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POTENTIAL OF INHIBITING THE RECEPTOR BINDING MECHANISM OF SARS-COV-2 USING PHYTOCHEMICAL EXTRACTS OF MEDICINAL HERB; MOLECULER DOCKING STUDY

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ABSTRACT

The Corona Viral Infective Disease (COVID-19), which leads to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is caused a pandemic situation globally. World Health Organization (WHO) declared that COVID-19 as a Public Health Emergency of International Concern (PHEIC) on January 30, 2020. Intend of this study is divulge the chemistry behind the phenomenon of viral (SARS-CoV-2) attachment on human epithelial cells as well as evaluate the receptor blocking abilities of selected herbal compounds. Significant anti-viral compounds were identified via review process of medicinal plants and Ferula asafetida, Glycyrrhiza glabra, Curcuma longa, Zingiber officinale etc. are widely used plant species for drugs against viral infectious diseases in Ayurveda medicine. Molecular docking prognosis have been carried out to demonstrate any possible secondary metabolites present in several anti-microbial herbs that could act as blocking agents of ACE2 and GRP78 receptors of epithelial cells to baffle the binding of receptor-binding domain (RBD) sections of SARS-CoV-2. Computational findings reveal that Phyto-chemicals such as Conferone, Samarcadin, Bdrakemin Farnesiferol A, Fernesiferol C and Galbanic acid isolated from Ferula asafetida have intensive binding energies for ACE2 receptor binding process. Apart from that Hederagenin and Ursolic also shows highest inhibitory potential towards human ACE2. When considering GRP78, almost all isolated compounds in oligo-gum resins of Ferula asafetida trot out perfect binding ability towards the active site of GRP78 receptor. Hence, it is worth to pay more attention on natural phytochemicals for mitigating of human viral infections.

INTRODUCTION

COVID ‘19 was first reported in Wuhan, Hubei province, China in December 2019 [1] and is now an intricate endemic in the world and as of today, 16th of April 2020, the number of coronavirus cases has exceeded 1,914,916 and more than 123,010 deaths were confirmed [2]. These numbers are increasing on a daily basis. The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) first occurred 17 years ago [3] and the novel coronavirus, SARS-CoV-2 is the seventh coronavirus known to infect humans. Among the other strains, SARS and MERS were the most widely known strains of coronaviruses, and each caused nearly 800 deaths [4]. WHO declared a public Health Emergency of international concern on 30th January 2020 for COVID ’19 [2]and it has become a burden to public health as well as to the world economy.

According to the Phylogenetic analysis, the SARS-CoV-2 virus belongs to lineage B of the beta-coronavirus [5]. The researchers analyzed genomic data available from SARS-CoV-2 and other coronaviruses, showing that the receptor-binding domain (RBD) sections of SARS-CoV-2 spike proteins are optimized for receptor binding [6]. The angiotensin-converting enzyme (ACE) - related carboxypeptidase, ACE2 is a type 1 (Hydrolase) integral membrane protein of 805 amino acids that contains one HEXXH + E zinc-binding consensus sequence [7]. Crystal structures of the native (PDB ID: 1R42) and inhibitor bound (PDB ID: 1R42) forms of ACE2 extracellular domains were solved to 2.2- and 3.3-Å resolution and deposited in RSCB protein data bank. When it considers tissue distribution of ACE2 protein, a remarkable expression found on lung alveolar epithelial cells and enterocytes of the small intestine. And also ACE2 present in arterial and venous endothelial cells, arterial smooth muscle cells [8] and, renal tissues [9]. It has proved a higher expression of ACE2 in the human failing heart [10].

The expression of ACE2 might provide possible routs of entry for SARS-CoV and can affect different organs of the body. Studies have provided genetic proof that ACE2 was a crucial SARS-CoV receptor in vivo [11].

The spike protein (S) of SARS coronavirus (PDB ID: 6ACD) attaches itself to its cellular receptor on ACE2. A defined receptor-binding domain (RBD) on S mediates binding interactions with ACE2. There are six RBD amino acids which have been identified as critical for binding to ACE2 receptors. They are L455, F486, Q493, S494, N501 and Y505 in SARS-CoV-2 [12]. The crystal structure at 2.9 Å resolution of the RBD, bound with the peptidase domain of human ACE2 in a study [13]. The structure of novel coronavirus (SARS-CoV2) spike receptor-binding domain complexed with its receptor ACE2 and the structure has deposited in RCSB PDB (PDB ID: 6LZG) at 2.50 Å of resolution.

Another study has predicted the binding of the SARS-CoV2 spike protein to GRP78 substrate-binding domain β (SBDβ) with binding affinities ranging from -9.8 up to -14 kcal/mol [14] and four regions of the spike protein were predicted as binding sites. According to literature, the active residues of GRP78 were identified as I426, T428, V429, V432, T434, F451, S452, V457, and I459 [15]. GRP78 is susceptible to virus recognition by its SBD. So it's possible to perform the virus entry in the cell. The overexpression of GRP78 initiated upon cell stress can enhance the escape of GRP78 from ER (Endoplasmic reticulum) and translocate to the cell membrane [16].

Using the computational model of the spike protein of SARS-CoV2 interacting with the Human ACE2 receptor, a large study has established to find interfering molecules that can disrupt the host-virus interactions using the world's most powerful supercomputer, SUMMIT [17]. A study demonstrated that human recombinant soluble ACE2 could significantly block the early stage of SARS-CoV2 infections [18].

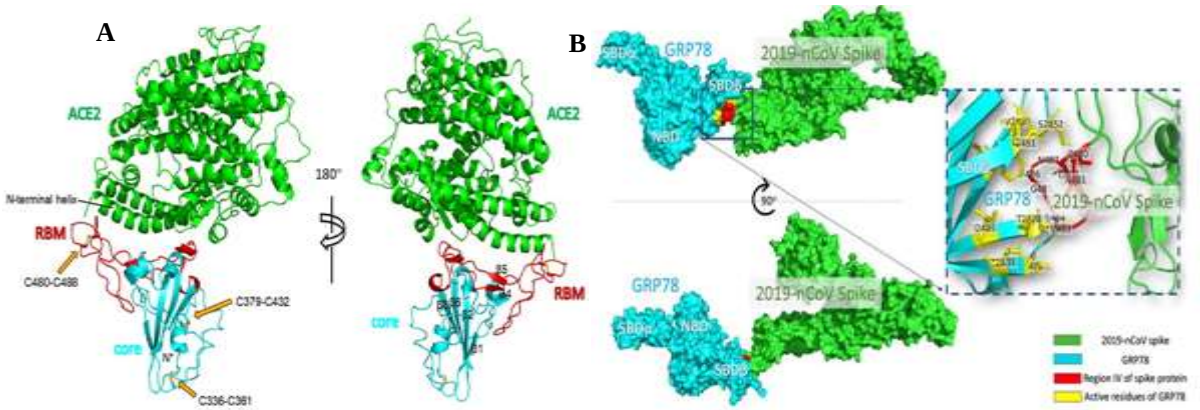

Figure 1. A, The SARS-CoV 2-spike protein and human ACE2 complex. Blue and red regions represent receptor binding region and core region of spike protein[19]***. B, The SARS-CoV 2-spike protein and GRP78 complex and the binding pocket [14].***

In the meantime, attempts are taken to address COVID '19 with Ayurvedic compounds and solutions as a preventive measure in south east Asian countries. However, they require laboratory and clinical testing. A pilot investigation of this issue with a computational eye would be faster and high throughput than laboratory testing for each plant material. This study focused on determining the binding energies and inhibition constants of 30 ligands with GRP78 and ACE2 receptor proteins. The 30 ligands were selected from the active ingredients in the ayurvedic products commonly used in South East Asian countries like India, Sri Lanka etc… Finally, our studies, as evident from the results presented below, show that indeed, few of the ligands of the Ayurvedic phytochemicals have reasonable binding energies and inhibition constants.

MATERIALS AND METHODS

Plant Review

In order to identify herbs that consist significant secondary metabolites which exhibits the anti-viral properties, a plant literature review was carried out.

Data Set

The two human receptor proteins, Angiotensin Converting Enzymes-2 (PDB ID : 6LZG) and Glucose Regulated Protein 78 (PDB ID : 5E84) structures were obtained from the https://www.rcsb.org/ website in .PDB format. The binding region of GRP78 and human ACE-2 to SARS- CoV-2 spike protein were obtained from literature. Additional binding pocket analysis was done by using Computed Atlas for Surface Topography of proteins (CASTp) (http://sts.bioe.uic.edu/castp/index.html?3igg). The 3-dimentional structures of analyzed ligands were obtained from the https://pubchem.ncbi.nlm.nih.gov/ website. The structural and molecular formulas of analyzed of ligands are listed in the Table 1.

Ligand Preparation and Molecular Docking

The ligand optimization was done by Avogadro version 1.2. The geometry optimization and energy minimization of ligands were optimized by using force field MMFF94, algorithm Conjugate gradient and 10000 steps. The output file was saved as mol2. The ligand files were converted to PDBQT file format by detecting the torsion root by using Autodocktools version 1.5.6.

The protein preparation prior to docking was done by using Autodocktools version 1.5.6. The water molecules were deleted, polar hydrogen atoms and kollman charges were added to the protein. The file was saved in the. PDBQT format for further analysis. The Autogrid 4.2 files were made in a such way that the grid box contained the binding region.

Autogrid 4.2 was used to generate the grid parameter files and map files. The genetic algorithm parameters were set as follows; the number of genetic algorithms (GA) runs: 10, population size: 300, the maximum number of evaluations: 25 000 000 and the other setting were set to default values. Autodock 4.2 was used for docking and generating result files in. dlg format.

Docking result analysis

The docking results were analyzed using AutoDock Tool version 1.5.6 to examine the binding energies. The binding pocket of the ligand was analyzed using PyMol and the protein ligand interaction profiler (https://plip.biotec.tu-dresden.de/plip-web/plip/index) was also used to validate the binding residues found by PyMol.

RESULTS AND DISCUSSION

In this analysis, 30 plant bioactive compounds were analyzed. Binding energy is a measure of the affinity of ligand-protein complex, or is the difference between the energy of complex and the sum of energies of each molecule separately. The threshold binding energy was set as -6.00 kcal/mol[20]. There were 10 and 21 plant compounds having binding energy equal for higher than the threshold value for human ACE2 and GRP78 respectively. Inhibition constant (Ki) is an indication of how potent an inhibitor is, it is the concentration required to produce half maximum inhibition [21]. The binding Energies and Ki of these compounds are listed in the Table

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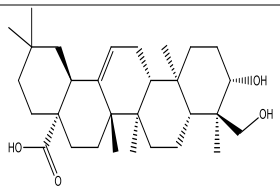
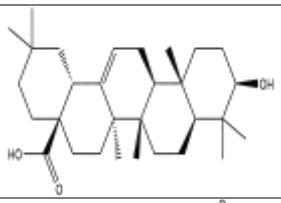
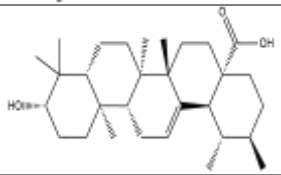
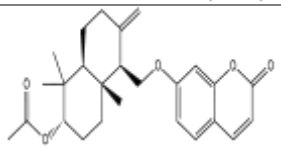
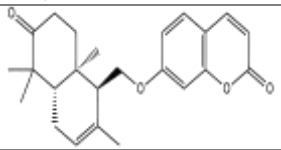
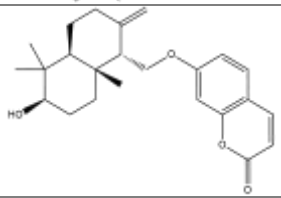
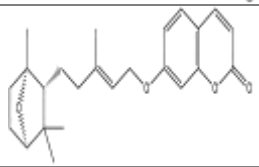
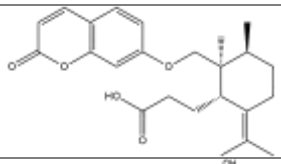
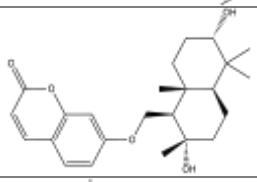
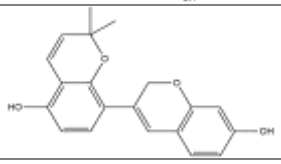
Table 1. Natural product compounds having binding energy ≤ -6 kcal/mol towards Human ACE2 and GRP78 along with their binding energies and inhibition constants (Ki). Also given is the chemical formula and chemical structure(2D).

Compound Name	Chemical formula	Chemical structure (2D)	Human ACE2		GRP78	
			Binding Energy (Kcal/mol)	Inhibition constant (μM)	Binding Energy (Kcal/mol)	Inhibition constant (μM)
Hederagenin	$C_{30}H_{48}O_4$		-6.15	31.13	-7.4	3.79
Oleanolic acid	$C_{30}H_{48}O$		-6.79	10.53	-7.69	2.29
Ursolic acid	$C_{30}H_{48}O_3$		-7.16	5.66	-7.67	2.4
Badrakemin	$C_{24}H_{30}O_4$		-6.22	27.76	-7.99	1.39
Conferone	$C_{24}H_{28}O_4$		-7.13	5.96	-8.28	0.853
Farnesiferol A	$C_{24}H_{30}O_4$		-6.21	28.01	-8.35	0.762
Farnersiferol C	$C_{24}H_{30}O_4$		-6.12	32.43	-7.12	6.01

Galbanic acid	$C_{24}H_{30}O_5$		-6	39.98	-7.64	2.52
Samarcadin	$C_{24}H_{32}O_5$		-6.23	27.1	-7.95	1.48
Glabrene	$C_{20}H_{18}O_4$		-6.06	36.3	-6.77	10.93

In the case of Human ACE2, all of the analyzed ligands were able bound to the spike protein binding site and some compounds were able to bind to this area with sufficiently high binding affinity and can be clearly seen in figure 2. These compounds may have ability to interfere and block protein-protein interactions which are necessary for the SARS- CoV-2 spike protein to bind to the human ACE2 receptor. In this case the binding energy varied between -6 kcal/mol (for Galbanic acid) and -7.16 kcal/mol (for Ursolic acid). The Ki for Galbanic acid and Ursolic acid ranged between 5.6 µM and 39.98 µM respectively. Ursolic acid has shown lowest Ki and the highest inhibitory potential towards human ACE2. Ursolic acid in found in natural source such as Apple peel (*Malus domestica*), Basil (*Ocimum basilicum*), Bilberries (*Vaccinium myrtillus*), Cranberries (V*accinium oxycoccos*), Rosemary (*Rosmarinus officinalis*), Thyme (*Thymus vulgaris*), Peppermint (*Mentha piperita*) and Prunes (*Prunus domestica*) [22]. Ursolic acid containing herbs are widely used in Sri Lanka and India as an Ayurveda medications for various ailments [23]. Glabrene (-6.06 kcal/mol) readily found in steam of welmee/Licorice (*Glycyrrhiza glabra*)[24] . Licorice shrub is a member of the pea family and grows in subtropical climates in rich soil. Below ground, the plant has an extensive root system with a main taproot and numerous runners. The main taproot which is soft and fibrous has a bright yellow interior color and harvested for medicinal [25] uses for treating upper respiratory immune enhancing effect and are used against upper respiratory tract infections [26]. Glycyrrhizin is major anti- viral agent found in licorice [27], but in this case it showed a binding energy below the threshold level (-4.36 kcal/mol). Badrakemin (-6.22 kcal/mol), Conferone (-7.13 kcal/mol), Farnesiferol A (-6.21 kcal/mol), Farnersiferol C (-6.12 kcal/mol) and Samarcadin (-6.23 kcal/mol) are bioactive compounds found in oligo-gum of Perumkayan (*Ferula asafetida*) [28]. *Ferula asafetida* is distributed from the Mediterranean region to Central Asia. It is also abundantly found in many parts of Afghanistan, India and Sri Lanka*. Perumkayan oligo-gum is proven to have anti-viral effect against viruses such as Rhinovirus (HRV) [29] and Influenza A (H1N1) [30]. Therefor it is widely used as Ayurveda medication against wide variety of viral infections throughout the south east Asia. In this region, it is also used for culinary purposes [31]. Perumakayan has shown promising results as 6 out of 9 tested phytochemicals have binding energies greater than the threshold value. Looking at the Ki of these compounds, Conferone has the lowest Ki (5.97 µM) and highest inhibitory action, followed by Samarcadin (27.1 µM), Bdrakemin (27.76 µM), Farnesiferol A (28.01 µM), Fernesiferol C (32.43 µM) and Galbanic acid (39.98 µM) respectively. Hederagenin (-6.15 kcal/mol) is found in many plant sources such Black cumin (*Nigella sativa*), which is used in Ayurveda [32]. Spiny gourd (*Momordica dioica*) and Sponge gourd (*Luffa cylindrica*) fruits also contain Hederagenin and are consumed by South East Asians.

When considering GRP78, most of the analyze ligands were able to bind perfectly with active site of GRP78. This can be clearly seen in Figure 3. The binding energies ranged from -6.09 kcal/ mol (for Liquiritigenin) to -8.38 kcal/mol (for Farnesiferol A). All of the test compound of Perumkayan oligo-gum showed exceptional binding to the active site of GRP78. Badrakemin (-7.99 kcal/mol), Conferone (-8.28 kcal/mol), Farnesiferol A (-8.38 kcal/mol), Galbanic acid (-7.64 kcal/mol), Methyl galbanate (-7.95 kcal/mol) and Samarcadin (-7.95 kcal/mol) show high binding energies when compared with other tested compounds. Perumkayan's phytochemical has significantly low Ki, which indicate their exceptional ability of inhibit GRP78 receptor. Farnediferol A (0.762

µM), Conferone (0.853 µM), Badrakemin (1.39 µM), Samarcadin (1.48 µM) and Galbanic acid (2.52 µM) are among them.

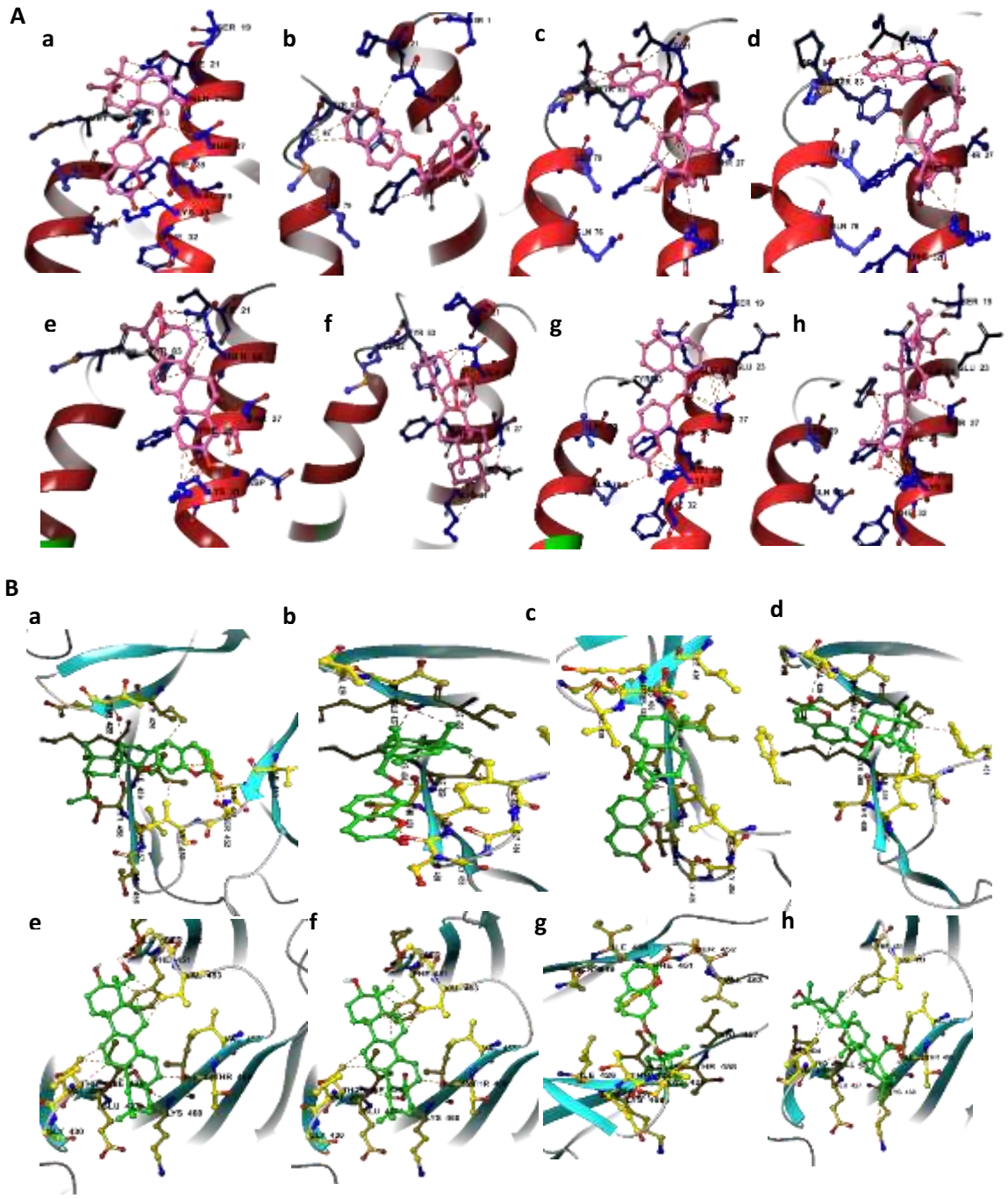


Figure 1. A, 3D visualization of docking analysis of human ACE 2 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g) and Ursolic acid (h). B, 3D visualization of docking analysis of GRP 78 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g) and Ursolic acid (h).

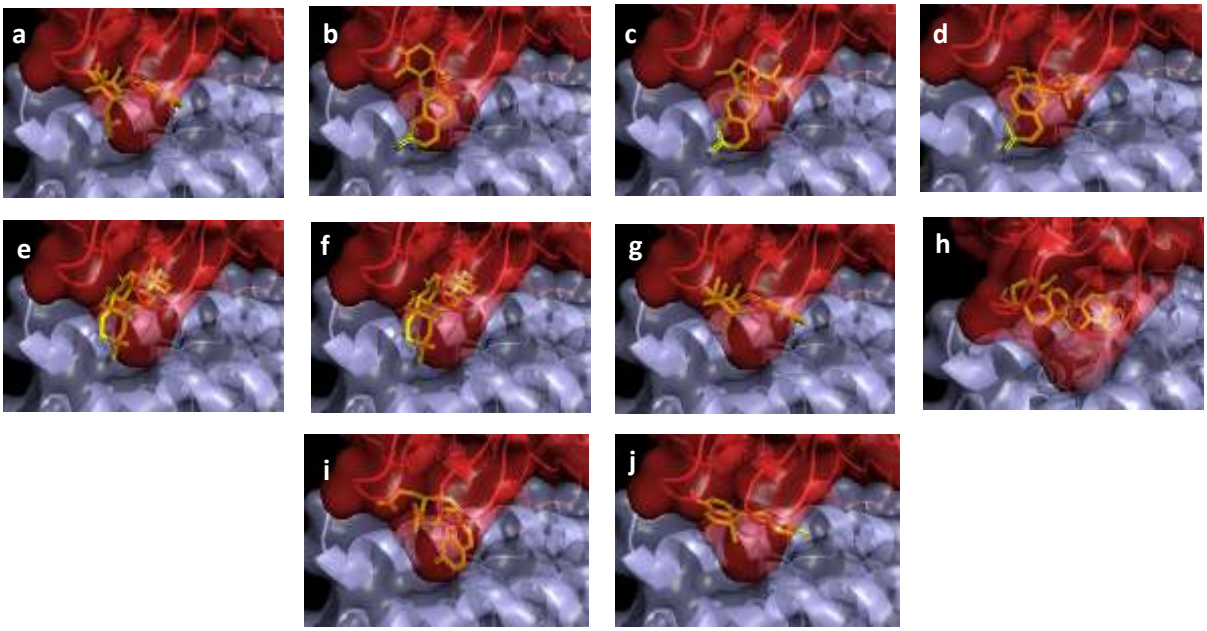


Figure 2. The Surface visualization of docking analysis of human ACE 2 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g) and Ursolic acid (h). B, 3D visualization of docking analysis of GRP 78 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g). Ursolic acid (h)., Galbanic acid (i) and Glabrene (j). The red color region indicates the SARS- CoV 2 spike protein and blue color region indicates the human ACE2 protein.

Out of the tested phytochemicals of Licorice, Hispaglabridin B (-7.43 kcal/mol) had the highest binding affinity and lowest Ki (.3.61 µM). Glycyrrhizin had a binding energy below the threshold level (-5.56 kcal/mol). Berberine (-6.56 kcal/mol) is ready found in yellow vine or veniwel geta (*Coscinium fenestratum)* [33] and is widely used in Ayurveda as a anti inflammation agent [34].The plant has been determined to be native to Sri Lanka and the Western Ghats in India. Curcumin (-6.33 kcal/mol) is present in Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*)[35] and are widely used in medicinal and culinary purposes throughout south east Asia. Berberine, Curcumin and Oleanolic acid had Ki of 15.46 µM and 23.04 µM respectively. Oleanolic acid (-7.79 kcal/mol) is present in Thyme (*Thymus vulgaris*), Rosemary (*Rosmarinus officinalis*), Olive (*Olea europaea*) and Java Apple (*Syzygium samarangense*) [36]. And has shown exceptional inhibitory action with Ki of 2.29 µM.

In summary, this study shows that several of the natural product compounds that are commonly used in Ayurveda and cuisines may have the potential ability bind to possible receptors of SARS-CoV-2 spike protein and inhibit the spike protein from binding to receptors. The results indicate that Perumkayan oligo-resin and licorice steam extract may have high prevention activity against COVID-19 and if confirmed by future studies could be used as a preventive medication.

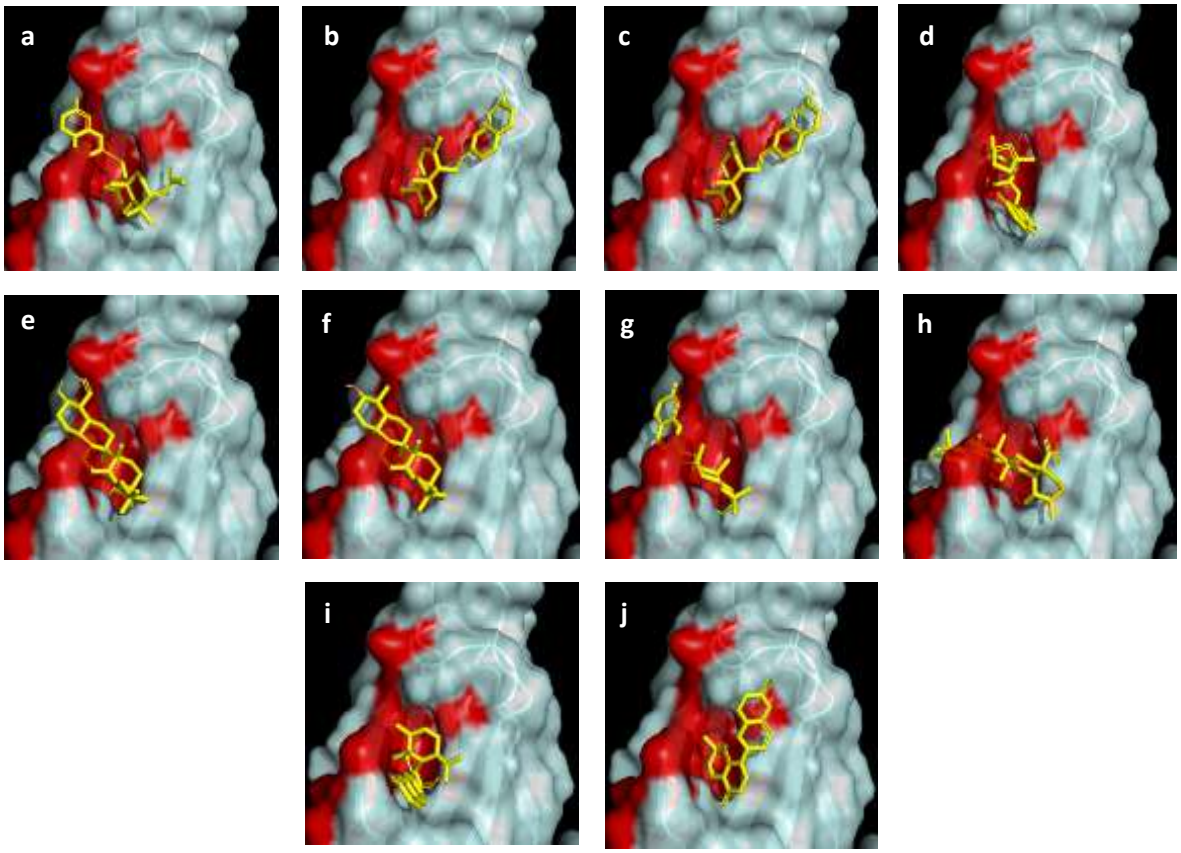


Figure 3. The Surface visualization of docking analysis of GRP 78 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g) and Ursolic acid (h). B, 3D visualization of docking analysis of GRP 78 binding with Badrakemin (a), Conferone (b), Fernesiderol A (c), Fernesiferol C (d), Henderagenin (e), Oleanolic acid (f), Samarcadin (g). Ursolic acid (h)., Galbanic acid (i) and Glabrene (j). The red color region indicates active site residues of GRP 78 which are responsible for SARS-CoV-2 spike protein binding.

CONCLUSION

At the time of writing COVID ’19 viral pandemic has become a major burden to human health and world economy. There is an urgent need for the rapid development of vaccines or anti-viral drugs against COVID 19. One approach is to use Insilco analysis to rapidly screen plant materials used in Ayurveda medicine to find phytochemicals that can be used against COVID ’19. As *Ferula asafetida* and *Glycyrrhiza glabra* compounds have a high ani-viral activity, they should be given a special attention. Our results have shown that 9 and 21 compounds were able to bind to Human ACE2 and GRP78 receptors at the SARS-CoV-2 spike protein binding region respectively. *Ferula asafetida*’s phytochemicals (Conferone, Samarcadin, Badrakemin, Farnesiferol A, Fernesiferol C and Galbanic acid) had exceptionally high binding energies and exceptionally low inhibitory constants. *Ferula asafetida* is a potential candidate suitable for further research. Admittedly the findings presented here are preliminary and the optimizations are needed to be done. More detailed molecular dynamic simulation studies should be done in order to have a deeper insight into the mode of inhibition.

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