



Receptor Study of Phytochemical Constituents Having Antiviral Activity Used Against Covid-19

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Abstract:-

In this study, an *in silico* molecular docking between the SARS-COV-2 four proteins (a)SARS-COV-2 nucleocapsid protein N-terminal RNA binding domain (6m3m),(b) nsp9 RNA binding protein of SARS COV-2 (6w4b), (c) the crystal structure of covid-19 main protease in APO form (6m03), and (d) structure of the 2019-ncov hr2 domain (6lvn)] available in the PDB (protein data bank), and the medicinal plant-based phytochemicals (retrieved from Pub Chem database) as ligand molecules i.e. Piperine (black pepper), eugenol (clove), Alliin (garlic), Gingerol (ginger) and Curcumin (turmeric). All these ligand molecules showed good docking with their respective receptor molecules and their scores range from -8.195 to -5.263. Dockthor with their respective receptor molecules and their scores range from -8.195 to -5.263. Dockthor portal (a receptor ligand- docking server) which was recently developed and published this year was used in the current study. The obtained results might help in the wet lab conditions to develop better antiviral compounds against sars-cov-2.

Keywords:-Sars-Cov-2, Covid-19, Docking, Phytochemical, Ligand.

Introduction:-

Currently, the entire world is facing the lockdown type situation caused by severe acute respiratory syndrome coronavirus-2 (SARS-COV-2), identified as the novel corona virus (nCoV, family: *coronoviridae*) responsible for causing the corona virus disease in 2019 designated as covid-19¹. The recent outbreak of novel corona virus (nCoV-2019) or SARS-COV-2 has raised a challenge to design a drug for this deadly virus. However, to date, not approved drug is available for this pathogenic virus (infectious pathogen transmitted through human to human). Computational drug discoveries against sars-cov-2 from natural sources has been suggested and tried by many scientists across the globe this year. This includes several *in silico* studies and few wet lab trials²⁻⁴. This novel corona virus marked its origin from Wuhan, the capital of central china's hubeiprovince. Bats, birds, camels, and pangolins are considered as the potential

natural reservoir of sars-cov-2 and the virus was later transmitted to humans. The covid-19 virus is fatal to humans in most of the cases and the mortality rate is increasing day by day (killed over 45,000 people and infected over 9,00,000)². Symptoms related to covid-19 includes sore throat, fever, pneumonia, sneezing, headache, cough, acute respiratory distress syndrome, acute cardiac injury, rhonorrhoea, fatigue, sputum production, haemoptysis, ground glass opacities, hypoxemia, dyspnoea, anaemia, lymphopenia and diarrhoea³. Every country is devising different ways to minimize the outbreak of sars-cov-2 responsible for the covid-19 pandemic⁴. Several medicines and vaccines are under trial to find the possible cure against the sars-cov-2⁵, meanwhile, precautionary measures and social distancing have been suggested and the only way to limit the spread of coronavirus^{6, 7}. The genome of sars-cov-2 consists of a positive single-stranded rna [(+) ssrna]. Various proteins present in the viral structure are spike (s) glycoprotein, nucleocapsid (n) phosphoprotein, membrane (m) glycoprotein and small envelope (e) glycoprotein. Team of doctors and scientists are working day and night to find a possible cure to tackle this virus. Keeping this in view, here we have presented a study which might be helpful for scientists and doctors. One such computational molecular docking approach has been taken up by us which might be helpful in developing antiviral compounds against this deadly.

Corona virus disease 2019 (covid-19) is a contagious disease caused by severe acute respiratory syndrome corona virus 2 (sars-cov-2). The first known case was identified in Wuhan, China, in December 2019. The disease has since spread worldwide, leading to an ongoing pandemic. Several testing methods have been developed to diagnose the disease. The standard diagnostic method is by detection of the virus' nucleic acid by real-time reverse transcription polymerase chain reaction (rt-pcr), transcription-mediated amplification (tma), or by reverse transcription loop-mediated isothermal amplification (rt-lamp) from a nasopharyngeal swab preventive measures include physical or social distancing, quarantining, and ventilation of indoor spaces, covering coughs and sneezes, hand washing, and keeping unwashed hands away from the face. The use of face masks or coverings has been recommended in public settings to minimize the risk of transmissions^{8,9}.

Name of Virus:-

The official names covid-19 and sars-cov-2 were issued by the, who on 11 February 2020. Tedros Adhanom explained:

- Co for corona,
- Vi for virus,
- D for disease,
- 19 for the outbreak was first identified (31 December 2019). The who additionally uses "the Covid-19 Virus".

Sign and Symptoms:-

Symptoms of covid-19 are variable, ranging from mild symptoms to severe illness. Common symptoms include in that:

1. Headache
2. Loss of smell taste
3. Nasal congestion
4. Runny nose
5. Cough
6. Muscle pain
7. Sore throat fever
8. Diarrhea
9. Breathing difficulties

One respiratory symptom cluster with cough, sputum, shortness of breath, and fever; a musculo skeletal symptom cluster with muscle and joint pain, headache, and fatigue; a cluster of digestive symptoms with abdominal pain, vomiting, and diarrhea.

Systemic Symptoms:-

Typical systemic symptoms include that:

1. Fatigue
2. Muscle
3. Joint pains
4. Some people have a sore throat.
5. Loss of smell
6. Loss of taste

Scope of Phytochemicals in the Management of Covid-19:-

Covid-19 is a declared pandemic caused by sars-cov-2 which has infected millions and took many lives across the globe, as no proven treatment is established so far. Researchers from various parts of the world are actively involved in developing suitable drugs, vaccines and any other possible methods to eradicate this pandemic. Alternative treatments are also gaining importance. China, where the covid-19 outbreak was first reported, strongly believes in natural drugs used in chinese system of medicine and they followed combination therapies. The phytochemicals or phytonutrients have proved its role in treatment of diseases including viral, bacterial, cardiovascular diseases, cancer etc. From the literature, few phytochemicals have shown significant antiviral activity against covid-19 through drug repurposing approaches like molecular docking. Also a wide range phytochemicals like alkaloids, tannins, polyphenols, flavanoids, proteins, lecithins, lignans, coumarins, and anthocyanidins possess antiviral properties against different types of corona virus including sars-cov. Since the development of new treatment with natural drugs requires time these phytochemicals also seen in common herbs can be incorporate in our daily diet

which would increase the immunity to fight covid-19. Ayurveda, oldest system of medicine have various documented traditional medicines which can be a source of antiviral phytochemicals and can be used for both management and treatment of covid-19. Ayurvedic system of medicine and ministry of ayush recommends the rational use of herbal drugs to combat covid-19⁵.

Pathophysiology of Corona Virus:-

Covid-19 can affect the upper respiratory tract (sinuses, nose, and throat) and the lower respiratory tract (windpipe and lungs).the lungs are the organs most affected by covid-19because the virus accesses host cells via the receptor for the enzyme angiotensin-convertingenzyme 2 (ace2), which is most abundant on the surface of type ii alveolar cells of the lungs. The virus uses a special surface glycoprotein called a "spike" (peplomer) to connect to the ace2 receptor and enter the host cell⁵.

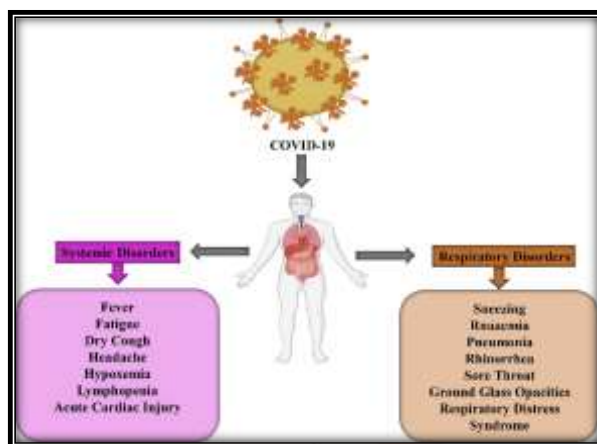


Figure No. 1: Pathophysiology of Corona Virus

Protein–Ligand Docking:-

Protein–ligand docking is a molecular modelling technique. The goal of protein–ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme. Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates. Several protein–ligand docking software applications that calculate the site, geometry and energy of small molecules or peptides interacting with proteins are available, such as auto dock and auto dockvina, rdock, flexaid, molecular operating environment and glide¹⁰.

Protein Flexibility:-

Computational capacity has increased dramatically over the last two decades making possible the use of more sophisticated and computationally intensive methods in computer assisted drug design. However, dealing with receptor flexibility in docking methodologies is still a thorny issue. The main reason behind this difficulty is the large number of degrees of freedom that have to be considered in this kind of calculations. However, in most of the cases, neglecting it leads to poor docking results in terms of binding pose prediction in real world settings. Using coarse grained protein models to overcome this problem seems to be a promising approach. Coarse-grained models are often implemented in the case of protein-peptide docking, as they frequently involve large-scale conformation transitions of the protein receptor.

Material and Methods:-

Protein and ligand interactions play a crucial role, wherein the latter is responsible for limiting the activity of former in many human cellular and biological functions. For docking, we have considered four sars-cov-2 proteins as receptor molecules and same mined from pdb database in pdf format i.e.(a) sars-cov-2 nucleocapsid protein n-terminal rna binding domain (6m3m), (b) nsp9 rna binding protein of sars-cov-2(6w4b), (c) the crystal structure of covid-19 main protease in apo form (6m03),(d)structure of the 2019-ncov hr 2 domain (6lvn).These four sars-cov-2 proteins were docked separately against five ligand molecules piperine (black pepper: *piper nigrum*), eugenol (clove: *syzygiumaromaticum*),alliin (garlic: *allium sativum*), gingerol (ginger: *zingiber officinale*) and curcumin (turmeric: *curcuma longa*)

These medicinal plant based phytochemicals were considered and their 3D structure files were retrieved from pub chem database in the sdf format, later converted to pdb format. There retrieved receptors and ligand files were uploaded to the dockthor portal for docking study.

Corona Virus Infection Mechanism:-

The SARS-CoV-2 shares some sequence homology with SARS-CoV but show different transmission potential and infection range. The ongoing COVID-19 disease is seen as more treacherous with still no treatment and could be potentially due to certain functional mutations occurring in the SARS-CoV-2. The major differences observed in SARS-CoV-2 are absent 8a, longer 8b and shorter 3b segments and different Nsp-2 and -3 proteins. Along with these, the open reading frames are also different in some places like in orf8 and orf10. The process of infection is totally dependent on the interaction between virus and the host cell and begins when the viral particles get attached to the cell surface receptor of the host cell and it delivers the nucleocapsid inside the target cell membrane. The main function is provided by S-protein in binding and fusion with the host cell membrane. SARS-CoV-2 binds with the same receptor ACE-2 present in respiratory epithelium and alveoli of lungs, as SARS-CoV^{11,12}.

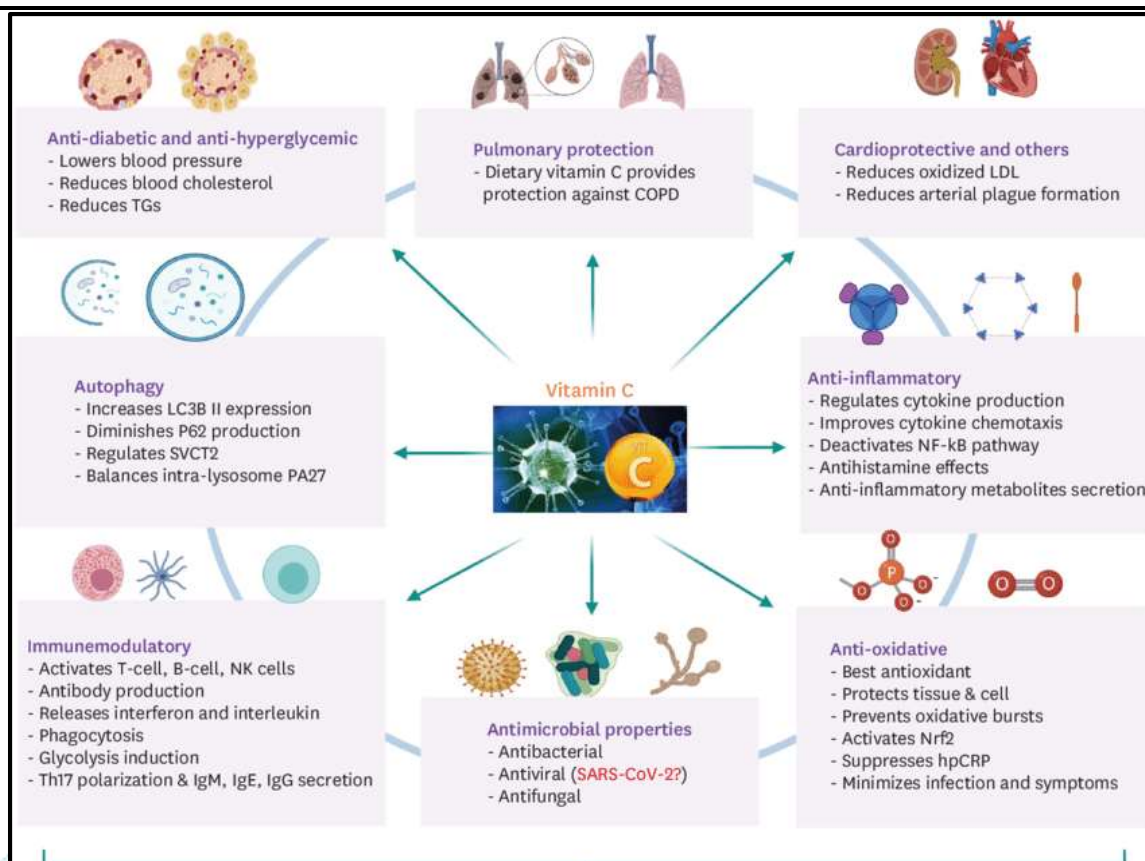


Figure No. 2: Corona Virus Infection Mechanism

***In silico* Studies Reveal Potential Antiviral Activity of Phytochemicals from Medicinal Plants for the Treatment of Covid-19 Infection:-**

The spread of covid-19 across continents has led to a global health emergency. Covid-19 disease caused by the severe acute respiratory syndrome corona virus 2 (sars-cov-2) has affected nearly all the continents with around 1.52 million confirmed cases worldwide. Currently only a few regimes have been suggested to fight the infection and no specific antiviral agent or vaccine is available. Repurposing of the existing drugs or use of natural products are the fastest options available for the treatment. The present study is aimed at employing computational approaches to screen phytochemicals from the medicinal plants targeting the proteins of sars-cov-2 for identification of antiviral therapeutics. The study focuses on three target proteins important in the life cycle of sars-cov-2 namely spike (s) glycoprotein, main protease (mpro) and rna-dependent rna-polymerase (rdrp). Molecular docking was performed to screen phytochemicals in medicinal plants to determine their feasibility as potential inhibitors of these target viral proteins. Of the 30 plant phytochemicals screened, silybin, an active constituent found in silybummarianum exhibited higher binding affinity with targets in sars-cov-2 in comparison to currently used repurposed drugs against sars-cov-2. With a ferina from *Withania somnifera* also showed significant binding to the targets proteins. In addition, phytochemicals from *tinospora cordifolia* and aloe barbadensis displayed good binding energetics with the target proteins in sars-cov-2. These results provide a basis for the use of traditional medicinal plants as alternative lines of treatment for covid-19 infect.

Molecular Docking Study of Potential Phytochemicals and Their Effects On The Complex of SARS-Cov-2 Spike Protein and Human ACE2:-

Angiotensin converting enzyme 2 (ACE2)(EC: 3.4. 17.23) is a trans membrane protein which is considered as a receptor for spike protein binding of novel corona virus (SARS-CoV2). Since no specific medication is available to treat COVID-19, designing of new drug is important and essential. In this regard, *in silico* method plays an important role, as it is rapid and cost effective compared to the trial and error methods using experimental studies. Natural products are safe and easily available to treat coronavirus affected patients, in the present alarming situation. In this paper five phytochemicals, which belong to flavonoid and anthraquinone subclass, have been selected as small molecules in molecular docking study of spike protein of SARS-CoV2 with its human receptor ACE2 molecule. Their molecular binding sites on spike protein bound structure with its receptor have been analyzed. From this analysis, hesperidin, emodin and chrysin are selected as competent natural products from both Indian and Chinese medicinal plants, to treat COVID-19. Among them, the phytochemical hesperidin can bind with ACE2 protein and bound structure of ACE2 protein and spike protein of SARS-CoV2 noncompetitively. The binding sites of ACE2 protein for spike protein and hesperidin, are located in different parts of ACE2 protein. Ligand spike protein causes conformational change in three-dimensional structure of protein ACE2, which is confirmed by molecular docking and molecular dynamics studies. This compound modulates the binding energy of bound structure of ACE2 and spike protein. This result indicates that due to presence of hesperidin, the bound structure of ACE2 and spike protein fragment becomes unstable. As a result, this natural product can impart antiviral activity in SARS CoV2 infection. The antiviral activity of these five natural compounds is further experimentally validated with QSAR study¹³.

Conclusion:-

We have applied docking studies on animal virus severe acute respiratory syndromecoronavirus-2 (sars-cov-2) which considered humans, bats and pangolins as its host. All the ligand molecules used in this *in silico* experiment (dry lab) have strong antioxidant and medicinal properties and they can be coupled/conjugated with drug molecules in wet lab conditions to develop better antiviral compounds. This might be a solution against the pandemic caused by the deadly novel corona virus (covid-19). This manuscript will help scientists and doctors to consider such ligands in their future drug development and targeting experiments against sars-cov-2.

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