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IN-SILICO DETERMINATION OF PHYTOCHEMICALS AGAINST SPIKE PROTEIN OF COVID-19

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ABSTRACT

Spike protein is present on the exterior of SARS-CoV-2 that mediates the binding of virus with human ACE2 receptor. S-protein has the ability to mutate in a short span of time. Using S-protein as a therapeutic target, Covid-19 infection can be prevented. Many plant-derived phytochemicals are found effective to treat viral infections. In this study, we selected top 10 phytochemicals following the Lipinski's rule of five from total 82 candidate phytochemicals. The binding energies were determined through molecular docking of the phytochemical ligands. Top three compounds having maximal interactions and lowest binding energies were visualized. We suggested Dictamnine, Deoxypodophyllotoxin and Deoxyartemisinin as therapeutic agents for Covid-19. The lowest binding energy was observed for Dictamnine (-20.4) followed by Deoxypodophyllotoxin (-20.1) and Deoxyartemisinin (-15.6). This study suggested the binding energies with the highest negative value are more effective to treat viral infection caused by SARS-CoV-2. Further studies on mechanism of actions, bioavailability and clinical trials can provide a clear way for the development of effective compounds that can pave the way for the discovery of the effective drug Covid-19 infection.

Key Words: Covid-19, drug designing, molecular docking, phytochemicals, spike proteins.

Abbreviations: ACE: Angiotensin Converting Enzyme, ADME: Absorption, Distribution, Metabolism and Excretion, WHO: World Health Organization, 3D: 3-Dimesional.

INTRODUCTION

SARS-CoV-2, the agent responsible for coronavirus infection was declared the global pandemic by WHO (He et al., 2020; Kannan et al., 2020; Lu et al., 2020; Park et al., 2020). It affects the respiratory epithelial lining of humans (Azkur et al., 2020; Guan et al., 2020). Club-shaped, type I glycoprotein spikes are present on the virus. Infection occurs due to the coupling of S-protein with the human Angiotensin converting enzyme 2 (ACE2) receptor (Cui and Shi, 2019; Wang et al., 2020).

Spike protein (S-protein) has two domains, one helps in recognizing and binding with host cell while other mediate the access in the host cell membrane by penetrating it (Li, 2016; Kakodkar et al., 2020; Mohamadian et al., 2021). Therapeutic targets help to prevent infections as it block the interaction between host receptors and infectious agents. Known therapeutic targets for combating this virus are S-protein and ACE2 receptors. Thus, manipulation of S-protein and ACE2 receptors can obstruct the binding, and as a consequence prevent viral entry into the host cell.

Phytochemicals are non-nutritive chemicals derived from plants *e.g.* alkaloids (block the active site of virus and results in reduced viral titers) and flavonoids (inhibits the reverse transcriptase and replication) (Edoga et al., 2005; Yadav and Agarwala, 2011). Phytochemicals have anti-inflammatory, anti-oxidant and anticancer properties (Han et al., 2007; Ali et al., 2008; Yadav and Agarwala, 2011). These are important component of ethnomedicines and ethnopharmacology (Govindappa et al., 2011) and play key role for development of many drugs. Phytochemicals do not harm the humans and help in regulating certain cellular pathways.

The need for the development of an effective drug to treat viral infections has always been a challenge for humans. Scientists are trying their best to find a cure for this viral infection and have found that some phytochemicals effective against viral infections including Covid-19 (Catanzaro et al., 2020). The use of pharmacological plants has a usual inclination for treatment purposes. Plants based medicines along with computational drug designing are considered an effective approach for development in drug discovery (Black, 1996; Sliwoski, et al., 2014; Dali et al., 2019).

There is no evidence for the availability of 100 % effective drugs treating this infection. The in-silico method of drug designing has gained significant importance as it provides an estimate of the effectiveness of the drug and is cost-effective. Contextually, in-silico determination of better drugs can be best method over laboratory procedures. We aimed for the use of in-silico method to design a cheap and serviceable drug for the treatment of Covid-19. For this purpose, plant derived phytochemicals and their effect on inhibiting the entry of virus were studied. Many phytochemicals were selected and their effect for inducing a conformational change and inhibition of spike protein and ACE2 complex was studied through molecular docking analysis. Present research can deduce huge pile of biomedical information for in-silico drug designing. Additional works to expound the possible mechanisms of phytochemicals against Covid-19 and bioavailability of these alkaloids are recommended.

MATERIALS AND METHODS

Data Retrieval and Visualization

The step-wise process that leads to the drug designing involves the identification of the target protein, comparative modeling, screening, molecular docking, and then the drug is subjected to clinical trials (Ou-Yang et al., 2012). All the data were retrieved by aforementioned steps. Phyre2 is an online tool based on the principles of homology modeling. It was used for the retrieval of 3D structures of proteins. Spike protein structure was downloaded from phyre2. PyMol was utilized for visualization of 3D structure of S-protein. It was also used to notice the interactions between protein and ligand.

The three-dimensional structure of ligands was retrieved from the online chemical database PubChem. The ligands were saved in SDF format along with the molecular structure and canonical smiles. PDB format of ligands is required because our software supports only PDB format. The conversion of SDF format into PDB format was done by online SDF to PDB convertor in the following order;

Open online SDF to PDB convertor > select PDB and 3D > Browse > select SDF file > open >Translate > Save

We installed the MGL tools and AutoDock Vina software for conversion of S-protein into PDBQT format for molecular docking (Linota et al., 2014; Joseph et al., 2017). In the AutoDock tools the PDB file of the S-protein was opened

through (*File > read molecule >destination >file name >open).*

After opening PDB file, water molecules were removed by *Edit >delete water* order and H atoms were supplemented via (*Edit > hydrogens > Add > polar only > ok).* Subsequently, protein structure (in PDBQT format) was saved. During target residue selection, PDBQT format of S-protein was opened into Auto Dock by executing (*File > Read Molecule > select file > open)* steps. Then the target amino acid residue was selected by (*Select > Select from string > Residue > Add > Dismiss).*

After residue selection, the grid box was generated by setting in *(Grid > Grid Box).* Then values for x, y, z dimensions were set by keeping spacing at 1.000 angstroms. The values for x, y, z dimensions were set in such a way that the target residues PRO330, CYS336, GLU340, ALA372, and CYS379 should have in the center of the grid box from all the sides. The aforementioned residues are active sites for the ligand to bind with. Auto Dock tools were also used for the format conversion of PDB files of ligands into PDBQT. (*Ligand > input> open> destination> file name (type PDB files) >open)*. In the final step, Molecular docking was performed using Auto-dock vina.

The ligands that followed Lipinski's Rule of Five and showing a negative sign for binding energy with high value were selected for Molecular docking (Linota et al., 2014; Joseph et al., 2017). Docking outcomes were observed by PyMol. Separate window was used to evaluate the protein-ligand interfaces and connected sites. Polar contact, angel, and dash view were set to observe the interaction between ligand-protein at the right place. All possible results obtained after docking was visualized in this way.

RESULTS

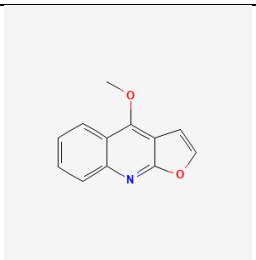
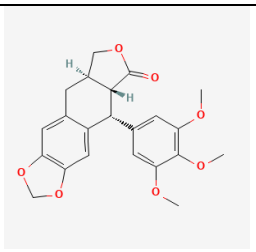
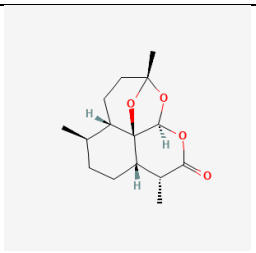
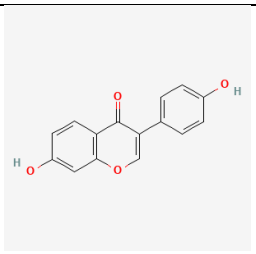
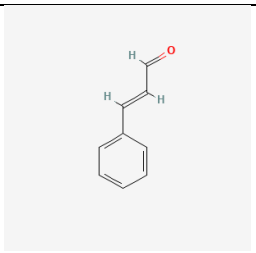
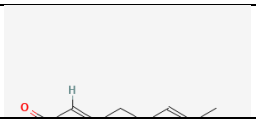
Present work includes the designing of an effective drug to treat covid-19. For selecting some candidate phytochemicals, 82 phytochemicals were studied on the basis of binding energies and interactions to the target proteins (Table S1 in Supplementary Material). Further, Lipinski's rule of five assisted in screening out 40 phytochemicals. The alkaloids were selected for their drug like properties, affinity with S-protein and number of binding residues (Table S2 in Supplementary Material). To avoid any failure in most probable drug selection; absorption, distribution, metabolism and excretion properties (ADME) were applied on 40 phytochemicals. We are suggesting following three lead compounds to be highly effective against Covid-19 (Table S3 in Supplementary Material).

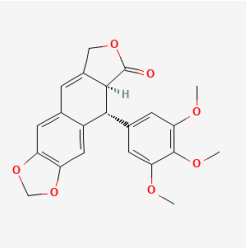
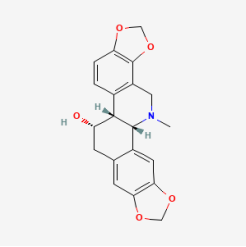
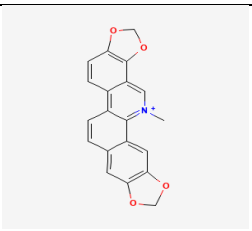
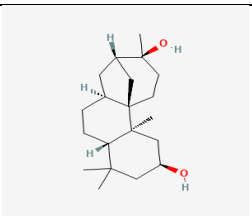
i. Dictamnine

The highest negative binding energy was observed by Dictamnine (-20.4). It interacted at THR 430 (CO-OC, 3.1), PHE 515 (CO-OH, 3.0), LEU 517 (CN-OC, 2.9), HIS 519 (CN-OC, 3.3), and HIS 519(CN-OC, 3.2) residues as shown in Fig 1 (b). Higher the negative binding energy of the ligand, the higher will be the suitability to interact with the target protein. The ADME properties of this alkaloid show that it can be used as a dominant therapeutic agent. This phytochemical interacted with five various residues and can be used effectually to block the activity of the target protein. The binding affinity and the interactions of other phytochemicals have been listed in the Table 1.

Dictamnine phytochemical is present in *Dictamnus angustifolius* (Wu et al., 1999) vernacularly known as Burning bush, Gas plant or Fraxinella

Table 1: Showing top 10 phytochemicals found effective against Covid-19, ADME properties and their affinity with S-protein.

Phytochemicals	ADME Properties		Drug Likeliness	Affinity	Amino Residue	Structure
DICTAMNINE	**Properties**	**Values**	Yes	-20.4	THR 430 PHE 515 LEU 517 HIS 519	
	Molecular weight (<500 Da)	199.21				
	H-bond acceptor (<10)	3				
	H-bond Donor (5)	0				
	LogP (<5)	2.38				
	Molar Refractivity (40-130)	58.01				
	Violations	**No**				
DEOXYPODOPHYLLOTOXIN	Molecular weight (<500 Da)	398.41	Yes	-20.1	THR 430 PHE 515 LEU 517 HIS 519	
	H-bond acceptor (<10)	7				
	H-bond Donor (5)	0				
	LogP (<5)	3.41				
	Molar Refractivity (40-130)	102.68				
	Violations	**No**				
DEOXYARTEMISININ	Molecular weight (<500 Da)	266.33	Yes	-15.6	ASP 428 ARG 355	
	H-bond acceptor (<10)	4				
	H-bond Donor (5)	0				
	LogP (<5)	2.74				
	Molar Refractivity (40-130)	69.29				
	Violations	**No**				
DAIDZEIN	Molecular weight (<500 Da)	254.24	Yes	-13.4	THR 430 ASP 428	
	H-bond acceptor (<10)	4				
	H-bond Donor (5)	2				
	LogP (<5)	1.77				
	Molar Refractivity (40-130)	71.97				
	Violations	**No**				
CINNAMALDEHYDE	Molecular weight (<500 Da)	132.16	Yes	-9.9	ASP 428 SER 514	
	H-bond acceptor (<10)	1				
	H-bond Donor (5)	0				
	LogP (<5)	1.65				
	Molar Refractivity (40-130)	41.54				
	Violations	**No**				
CITRAL	Molecular weight (<500 Da)	152.23	Yes	-9.9	SER 514 ASP 428	
	H-bond acceptor (<10)	1				

	H-bond Donor (5)	0				
	LogP (<5)	2.47				
	Molar Refractivity (40-130)	49.44				
	Violations	**No**				
ALPHA-APOPICROPODOPHYLLOTOXIN	Molecular weight (<500 Da)	396.39	Yes	-8.6	THR 430 SER 514	
	H-bond acceptor (<10)	7				
	H-bond Donor (5)	0				
	LogP (<5)	3.32				
	Molar Refractivity (40-130)	103.00				
	Violations	**No**				
CHELODONINE	Molecular weight (<500 Da)	353.37	Yes	-8.0	ARG 355 THR 430 SER 514 HIS 519	
	H-bond acceptor (<10)	6				
	H-bond Donor (5)	1				
	LogP (<5)	3.21				
	Molar Refractivity (40-130)	96.12				
	Violations	**No**				
SANGUINARINE	Molecular weight (<500 Da)	332.33	Yes	-8.0	ASN 343	
	H-bond acceptor (<10)	4				
	H-bond Donor (5)	0				
	LogP (<5)	-0.04				
	Molar Refractivity (40-130)	94.68				
	Violations	**No**				
STEMODIN	Molecular weight (<500 Da)	306.48	Yes	-7.7	ARG 355 THR 430 THR 430 SER 514	
	H-bond acceptor (<10)	2				
	H-bond Donor (5)	2				
	LogP (<5)	3.2				
	Molar Refractivity (40-130)	91.38				
	Violations	**No**				

It belongs to Rutaceae family, commonly found in temperate and open forest habitats of Southern Europe, North Africa, and mostly in Asia. More than a hundred chemicals have been extracted from this species like alkaloids, flavonoids, phenylpropane, limonoid triterpenoids, and coumarins.

In Pakistan, it is found in Western Himalayas. Moreover, Dictamnine can be extracted from three species of genus Hortia (Rutaceae), namely H. brasiliana, H. longifolia, H. superba (Portela et al., 2020).Dictamnine can cause cytotoxicity in women's cervix, colon, and oral carcinoma. Despite all these harmful side effects, it can combat bacteria and fungi like Saccharomyces cerevisiae, with a very low inhibitory concentration. Its interaction has been depicted in Figure 1.

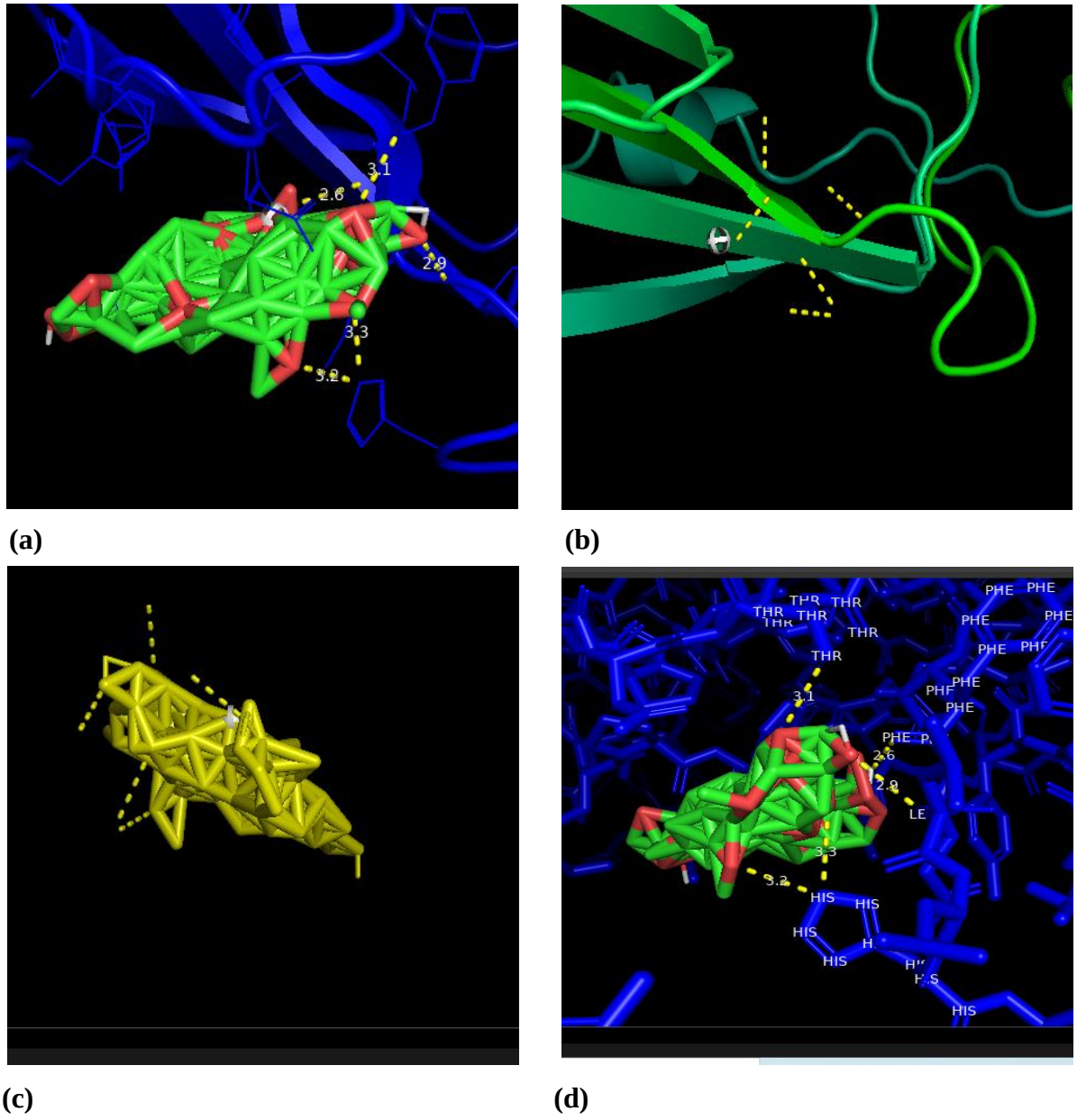


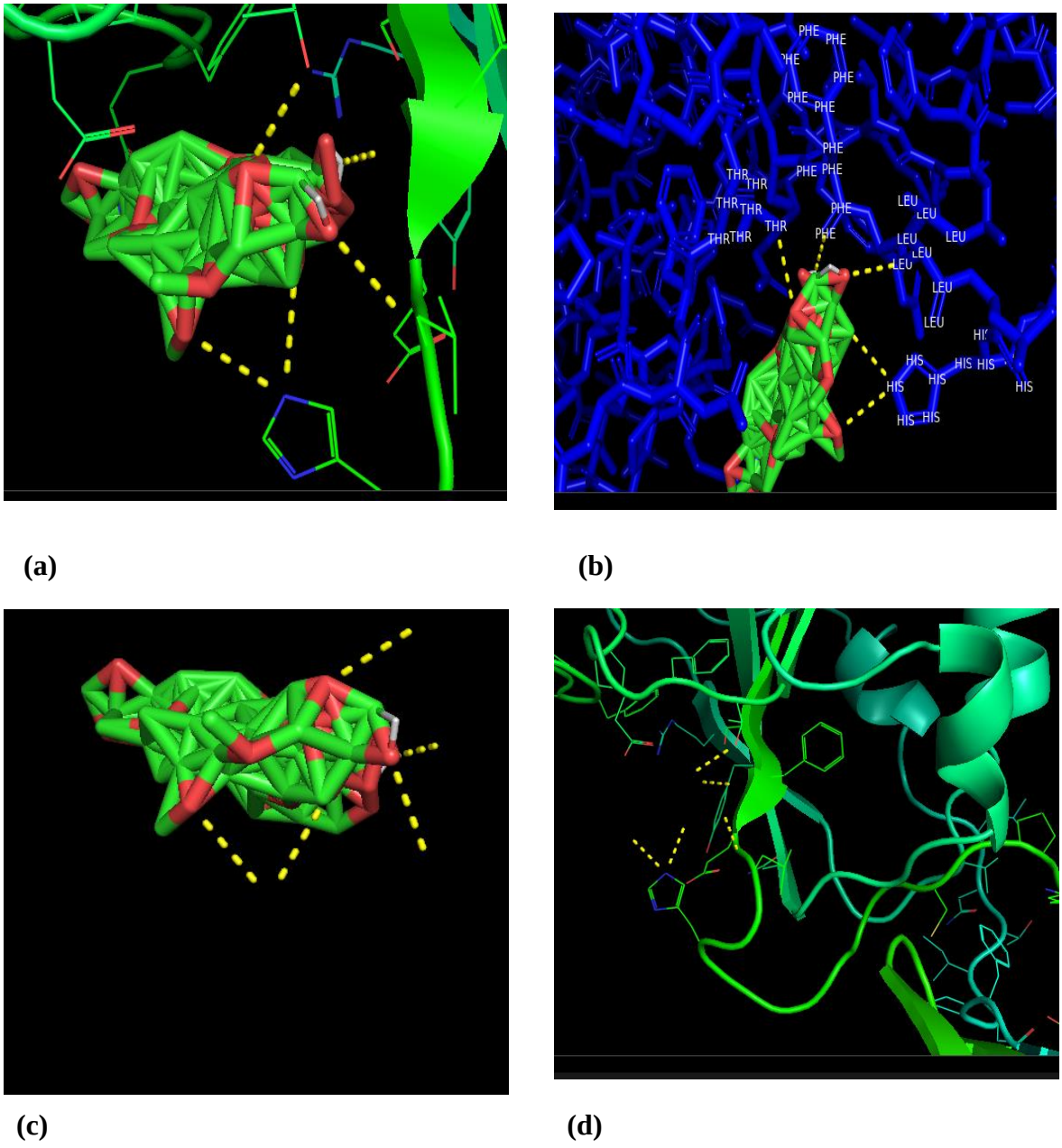
Figure 1: (a) Shows the cartoon view of interactions between Dictamnine and Covid S-protein, (b) Dictamnine showing the greatest interaction with S-protein residues: THR 430, PHE 515, LEU 517, HIS 519 and HIS 519, (c) Showing the Dictamnine ligand which have maximum potential to bind with S-protein, (d) Covid S-protein domain that is the active site for phytochemicals to bind with.

i. Deoxypodophyllotoxin

The second highest (negative) energy was shown by Deoxypodophyllotoxin (-20.1) at THR 430 (CO-OC, 3.1), PHE 515 (CO-OH, 2.6), LEU 517 (CN-OC, 2.9), HIS 519 (CN-OC, 3.3) and HIS 519 (CN-OC, 3.2) as shown in Table 1.

Deoxypodophyllotoxin is alignin-type chemical with the potential to treat tumors and inflammation, Figure 2 (c). This phytochemical is highly bioactive as antiviral agent (Hu et al., 2016). It has been used for cancer treatments of human prostate gland (Hu et al., 2016), cervix (Yong et al., 2009) and breasts (Benzina et al., 2015). Deoxypodophyllotoxin is extracted from *Anthriscus sylvestris* (Apiaceae), *Podophyllum hexandrum* (Berberidaceae), *Pulsatilla koreana* (Ranunculaceae), *Callitris intratropica Juniperus chinensis* and *J. procera*

(Cupressaceae) (Muhammad et al,, 1995; Ali et al., 1998; Giri and Narasu, 2000; Kim et al., 2002). Among above mentioned Deoxypodophyllotoxin sources, *P. hexandrum* is native to Northern areas of Pakistan including Himalayan region and Gilgit Baltistan, South Asia, Eastern United States and Southeastern Canada.



(a) **(b)**

(c) **(d)**

Figure 2: (a) Cartoon view of Deoxypodophyllotoxin and S-protein of Covid-19, (b) Shows the sencond maximal interaction of Deoxypodophyllotoxin with THR 430, PHE 515, LEU 517, HIS 519 residues of S-protein, (c) Separate view of Deoxypodophyllotoxin ligand, (d) The active site of Covid-19 S-protein domain that have affinity for different pytochemicals.

In Pakistan it is hard to find it due to overexploitation for ethnomedicinal purposes. The whole plant is very poisonous except some ripened parts. It is also used as purgative and cytostatic. Due to its high toxicity, it has not yet been used clinically. Its interaction S-protein is being depicted in Figure 2 (a).

ii. Deoxyartemisinin

Phytochemical Deoxyartemisinin showed binding energy -15.6 and interaction at ASP 428 (CO-OH, 2.1) and ARG 355 (CN-OC, 3.3) as shown in Figure 3 (a). It has been found effective to block the replication of the Covid-19 and its recent variants.

It shows anti-malarial, anti-scabies, anti-*Candida albicans* infections, anti-bacterial capabilities like *E. coli*, Salmonella, and other bacterial infections along with plasmodial parasites including *Schistosomas* (Cai et al., 2004; Mishina et al., 2007). Its interaction with S-protein has been depicted in Figure 3 (b).

Deoxyartemisinin is extracted from *Artemisia spp.* especially *A. annua* belonging to Asteraceae family. It is also called Sweet wormwood, Sweet annie and Sweet sagewort. It is native to temperate Asia including Pakistan and China, but established itself in many countries like North America. In Pakistan, total 25 species of genus *Artemisia* are extensively found (Ghafoor, 2002). In traditional remedies of many diseases these plants and their extracts are commonly used across the Pakistan. *A. annua* also has flavonoids which can block the replication of Covid-19.

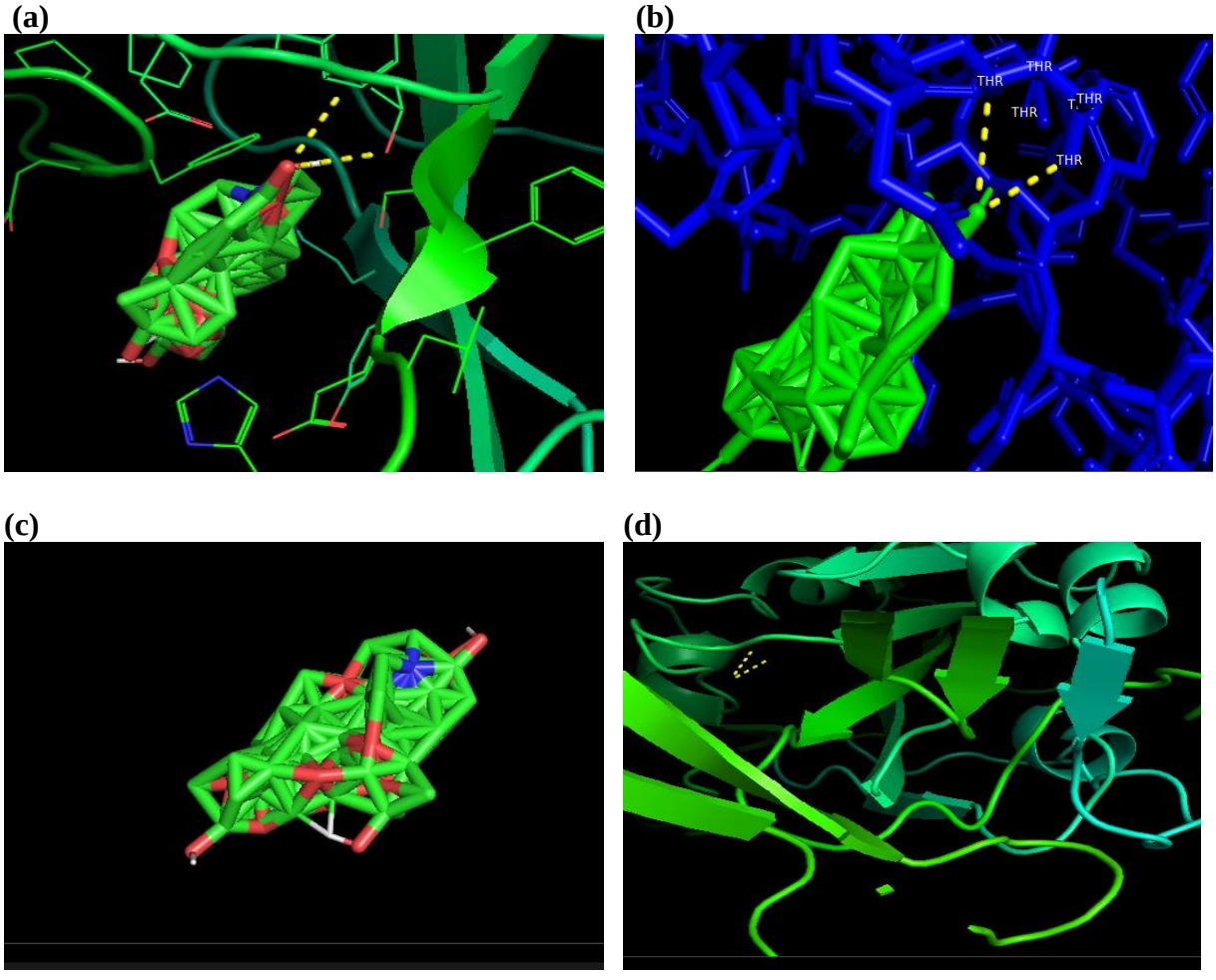


Figure 3: (a) Deoxyartemisinin and S-protein (cartoon view) interactions at two binding sites namely: ASP 428 and ARG 355, (b) Deoxyartemisinin showing interaction with S-protein residues, (c) 3-D view of phytochemical Deoxyartemisinin ligand, (d) Covid-19 S-protein domain that provide active binding sites to the phytochemicals.

DISCUSSION

Antiviral agents are used to reduce viral loads and stop the development of other respiratory infections. Plasma therapy, differential efficiency of vaccines and side-effect, and intensive mutation in the spike proteins of Covid-19 are emphasizing for the development of other chemo-active and inexpensive drugs (Basiri et al., 2021; Tenforde, 2021). Regenerative medicine are different cell-tissue therapeutics (Basiri et al., 2021). The most effective drug can be made utilizing all the information regarding the biological and structural aspects.

In this era of advancement in technology, screenings of drug-like small molecules has become very efficient by using various time efficient techniques and computer aided programs (Lim, et al. 2012). Different types of parameters related to drug like molecules are optimized for successful drug discovery (Sliwoski, et al., 2014). In-silico drug designing and molecular docking involves determination of ligand-receptor interactions which depends on binding of bioactive lead compounds to desired receptor (Joseph et al, 2017). This approach for drug design has good clinical rates (Sehgal et al., 2018). For lead compound some explicit threshold energy to bind is must and conclusively shows drug characteristics.

These lead bioactive compounds can easily be extracted from plants by using different techniques like High Performance Liquid Chromatography-HPLC (Ingle et al., 2017). Isolated concentrated compounds have greater efficiency, lesser side effects and similar contrivances of mechanisms as synthetic drugs (Yadav and Agarwala, 2011). Most of the phytochemcials are toxic as *Deoxyartemisinin* (Sabir et al., 2010), the determination of proper dosage and administration is of utmost importance, All of our suggested phytochemicals can be used against Covid-19, after improving these compounds for ADME, enhancing their potency, bioavailability and reducing their toxicity (Ou-Yang, et al., 2012; Tung, 2014). These tests with improvements can the lead drug into preclinical development.

Phytochemicals are extracted from wild and cultivated plants that help against pathogenic microorganisms including viruses (Marjorie, 1996; Govindappa, et al., 2011). The evasion strategies used by the viruses are an important tool in giving insight for developing anti-viral drugs against them. Most of the phytochemicals have the ability to affect various steps of viral attack and replication so that their antiviral property is enhanced due to their specified mechanism.

Phytochemical on binding with receptors induces some conformational changes in their structures. Further, blocks the progression of viral attack on the host cells. Molecular docking analysis involves the visualization of interaction between ligand (phytochemical) and receptor protein (Linota, et al., 2014). It clearly shows these induced conformational changes on the binding of ligand and elucidates the drug action. (Russo et al., 2020). The allosteric modulation by phytochemical ligands hinders the interaction of the S-protein and ligand is considered specifically in molecular studies.

Several studies confirmed the application of useful phytochemicals as drugs for the treatment of potentially harmful ailments at no or minimum cost (Loew, et al, 1993; Dutta, et al, 2010; Leelananda and Lindert, 2016). Whereas, synthetic drugs require a large span of time to be made and are economically expensive (Mishra and Tiwari, 2011). More than 50 % of the world medicines are made from flora or their extracts (Ghani, 1998). Plants or their extracts are used for curing different conditions in human and animals. The important factor to utilize plants in sickness is high costs of allopathic medications and unapproachable

medical centers (Qureshi et al., 2006; Mahmood, et al., 2011; Mishra and Tiwari, 2011).

CONCLUSION

From this study, it can be concluded that phytochemicals have great potential to inhibit S-protein to cure Covid-19 infection. In summation, present study aimed to screen or design a drug by using phytochemicals with no or less harm to block the association between S-protein of Covid-19 and ACE2 receptor of human cells. The selected residues for docking were *PRO 330, CYS336, GLU340, ALA372, and CYS379.* Some phytochemicals showed the lowest binding energy with the target protein. This binding energy shows suitable interactions of ligand to the target protein active site. These phytochemicals can block the viral binding site and reduce viral titers.

Many phytochemicals like Dictamnine, Deoxypodophyllotoxin and Deoxyartemisinin showed the lowest binding energies and their efficacy was confirmed by molecular docking as well. The source plants of these phytochemicals are *Dictamnus albus Anthriscus sylvestris* or *Podophyllum spp.*and *Artemisia annua*, respectively. They have anti-Covid-19 and anti-inflammatory agents. These phytochemicals are under trial and might be used in future to prevent and cure Covid-19. Although, these studies have revealed promising results but there is a lot to be done in this regard to check the effectiveness of phytochemicals in combating viral infections, their success in clinical trials, and the cost-effectiveness. To that end, future works must focus for collecting, purifying and characterizing these suggested chemicals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Muhammad Irfan conducted the study as a part of the thesis; Muhammad Adil, Areej Fatima and Arooj Fatima partially retrieved and visualized the data; Ammara Khalid gave valuable inputs and suggestions for the drafts; and Muhammad Bilal gave the feedbacks during the whole study, wrote the present manuscript and revised it.

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