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TARGETING COVID-19 (SARS-COV-2) SPIKE GLYCOPROTEIN THROUGH ACTIVE PHYTOCHEMICALS OF AYURVEDIC MEDICINAL PLANTS USING COMPUTATIONAL BIOLOGY APPROACH

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ABSTRACT

Background: Recent outbreaks of novel coronavirus (COVID-19) disease-causing respiratory infection have hampered public health significantly. Currently, drugs and vaccines are being developed to combat this novel virus, and those vaccines have yielded positive and satisfying results. However, those vaccines have a slew of unexpected side effects that can harm and disrupt an individual's normalcy. An attempt was made here to recognise natural phytochemicals from medicinal plants and repurpose them against COVID-19 using computer-aided drug design. ***Methods:*** The target and ligands were screened virtually. Furthermore, molecular docking studies with 202 phytochemicals were conducted, and nine compounds were discovered to be potent inhibitors of the SARS-CoV-2 Spike glycoprotein (PDB ID 6VXX). ***Results:*** We have found that the nine phytochemical compounds giving better binding conformations were as follows three from *Withania somnifera* (*Ashwagandha*), two from *Azadirachta indica* (*Neem*), one from *Adhatoda vasica* (*Adulsa*), two from Ocimum. sanctum (*Tulsi*), and one from T. cordifolia (*Giloy*). ***Conclusion:*** Hence from the present study, it could be suggested that active phytochemicals from medicinal plants could potentially inhibit the spike glycoprotein of SARS-CoV-2, thereby strengthening the management strategy against COVID-19, a global contagion.

KEYWORDS: Novel coronavirus (SARS-CoV-2), Phytochemicals, Virtual Screening, Molecular Docking.

INTRODUCTION

The world has stopped and people are quarantined the World Health Organization (WHO) has declared the corona virus disease 2019 (COVID-19) a pandemic. A global coordination and unity effort is needed to stop the further spread of the virus. A pandemic is something which occurs over a broad geographical area and it affects an exceptionally high proportion of the population. According to the current statistics, as of 12 May 2021, there have been 159,784,683 confirmed cases of COVID-19, including 3,320,036 deaths, reported to WHO. It is a common pneumonia which is caused by an individual standard virus, SARS CoV-2. It was named Corona Viruses Disease 2019 (COVID-19) by the World Health Organization (WHO) on January 30, 2020. (Mehmood *et al.*, 2021)

Corona viruses are a family of viruses that cause illnesses such as respiratory diseases or gastrointestinal diseases. Respiratory diseases can range from moderate to severe disease including common cold e,g.

- Middle East Respiratory Syndrome (MERS-CoV)
- Severe Acute Respiratory Syndrome (SARS-CoV)

Symptoms of COVID-19 are non-specific range from no symptom to severe pneumonia, loss of taste and smell, breathlessness, low oxygen level, and death.

The complete sequence of SARS-2 has been obtained and the disease can be diagnosed by RT- PCR and the sample are collected from both the lower or upper respiratory tract or by counting the total number of leucocytes and lymphocytes. (Mehmood *et al.*, 2021).

The SARS-CoV-2 S protein is highly conserved among all human corona viruses (HCoVs) and is involved in receptor recognition, viral attachment, and entry into host cells. (Huang *et al.*, 2020)

The S protein represents a perfect target for vaccine and antiviral research endeavors. It also plays a role in penetrating into cells i.e S protein of viruses, specifically the SARS-CoV-2 virus, It is a major inducer for neutralizing antibodies (NAbs). Nabs are protective antibodies that are naturally produced by our humoral immune system. Vaccines are developed against this novel virus and those vaccines have shown positive and satisfying outcomes.

But those vaccines have multiples unexpected side effects as well which may harm and alter the individual's normalcy. So hence to find out the best solution with the least side effects I

explored the Ayurvedic medicinal plant, there are countless benefits over medicinal plant such as naturally available, alleviates hormones and metabolism, anti-inflammatory, and boosting up of the immune system. Perceiving in the immunity-boosting actions during the COVID-19 situation is necessary, it is essentials to consume supplements in the form of immune nutrients such as vitamin C, B-complex, zinc, and copper which will support our body to fight against the SARS-CoV-2. (Tripathi *et al.*, 2020)India's Ministry of AYUSH has also stated, "Ayurveda's immunity-boosting measures for self-care during COVID 19 crisis". ('Ministry of Aayush', 2020) Ayurveda means "The Science of Life" Ayurvedic knowledge originated in India more than 5,000 years ago and is often called the "Mother of All Healing"(Mishra *et al.*, 2010). It adopts a unique holistic approach to the entire science of life, health, and cure. A group of rejuvenative methods that impart biological nourishment to the body tissues was described in Ayurvedic text (Singh, Narsimhamurthy and Singh, 2008)It describes many medicinal plants with a wide range of therapeutic potentiality in treating the ailment of the respiratory system; some of the notable ones but not limited to are *Withania somnifera* (*Ashwagandha*), *Tinospora cordifolia* (*Giloy*), *Ocimum sanctum* (*Tulsi*) *Azadirachta indica* (*Neem*), *Adhatoda vasica* (*Adulsa*) used in the present study. They are all immunomodulators (a group of drug that mainly target the pathway) and makes the body strong against infections.

Withania somnifera (*Ashwagandha*) is one of the most valued Indian ayurvedic medicinal plants that are revered as a biologically active immunomodulator and their ability to balance, energize, rejuvenate, and revitalize. *Ashwagandha* comprises a rich source of various phytoconstituents such as Withaferin A, steroidal lactones of the withanolide series, steroidal alkaloids of the withanoside series, and some other compounds Active constituents of *Ashwagandha* are known to possess promising anti-influenza activity, against chikungunya, inhibit infectious Bursal Disease virus and are beneficial for the treatment of genital disease caused by Herpes Simplex Virus among African tribes, etc. (Tripathi *et al.*, 2020)

T. cordifolia (Guduchi or *Giloy*), is a medicinal plant which has been used for its remedial purpose from last thousands of year in Ayurvedic system of medicine. Its extracts have alkaloids, glycosides, steroids, and polysaccharides. It is documented for its immunomodulatory, antidiabetic, antioxidant, antihepatotoxic, and cytotoxic effects. The active phytoconstituents, Tinocordioside, Cordifolioside A, Magnoflorine, and Syringin are known for their immunomodulatory effect. (Goyal *et al.*, 2011)

O. sanctum (*Tulsi*), it is a sanctified herb used because of its medicinal property as per Ayurvedic scriptures. Its multiple therapeutic actions comprises adaptogenic, immunomodulatory, antimicrobial, cardioprotective, and anti-inflammatory effects, anti-viral, anti-fungal, and anti-bacterial activity, also possess anti-diabetic, analgesic, antifertility, anticancer, antispasmodic, antiemetic, diaphoretic and hepatoprotective actions. Their leaves are beneficial for the treatment of rheumatism, bronchitis, and pyrexia and are considered as 'Elixir of life' for its healing power. By the enhancement of both cellular and humoral immunity, it strengthens the immune response Eugenol and Ursolic acid are its main phytoconstituents.(Shree *et al.*, 2020)

Azadirachta indica (*Neem*), a member of the Meliaceae family, is a well-known medicinal plant, especially in the Indian subcontinent. The chemical constituents of *Neem* include azadirachtin, 7-desacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, 7-desacetyl-7-benzoylgedunin, Nimbin, nimbiol, polyphenolic flavonoids, etc. Ethanol extracts of *Neem* leaves have been shown to exhibit anti-microbial properties, and *Neem* components have demonstrated free radical scavenging and anti-inflammatory activities.(Borkotoky and Banerjee, 2020)

Adhatoda vasica, an ayurvedic medicinal plant has been used in various chest and respiratory tract infections viz.- whooping cough, chronic bronchitis, asthma and has been employed as a sedative expectorant in the treatment of excessive phlegm (mucus with bacteria, debris, and sloughed-off inflammatory cells). (Sharma, Bhardwaj and Singh Cannoo, 2018)

Vasicine is a major bioactive pyrroquinazoline alkaloid of vasaka present within the concentration of 1.3%. Minor alkaloids include adhatonine, vasicinol, and vasicinolone.

Various activities reported are: Antibacterial, Anticholinesterase, Wound healing, Hypoglycaemic, Anti-inflammatory, etc. (Ahmad *et al.*, 2009)

In the present study, insilico studies were performed, which is an initial step in the drug discovery and development process, to identify the most potent compound by targeting the novel sites of the spike glycoprotein of SARS-CoV-2, a viral protein required for binding and its entry into the host cells. Currently, only a few FDA- approved drugs like Chloroquine, Hydroxychloroquine, Remdesivir, Bevacizumab, Fluvoxamine, etc., either alone or in

combination with other drugs, are being used to treat the infection. By considering this, Molecular docking approach was performed on various phytochemicals.

MATERIALS AND METHODS

Target identification

To study the mode of interaction of different phytochemicals with protein SARS-CoV-2 spike glycoprotein molecular docking was performed. RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (https://www.rcsb.org/) was used to retrieve the Three-dimensional crystal structure of COVID-19 (SARS-CoV-2) spike glycoprotein (PDB ID:6VXX) with a crystal resolution of 2.80 Å. Protein preparation was done with the help of the 'Prepare protein' protocol of Discovery studio 4.0 (DS 4.0). Heteroatoms, ligands, and water molecules present in the crystal structure were removed. (Shree *et al.*, 2020)

Protein-Protein interaction

For network analysis, the STRING v.11.0 https://string-db.org/ (Szklarczyk *et al.*, 2019)(Szklarczyk *et al.*, 2017)database was used for determining the possible protein-protein interactions (PPIs) with other protein partners.

Protein structure validation

The PROCHECK of the SAVES v.6.0 program (https://saves.mbi.ucla.edu/) was performed to predict the Ramachandran Plot(Saikat, 1369)

Prediction of active binding site

The active binding sites for spike glycoprotein was predicted by using the CASTp http://sts.bioe.uic.edu/castp/ web server(Tian *et al.*, 2018)

Ligands Identification and Selection

The potential inhibitors of SARS-CoV-2 spike glycoprotein were taken from various medicinal plants. The total number of 202 active phytochemicals were consider in this study that comprising of 48 from *W. somnifera* (*Ashwagandha*) (Maurya and Sharma, 2020), 27 from *T. cordifolia* (*Giloy*) (Maurya and Sharma, 2020), 48 from *O. sanctum* (*Tulsi*) (Maurya and Sharma, 2020), 64 from *Azadirachta indica* (*Neem*), and 15 from *Adhatoda vasica* (*Adulsa*). This phytochemicals were retrieved from database like PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://www.chemspider.com/), and ZINC15 (https://zinc15.docking.org/).

Molecular docking

PyRx virtual screening tools was used for the preparation of the input files and performing molecular docking using Vina wizard. For the preparation of protein input files, all water molecules, ligands, and ions were removed from *.pdb files. The polar hydrogens were added to the protein structure and prepared files were saved in *.pdbqt format. The molecule's energy was minimized using energy minimization tools of PyRx virtual screening tools and ligands were saved in *.pdbqt format for further docking process. Region-specific docking was performed against SARS-CoV-2 spike protein. The active binding sites for spike glycoprotein was predicted by using the CASTp web server. The grid center for docking was set on the active sites of the spike protein. All docking results were sorted by the binding energy. 2D interaction of the ligand and protein was visualized using Discovery Studio Visualizer.

RESULTS

Protein-protein interaction

The primary focus of protein-protein interactions is confessing how cellular systems operate. Such connections allow the filtering, evaluating, and validating of functional genomics data and offering an insightful platform for annotating functional, structural, and evolutionary features of proteins. The platform can serve predictions for possible experiments and map the interactions between different species (Saikat, 1369). The STRING v.11.0 database was performed to determine the protein-protein interaction (PPIs). This database collects and combines a large number of predicted and experimentally determined PPIs using network-based methods and evaluates PPIs at physical (direct) and functional (indirect) levels. The STRING database determined the functional fellows with scores as of AR (0.940), NKX3-1 (0.856), TMPRSS4 (0.851), ETV1 (0.803), SLC45A3(0.774), ETV4(0.726), ERG (0.726), FKBP5 (0.717), FAM3B (0.706), and PTEN (0.690) respectively. Here the input protein query TMPRSS2 red-colored showed direct interaction and the first shell of interaction is shown in **Figure 1**. TMPRSS2 (Transmembrane Serine Protease 2) is a Protein Coding gene. Diseases associated with TMPRSS2 include Covid-19 and Influenza. It facilitates human coronaviruses SARS-CoV and SARS-CoV-2 infections via two independent mechanisms, proteolytic cleavage of ACE2 receptor which promotes viral uptake, and cleavage of coronavirus spike glycoproteins which activates the glycoprotein for host cell entry.

Protein structure validation

The SAVES server's PROCHECK program was utilized for structural quality assessment of the protein, **(Figure 2)** where the arrangement of the ψ angle and the φ angle were studied. Residues in most favored regions engulfed 86.4% that includes residues in additional allowed regions- 133 (11.8%), generously allowed regions- 13 (1.1%), disallowed regions- 8 (0.7%), non-glycine and non-proline residues- 1131, number of end-residues (excl. Gly and Pro)- 2, number of glycine residues- 82 (shown as triangles), number of proline residues- 58 respectively. A total number of (1273) residues found in the protein 3D structure.

Molecular docking

The Molecular docking study revealed different 202 active phytochemicals present in *Ashwagandha*, *Giloy*, *Tulsi*, *Adulsa*, *Neem* exhibit significant binding affinity. Among those 202 phytochemicals, the 9 best phytochemicals were shown predominantly in our study and they mainly belong to plants like *Ashwagandha* followed by *Neem>Adulsa>Tulsi>Giloy* and that is depicted in **Table 1.**

Potential inhibitors from W. somnifera (*Ashwagandha*) against SARS-CoV-2 Spike glycoprotein

Molecular docking analysis of 48 compounds of W. somnifera (*Ashwagandha*) **(Table 2)** gave three best compounds namely Withanoside VIII (CID_101168805), *Ashwagandha*nolide (CID_16099532) and Sitoindoside IX (CID_ 189586) that showed significant binding affinity towards SARS-CoV-2 Spike glycoprotein.

Withanoside VIII **(Figure 3 (a))** showed various pharmacophoric interactions which include conventional hydrogen bond interaction with residue ASN 99, ARG 190, and GLU 82, pi-alkyl interaction with LEU 226, ILE 119, and VAL 126. Numerous Van der Waals interactions were also showed by many residues. *Ashwagandha*nolide **(Figure 3 (b))** showed various pharmacophoric interactions which includes conventional hydrogen bond interactions with the residues LEU 293, ALA 292, ARG 634, ASP 287, ASN 606, and pi-alkyl interaction with VAL 289 and also few Van der Waals interactions were observed. Sitoindoside IX **(Figure 3 (c))** showed various pharmacophoric interactions which includes conventional hydrogen bond interactions with the residues ASN 121, ARG 190, ILE 101, GLU 96, and alkyl VAL 126 and some Van der Waals interactions.

Potential inhibitor from *Azadirachta indica* (*Neem*) against SARS-CoV-2 Spike glycoprotein

Molecular docking analysis of 64 compounds of *Azadirachta indica* (*Neem*) **(Table 3)** gave two compounds namely Nimocin (ZINC00257568423) and Nimbaflavone (CID_14492795) that showed significant binding Affinity towards SARS CoV-2 spike glycoprotein.

Nimocin **(Figure 4(a))** showed various pharmacophoric interactions which include conventional hydrogen bond interaction with the residues GLY 103, ILE 101, and SER 172. Pi-pi stacked interaction was formed with PHE 175 also Many van der Waals interactions were formed by remaining residues. Nimbaflavone (**Figure 4(b))** showed various pharmacophoric interactions which include conventional hydrogen bond interactions with the residue TRP 104 pi-alkyl interaction with LEU 226, VAL 126 and pi-pi stacked interaction was formed with PHE 175 and Many van der Waals interactions were formed by remaining residues.

Potential inhibitor from *Adhatoda vasica* (*Adulsa*) against SARS-CoV-2 Spike glycoprotein

Molecular docking analysis of 15 compounds from *Adhatoda vasica* (*Adulsa*) **(Table 4)**, only one compound namely alpha-amyrin (CID_ 73170) that showed significant Affinity towards SARS CoV-2 spike glycoprotein.

Alpha- amyrin **(Figure 5)** showed various pharmacophore interactions include pi-sigma PHE 175 and pi-Alkyl interaction TRP 104 and PHE 192 and many van der Waals interactions were formed by remaining residues.

Potential inhibitors from O. sanctum (*Tulsi*) against SARS-CoV-2 Spike glycoprotein

Molecular docking analysis of 48 compounds of O. sanctum (*Tulsi*) **(Table 5)** gave two best compounds namely vitexin (CID_ 5280441) and Ursolic acid (CID_64945) that showed significant binding Affinity towards SARS CoV-2 spike glycoprotein.

Vitexin **(Figure 6 (a))** showed various pharmacophore interactions which include conventional hydrogen bond interaction with the residues TRP 104, GLY 103, and HIS 207, pi- alkyl interaction with ILE 119, ILE 203, and LEU 226, and one unfavored interaction was seen with SER 205. There could be multiple reasons for such unfavorable interactions and it will be clear on doing in vivo analysis. Ursolic acid **(Figure 6 (b))** showed various

pharmacophore interactions which include conventional hydrogen bond interactions with the residues ALA 228. Also, many van der Waals interactions were formed by remaining residues.

Potential inhibitor from T. cordifolia (*Giloy*) against SARS-CoV-2 Spike glycoprotein

Molecular docking analysis of 27 compounds of T. cordifolia (*Giloy*) **(Table 6)** gave only one compound namely Aporphine (CID_114911) that showed significant binding Affinity towards SARS CoV-2 spike glycoprotein.

Aporphine **(Figure 7)** showed various pharmacophore interactions which include pi-Alkyl interaction with VAL 126, ILE 201, ILE 119 also Many van der Waals interactions were formed by remaining residues.

On performing Molecular docking, looking at pharmacophoric interactions and binding affinity scores, it signifies that phytochemicals from W. somnifera (*Ashwagandha*), T. cordifolia (*Giloy*), and O. sanctum (*Tulsi*), *Azadirachta indica* (*Neem*), *Adhatoda vasica* (*Adulsa*) are predicted to acts against potent inhibitors of SARS-CoV-2 spike glycoprotein.

DISCUSSION

Currently, in modern medicine, both individual and combination of anti-malarial, anti-viral, and corticosteroid therapy are being used for treating COVID-19. The medical fraternity is divided on its use amid a lack of strong evidence of its success in treating COVID-19. The raging second wave of the novel coronavirus disease (COVID-19) in India has made one drug nearly a household name: Ivermectin is being prescribed much more liberally than national guidelines suggest. And there is no discernible evidence of how effective this common antiparasitic is in combating the pandemic. Researchers across the world are studying several different therapies to learn whether they might work to treat COVID-19. They are Remdesivir, Dexamethasone, Hydroxychloroquine, etc Drugs like hydroxychloroquine and antibiotics (like azithromycin) were no longer recommended, and Ivermectin was introduced. Remdesivir (Veklury) is an antiviral medication approved to treat COVID-19 in hospitalized patients, but it is not 100% effective. Also, the vaccines like Covishield and Covaxin Vaccines are developed against this novel virus and those vaccines have shown positive and satisfying outcomes. But all these vaccines have multiples unexpected side effects as well, which may harm and alter the individual's normalcy. So hence to find out the best solution with the least side effects The present study have been explored the Ayurvedic medicinal

plant, there are countless benefits over medicinal plant such as naturally available, alleviates hormones and metabolism, anti-inflammatory, and boosting up of the immune system. Natural products with medicinal properties can be used, as the toxicity profile is less in comparison to synthetic compounds. Consulting Ayurveda for treating COVID-19 provides reliable evidence-based medicinal plants for managing the ailment of respiratory disorders.

In silico approaches such as molecular docking and molecular dynamic simulation studies provides the fundamental starting point in terms of binding energy and stability of ligands with proteins for further research. The present work describes the role of active phytoconstituents from Ayurvedic medicinal plants *W. somnifera* (*Ashwagandha*), *T. cordifolia* (*Giloy*), and *O. sanctum* (*Tulsi*) *Azadirachta indica* (*Neem*), *Adhatoda vasica* (*Adulsa*) for treating the COVID 19 pandemic. some of the phytoconstituents from these medicinal plants have shown promising effects against COVID-19 targets.

Since spike glycoprotein protein plays an essential role in virus entry, designing a new drug or inhibitor that can bind to this spike protein and inhibiting its access can be a solution against COVID-19 From present work, it has been suggested that the best-docked compounds obtained from Ayurvedic medicinal plants Nine phytochemicals namely Withanoside VIII, *Ashwagandha*nolide, Sitoindoside IX, Nimocin, Nimbaflavone, Alpha-amyrin, Epitaraxerol, Vitexin, Ursolic acid, and Aporphine obtained from medicinal plants, have predicted the Highest binding energy. These phytoconstituents not only impede the interaction of viral protein to the host cell to transmit and propagate inside the human body but are also safe to repurpose against COVID-19 without any toxicity. Despite WHO declared a pandemic, only a few specific antiviral drug or vaccine has been approved for Coronavirus treatment. Thus, creating an intense need to develop an effective antiviral agent against SARS-CoV-2. The discovery and development of the new drug or vaccine are challenging and time-consuming processes that may vary from few months to years. Therefore, repurposing the existing drugs or using phytochemicals from various medicinal plants can be an alternative to fight the infection. Molecular docking is a crucial tool to understand drug biomolecular interactions in the field of Computer-assisted drug design. The main objective of ligand-receptor docking is to predict the predominant binding mode of a ligand with a receptor (protein) of a known 3-Dimensional structure. Binding energy (B.E) is the capability of a specific ligand (small molecule) and the strength by which a compound interacts and binds to a target molecule's active sites.(Rathan Kumar, 2021) This data is used to study and compare the binding affinity

of different ligands with their corresponding receptor molecule. Lower the binding energy, greater the affinity of a ligand towards the receptor molecule. Thus, a compound with a higher negative value can be chosen as a viable drug candidate.

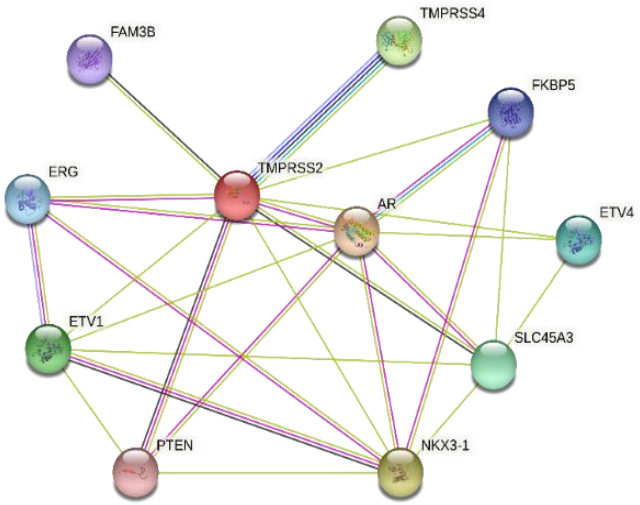


Figure 1: String network of the protein determines the protein-protein interactions.

The color edges represent the line of evidence: cyan color-curated databases, pink - experimentally determined, black - co-expression, light blue - protein homology, and light green- text mining.

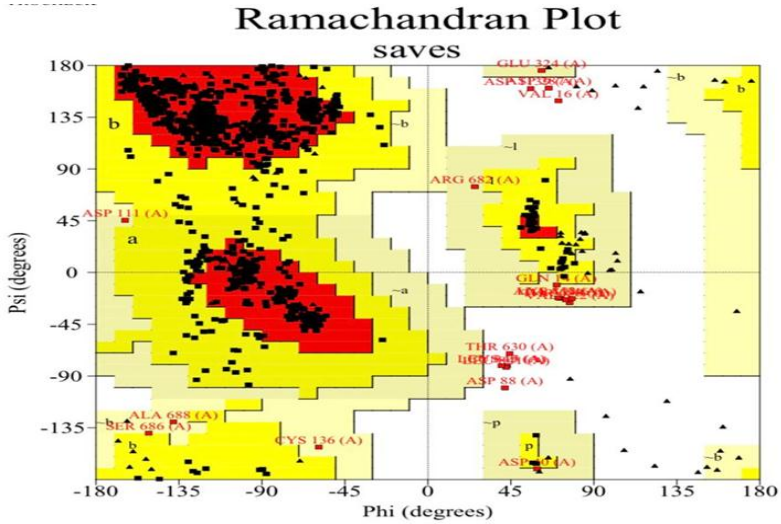


Figure 2: The Ramachandran Plot statistics of the protein structure validated by the PROCHECK program.

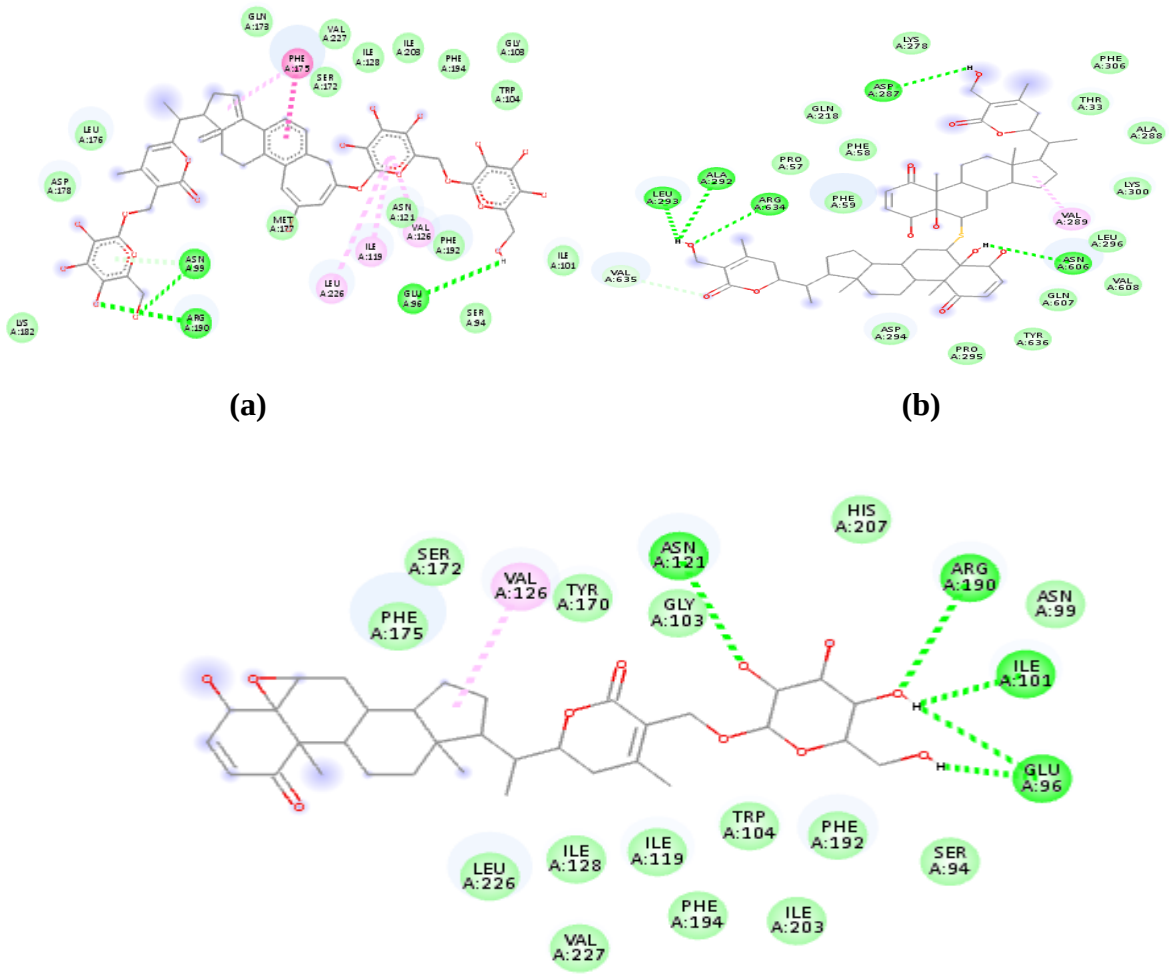


Figure 3: **2D interactions of W. somnifera (*Ashwagandha*) plant phytochemicals with SARS-CoV-2 Spike glycoprotein where (a) shows pharmacophore of Withanoside VIII, (b) shows pharmacophore of *Ashwagandha*nolide, and (c) shows pharmacophore of Sitoindoside IX.**

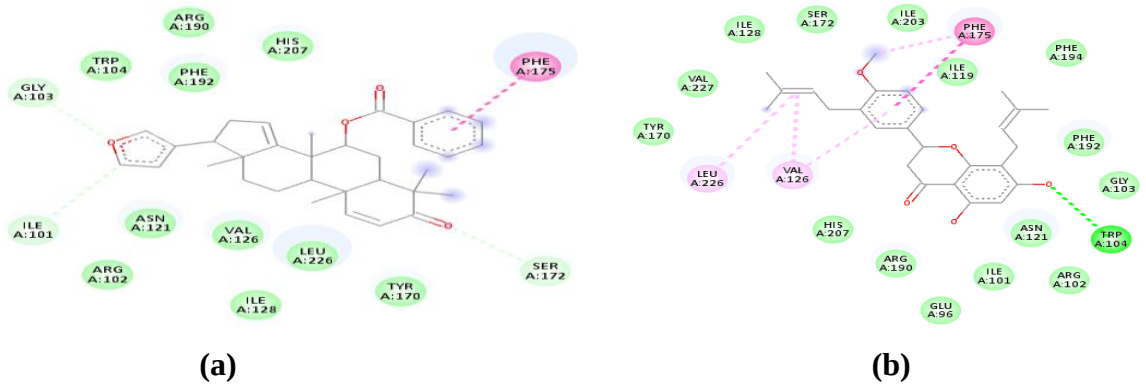


Figure 4: 2D interactions of *Azadirachta indica* (*Neem*) plant phytochemicals with SARS-CoV-2 Spike glycoprotein where (a) Shows pharmacophore of Nimocin, (b) Shows pharmacophore of Nimbaflavone.

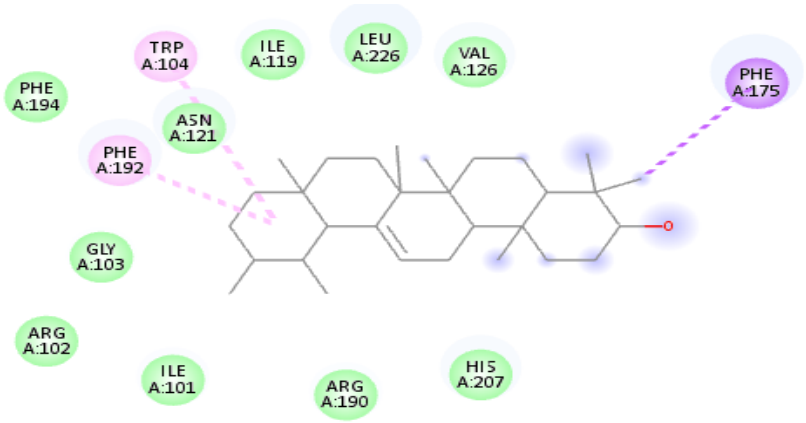
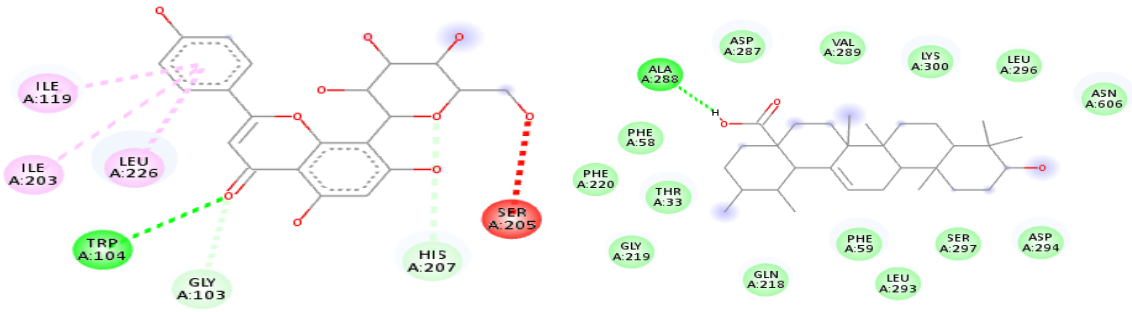


Figure 5: 2D interactions of *Adhatoda vasica* (*Adulsa*) plant phytochemical Alpha-amyrin with SARS-CoV-2 Spike glycoprotein.



(a) **(b)**

Figure 6: 2D interactions of O. sanctum (*Tulsi*) plant phytochemicals with SARS-CoV-2 Spike glycoprotein where (a) shows pharmacophore of Vitexin, (b) shows pharmacophore of Ursolic acid.

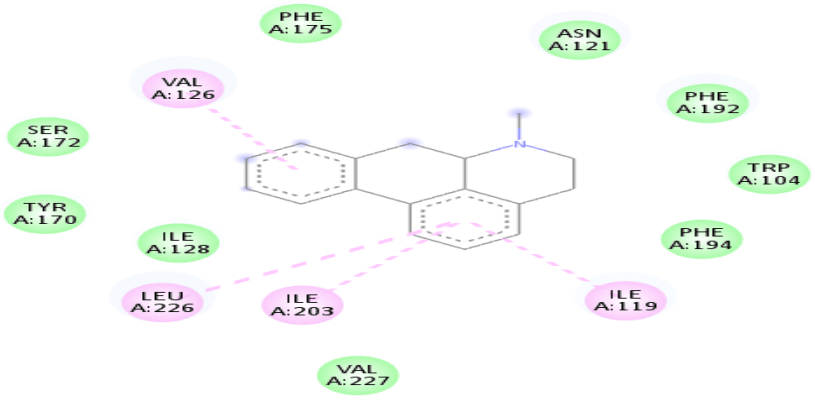


Figure 7: 2D interactions of T. cordifolia (*Giloy*) plant phytochemical Aporphine with SARS-CoV-2 Spike glycoprotein.

Table 1: Top Nine best docked phytochemicals with SARS-CoV-2 Spike glycoprotein.

Sr. no.	Medicinal Plant	Phytochemicals	PubChem / ZINC ID	Binding Affinity (kcal/mol)
1	*Ashwagandha*	a. Withanoside VIII	PC 101168805	-10.7
		b. Ashwagandhanolide	PC 16099532	-10.3
		c. Sitoindoside IX	PC 189586	-10
2	*Neem*	a. Nimocin	ZINC00257568423	-10.1
		b. Nimbaflavone	PC 14492795	-9.2
3	*Adulsa*	a. Alpha-amyrin	PC 73170	-9.6
4	*Tulsi*	a. Vitexin	PC 5280441	-9
		b. Ursolic acid	PC 64945	-9
5	*Giloy*	a. Aporphine	PC 114911	-9

Table 2: Docking output of active phytochemicals from *Withania somnifera* (*Ashwagandha*).

Compound Name	Binding Affinity (Kcal/mol)
Withanoside VIII	-10.7
*Ashwagandha*nolide	-10.3
sitoindoside IX	-10
Beta-Amyrin	-9.8
24,25-dihydrowithanolide D	-9.2
somniferine	-9.1
Withanolide	-9.1
Withaferine A	-9
Withanoside II	-9
Ergostane	-8.9
Withanolide A	-8.9
Withanolide M	-8.9
Withanoide IV	-8.9
Withanolide D	-8.7
Withanolide E	-8.7
coagulin Q	-8.5
Withanolide J	-8.5
Withanolide G	-8.5
Withanolide P	-8.5
Withanoside XI	-8.4
Withanolide S	-8.4
27-deoxywithaferin	-8.4
2,3-dehydrosominifericin	-8.4
27-hydroxywithanolide B	-8.3
17 alpha-hydroxywithanolide D	-8.3
Withanoside III	-8.3
Withanoside Q/R	-8.3
Somnifericin	-8.3
Withanolide B	-8.2

Withanolide O	-8.2
27-deoxy-14-hydroxywithaferin A	-8.2
withanone	-8.1
Withanoside X	-8.1
3-b-hydroxy-2,3-dihydrowithanolide F	-7.9
Withanolide N	-7.9
Withanolide F	-7.9
Withanolide C	-7.5
Chlorogenic acid	-7.2
Withasomnine	-7
Scopoletin	-6.3
Anaferine	-6.2
Anahygrine	-6
Hentriacontane	-5.1
Cuscohygrine	-5
Isopeuetierine	-4.9
Pseudotropine	-4.8
Hygrine	-4.8
Choline	-3.2

Table 3: Details of active phytochemicals from *Azadirachta indica* (*Neem*).

Compound Name	**Binding Affinity (Kcal/mol)**
Nimocin	-10.1
Nimbaflavone	-9.2
Lupeol	-8.9
1 beta, 2 beta diiepoxyazadiradione	-8.8
campesterol	-8.8
Epoxyazadiradione	-8.7
Nimbidinin	-8.7
Melianin A	-8.7
Isoazadirolide	-8.6
phtosterol	-8.6
Beta-amyrin	-8.5
cycloeucalenone	-8.5
7-deacetyl-7-benzoylgedunin	-8.4
7-deactyl-7-benzoyl derivative of azadirone	-8.3
7-deacetyl-7-oxogedunin	-8.2
meliani B	-8.2
nimocinolide	-8.1
kulactone	-8.1
17-hydroxyazadiradione	-8
kaempferol	-8
Isonimocinolide	-8
Azadirone	-8
Isomeldenin	-7.8

6-acetyl nimbandiol	-7.8
beta-carotene	-7.8
Meldenin	-7.8
Isoquercitin	-7.7
17-epi-17-hydroxyazadiradione	-7.6
fraxinellone	-7.5
gedunin	-7.5
24-methylenecycloartanol	-7.4
Isonimolicinolide	-7.3
Diacetylvilasinine	-7.3
Azadirachtol	-7.3
Azardiradione	-7.3
vilasinin	-7.3
nimbidic acid	-7.2
17-epiazadiradione	-7.2
meliaonone	-7.1
azadirachtanin	-7.1
Desacetylnimbin	-7
Azadirachtin	-6.9
salannol acetate	-6.9
4-campsesten-3-one	-6.9
nimbidiol	-6.9
elemol	-6.8
nimbocinolide	-6.8
nimbidinin	-6.8
6-beta-hydroxy-4-stigmasten-3-one	-6.7
Esculetin	-6.7
kulinone	-6.7
24-methylenecycloarton-3-one	-6.7
6-deactylnimbinene	-6.7
nimbinene	-6.6
2,3 dehydrosalannol	-6.4
nimbandiol	-6.4
deactylsalanine	-6.4
salannin	-6.3
cycloeucalenone	-6.2
cinnamic acid	-6.1
benzylalcohol	-4.8
Arachidic acid	-4.1
Docosanoic acid	-3.7
Thioamylalcohol	-3.2

Table 4: Details of active phytochemicals from *Adhatoda vasica* (*Adulsa*).

Compound Name	Binding Affinity (Kcal/mol)
Alpha amyrin	-9.6
Epitaraxerol	-9.2
Sitosterol	-8.4

Vasicoline	-7.5
Vasicolinone	-7.2
Adhatodine	-7.1
Vasicinolone	-6.8
Deoxyvasicinone	-6.7
Vasicine	-6.5
Anisotine	-6.5
Vasicol	-6.3
Carotene	-6.2
Vasicinone	-5.9
Vasicinol	-5.6
Deoxyvasicine	-5.5

Table 5: Details of active phytochemicals from *Ocimum sanctum (Tulsi).*

Compound Name	Binding Affinity (Kcal/mol)
ursolic acid	-9
vitexin	-9
cirsilineol	-8.9
orientin	-8.9
molludistin	-8.9
apigenin	-8.6
isothymonin	-8.6
isothymusin	-8.5
luteolin	-8.5
sitosterol	-8.4
galuteolin	-8.2
isovitexin	-8
vicenin-2	-8
Aesculin	-8
luteolin-7-o-glucuronide	-8
oleanolic acid	-7.9
cadinene	-7.6
vicenin	-7.3
alpha-cadinol	-7.1
cirsimaritin	-6.7
chlorognic acid	-6.6
caryophyllene	-6.5
Gallic acid ethylester	-6.1
bergamotene	-6.1
Methyl cinnamate	-6.1
Eugenol	-6
Gallic acid methylester	-5.8
vanillic acid	-5.8
carvacrol	-5.8
Rosmarinic acid	-5.7
linolenic acid	-5.6
terpinene-4-ol	-5.5

steric acid	-5.4
3-carene	-5.4
Gallic acid	-5.3
Eucalyptol	-5.3
Beta-pinene	-5.3
citral	-5.3
ocimene	-5.3
4-hydroxybenzoic acid	-5.2
alpha-pinene	-5.2
methyl eugenol	-5.2
Estragole	-5.2
oleic acid	-5.1
4-hydroxybenzaldehyde	-5
ascorbic acid	-4.9
linoleic acid	-4.8
Linalool	-4.6

Table 6: Details of active phytochemicals from *Tinospora cordifolia (Giloy).*

Compound Name	**Binding Affinity (Kcal/mol)**
Aporphine	-9
Berberine	-8.7
Pregnane glycoside	-8.6
Jatrorrhizine	-8.6
cordioside	-8.2
beta-sitosterol	-8.2
columbin	-8
palmatine	-8
tinosponone	-7.7
isocolumbin	-7.6
tetrahydropalmatine	-7.6
tinocordioside	-7.3
ecdysterone	-7.3
tinosporide	-7.3
chasmnthin	-7.2
tinosporin	-7
magnoflorine	-7
palmarin	-6.9
bergenin	-6.8
20 β-hydroxyecdysone	-6.7
Tinocordifolin	-6.5
Tembetarine	-6.4
Sinapic acid	-5.8
Syringin	-5.5
Heptacosanol	-4.9
Octacosanol	-3.9
Choline	-3.2

CONCLUSION

Currently, the outbreak of the COVID-19 has become the biggest threat to human health, and the lack of any specific antiviral drug has created a global challenge for developing an effective drug or inhibitor against SARS-CoV-2 that can be quickly produced and easily distributed at an affordable cost. In this situation, the in silico approach can be a useful tool for identifying phytochemicals that can inhibit its entry into the host cells, which could be the potential therapeutic solution for the disease. Protein networking, protein coding genes, disease-associated genes, and the mechanism of the protein-protein interaction were all demonstrated. This aided in determining the association of the SARS-CoV-2 protein pathway and whether those genes are associated with any other diseases, learning about its function, and identifying potential spike-glycoprotein inhibitors. Using various bioinformatics tools, we identified potential inhibitors of the spike glycoprotein of SARS CoV-2 in this study. In this study, 9 best phytochemicals from various medicinal plants were discovered to be capable of binding and inhibiting SARS-CoV-2 spike glycoprotein. Those 9 best phytochemicals with the highest binding affinity are from *Ashwagandha>Neem>Adulsa>Tulsi>Giloy* (in descending order) can serve as effective alternative therapeutics against the COVID-19 infection. Furthermore, these findings can be taken in powder form or in the form of ‘kadha,' which will boost an individual's immunity and inhibit viral severity by interfering at the various stages of virus multiplication in the infected person.

ABBREVIATIONS

SARS CoV: Severe acute respiratory syndrome corona virus

MERS CoV: Middle East respiratory syndrome

WHO: World Health Organization

RT-PCR: Real-Time polymerase chain reaction (PCR)

HCoVs: Human corona virus

NABs: Neutralizing antibodies

RCSB: Research collaborator for structural Bioinformatics

SDF: Structure Data file

PDB: Protein data bank

CASTp: Computed Atlas of surface Topography of proteins

PPIs: Protein-Protein Interactions

ACE2: Angiotensin-converting enzyme 2

TMPRSS2: Transmembrane serine protease 2

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CONFLICT OF INTEREST

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