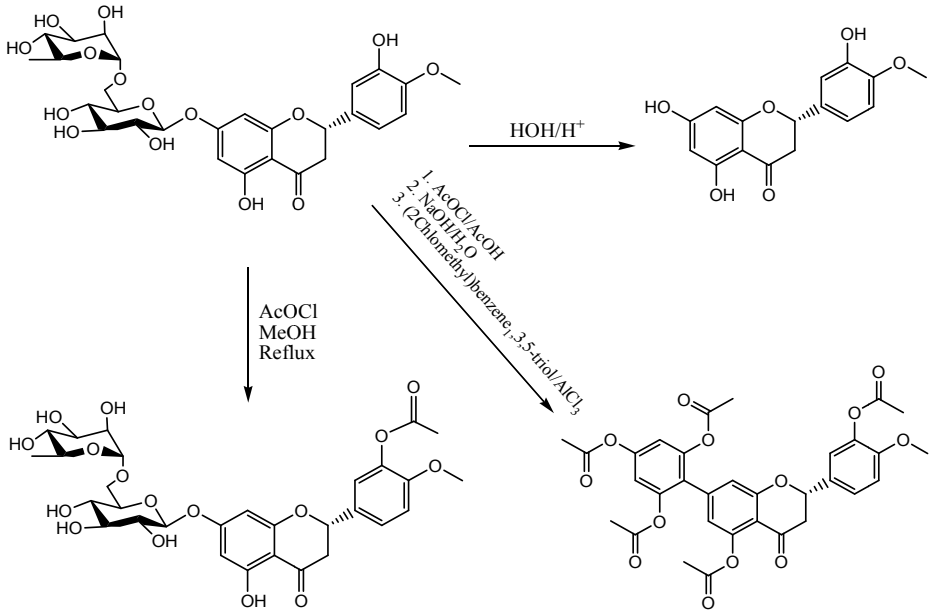


Fig. 3. Plausible scheme for synthesis of hesperidin derivatives

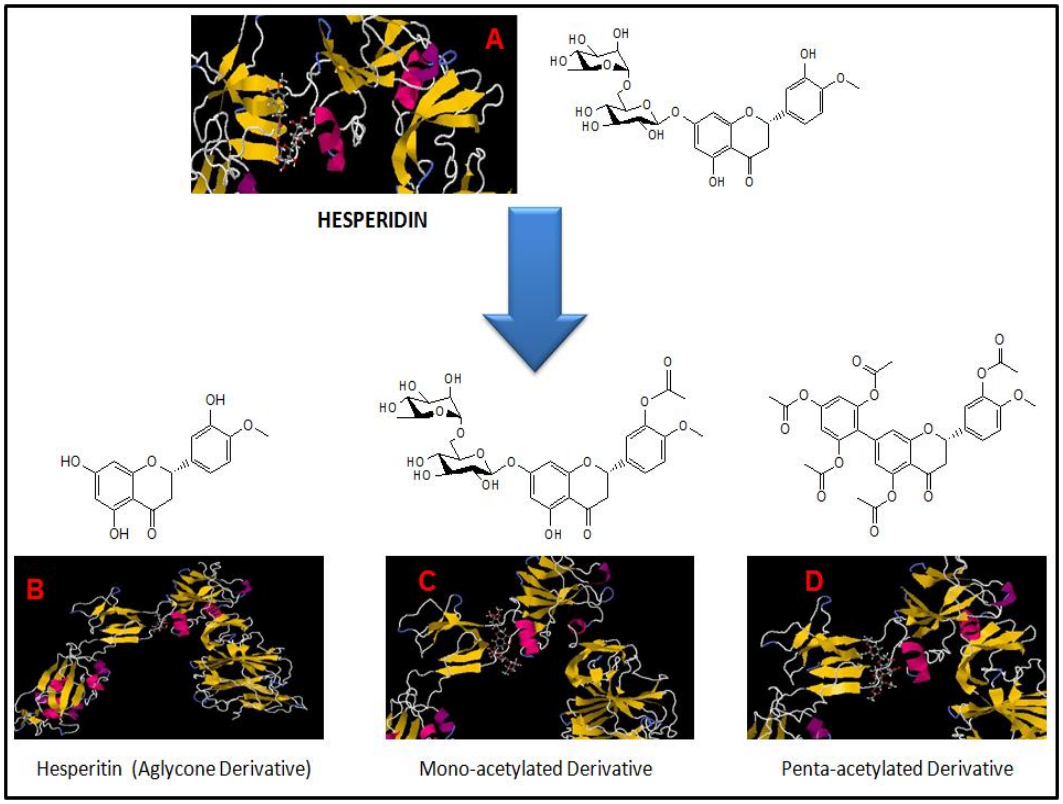


Fig. 4. Hesperidin binding affinities (ΔG) with SARS-CoV-2 spike protein: (A) The binding affinity of hesperidin = -7.57 Kcal/mol; (B) its aglycone derivative (hesperitin) = -6.93 Kcal/mol; (C) Mono-acetylated derivative = -7.82Kcal/mol); and (D) Penta-acetylated derivative = -8.39 Kcal/mol

The rationale to choose hesperidin as it has a reasonable binding affinity and five sites for the acetylation process. In addition, hesperidin derivatives are known to help in diabetes [32]. The plausible scheme for synthesis is given in Fig. 3. The acetylated derivative might increase the number of hydrogen bonding with the spike protein which can further stabilize the ligand-protein interactions. As a result, we found a significant increase in the binding affinity on the ligand acetylation. These results are extremely important as it opens an avenue to study the efficacy of natural compounds and their derivatives in inhibiting coronavirus. Next, we compared the effectiveness of mono-and penta acetylated derivative of hesperidin. We did not find any significant difference in mono- and penta acetylated derivatives attributed to the maximum number of stable interactions generated in mono acetylated form. Swiss-dock and Autodock-vina of Hesperidin derivatives, mono-acetylation, and Penta-acetylation with spike protein show binding affinities of -7.82 and -8.39Kcal/mol respectively (Fig. 4). A significant increase in binding affinity values in derivatives compares to hesperidin molecule shows derivatives could be better at combating SARS-CoV-2 than their parent compounds.

The emerging field, *in silico* study, could save time in the dugs manufacturing process basically in the selection of hit compounds during pharmaceutical manufacturing. To summarize we have investigated the potency of 16 natural compounds in restraining coronavirus infection using the molecular docking technique. We further generated acetylated derivative of hesperidin which can have up to 5 acetylated sites and analyzed its potential to bind spike protein. A huge difference was observed on acetylation which is due to the increased number of hydrogen bonding network stabilizing ligand-protein interaction. A difference between mono and penta acetylated form of hespiridin derivatives is probably attributed to the increase in the number of stable interactions generated in penta acetylated form. The aglycone derivative which is a truncated version of hesperidin cannot form as many interactions so it has considerable less affinity of -6.93 Kcal/mol. It was assumed that the interaction occurred due to the hydrogen bonding that stabilizes the docked structures. Virus spike protein is the target for drugs and vaccines because it interacts with human ACE2 protein. Hesperidin can form hydrogen bonds with the spike protein as shown in a previous study [33]. In this way, hesperidin can inhibit the spike-ACE2 interaction. The docking structure of hesperidin can be stabilized mainly by 2 hydrogen bonds. These hydrogen bonds are formed between spike protein's F457 of O7 of hesperidin. The second hydrogen bond is formed between E455 and the hesperidin's O atom. In future, we will generate the acetylated and other active moieties and study their interactions with other natural compounds with higher affinities. We will also study the ligand-protein interactions involved in other pathways like mTOR pathway. Our study showed that the synthetic drugs from pharmacological sectors exhibit greater promiscuity differences than do natural products as similar results were noticed by the work of Medina-Franco [34].

Medicinal plants and herbs and their derived active phytochemicals/compounds, especially flavonoids have long been used for the treatment of various disorders and diseases [35] due to their enhanced unique abilities of active compounds [20,21,36] such as antimicrobial, anticancer, antioxidant and anti-neuroinflammatory and many more. Hesperidin, a flavonoid also reported to have above given biological activities [37] and as a promising agent for the management of neurodegenerative diseases [33]. Our study indicated that the acetyl derivatives can be further consideration to combat highly infectious disease like COVID-19. In the present scenario of ecological and drug safety, naturally-derived medicines may surely provide better functionality as well as compatibility.

4. CONCLUSION

The development of vaccines and emergency authorization has been approved by several governments to combat COVID-19 infections. Therefore, there has been great concern about its side effects for the long term and its effectiveness against new mutated strains. Vaccines are provided approval for emergency authorization, however, the long term effects of the vaccines as well as the availability are questionable. The natural compounds on the study on the other hand have a high safety profile and availably in plenty through natural resources. A resurgence of COVID-19 or related disease can be catastrophic. There is an urgent need to look for effective antiviral agents for many coronavirus strains with minimum side-effects, and maximum efficacy globally. The present work showed that natural compounds can act as an efficient therapeutic with high

efficacy. The natural compounds utilized in the study have multiple benefits. Hesperidin is also known for its neuroprotective functions and anticancer potential. Hence using these natural compounds will provide pleiotropic protection against multiple diseases all at once. Natural products and their derivatives should be extensively studied further as they have a very high safety profile and the potential to resist coronavirus infection. Furthermore, *in-vitro* and *in-vivo* studies (in cell lines) should be extensively conducted to examine the efficacy of natural compounds which can serve as additional alternatives to vaccines.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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