International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Plumbagin's Molecular Docking Studies as a Possible Target for the SARS-Cov-2 Spike Receptor Protein

Murugesan Raman[1], Girija Shanmugam[2], Ashokkumar Jayavel[3]

[1,2]Plant Biotechnology Laboratory, Department of Biotechnology, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India
[3]Bioinformatics & Data Management, ICMR – National Institute of Virology, Pune, Maharashtra, India.

Abstract
Around the end of 2019, reports of unusual cases of pneumonia with specific symptoms started to arrive in Wuhan, China. Within a few weeks, the atypical pneumonia spread throughout China, and a few months after that, it became a global pandemic. The etiological agent of that pandemic, which is officially known as COVID-19, is Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In an attempt to find an efficient therapy to either fight the spread of COVID-19 or to cure infected people from its tissue damage, researchers around the world have been studying several plants, herbs, and natural products. Abundant studies have revealed that organic compounds can be very operative in averting virus-mediated infection. The purpose of this study was to accomplish molecular docking studies among plant-derived naphthoquinone (Plumbagin) and spike receptor (THR304) proteins of coronavirus. MGL virtual screening tool and Biovia Discovery Studio were utilized in the current molecular docking investigations. Outcomes of docking studies exposed that selected organic compounds have interacted with targeted spike receptor protein with binding energies in the range of -4.0 to -9.5 kcal, which means the binding energy of the target and ligand is -5.78. In summary, plumbagin seems to be a more effective primary protease inhibitor than the other chosen ligands for deactivating the SARS-Coronavirus.

Keywords: SARS-CoV-2; Plumbagin; Molecular docking.

1. Introduction

Plants play a vital role as valuable sources of medicine, with a considerable number of drugs being derived from them. One such plant commonly used in the traditional Indian system of medicine is Chitraka, scientifically known as *Plumbago spp*. It belongs to the Plumbaginaceae family. *Plumbago spp.* is a perennial, sub scandent shrub and is widely found in the wild as well as in cultivation due to its numerous therapeutic benefits (Nguyen, et al. 2004). The roots of *Plumbago spp.* exhibited to have antiviral, antioxidant, hypolipidemic, anti-atherosclerotic, central nervous system stimulant, and anti-fertility properties (Bisht et al 2023). The SARS-CoV-2 is a highly infectious virus that causes COVID-19, a serious respiratory infection that has caused over 57 million infections and over 1.3 million deaths worldwide, as of 11/20/20 (kent et al 2020). SARS-CoV-2 causes infected cells to express a main protease that is responsible for site-specifically cleaving the polyprotein, which is translated from viral mRNA within human cells. The proteolytic activity of the main inhibiters is essential for the virus to generate the

individual proteins that are necessary for replication and infection. The essential role of M^{pro}, as well as the success of HIV protease inhibitors in the treatment of HIV/AIDS, make M^{pro} an attractive therapeutic target to treat Covid-19 (Dai et al 2020, Jin et al 2020, Zhang et al 2020) Corona-viruses are a diverse group of viruses that belongs to the family coronaviridae. They are composed of a long RNA strand. Their genome is the largest among all RNA viruses. They are named after the crown-like spikes present on their surface. The main infection is caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) (Sood et al. 2021; Vashishth and Tehri 2020). SARS-CoV-2 Ibeta coronavirus belongs to the coronaviridae family, similar to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). It is one among the 36 coronaviruses in the family of coronaviridae within the order Nidovirals. Members of this family are mainly known to cause respiratory or intestinal diseases in various creatures including humans (Tuli et al. 2021a). Recent research suggests that natural plant-based compounds such as phytochemicals including flavonoids, alkaloids, and others may be useful in the development of safe SARS-CoV treatments (Talwar et al. 2020; Vardhan and Sahoo 2020; Silveira et al., 2020; Tuli et al. 2021a; Tuli et al. 2021b;). In the year 2020, Vardhan and Sahoo 2020) pursued in silico computational analysis of phytochemicals including glycyrrhizic acid, limonin, 7-deacetyl-7-benzoylgedunin, maslinic acid, corosolic acid, obacunone, and ursolic acid and suggested their utility as promising drugs to target proteins of SARS-CoV-2. Similarly, (Hall and Ji 2020) explored the in-silico potential of Zanamivir, Indinavir, Saquinavir, and Remdesiviras proteinase inhibitors.

Nowadays, one of the most widely used computer programs on the molecular docking realm is AutoDock Vina, a software developed by (Trott and Olson 2010) and whose main application is to perform rigid-flexible molecular docking screenings. In this type of in silico methodology, the chosen target remains rigid (with no rotation, no translation and no torsion), while its associated ligand has enough flexibility to generate distinct conformations (positions), out of which a favorable interaction can be established (Trott & Olson 2010).

Many pathogens have been fought off with the help of medicinal plants. This significant finding might be explained by the presence of plumbagin in naturally occurring plants (Figure 2). This has led to the emergence of a wide range of drug classes derived from natural plants. For centuries, people have utilized teas, poultices, decoctions, and a plethora of other methods to extract and administer the medicinal properties of herbs. Furthermore, assessments have been carried out in the past few decades to determine their potential therapeutic applications (Atanasov et al 2015).

The advantages of natural compounds over synthetic ones are astounding. Compounds derived from medicinal plants do not require synthesis, even though both of these chemical entities have the same structure and, consequently, the same physicochemical and biological properties. As a result, they have a positive environmental impact because extraction methods may use less organic solvent which is generally known to be toxic in smaller amounts. (Fuzimoto & Isidoro, 2020). For this reason, we analyzed plumbagin compounds to verify their efficacy against SARS-CoV-2 proteins through in-silico investigations.

2 Materials and methods

We have used different online bioinformatics servers, databases such as PDB, PubChem and tools and PYMOL, Discovery studio in this in silico docking study to examine the naphthoquinone docking interaction against the SARS-CoV2 spike protein with plumbagin

2.1 Software's Used in Docking Interaction

Our MGL virtual screening tool has been applied to receptors ranging from small molecules to macromolecules. MGL is a free and open-source virtual screening tool. It is a mixture of numerous software like Autogrid4, Open Babel, and others. MGL includes a docking wizard with an easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. Biovia Discovery Studio is a software suite that enables life science researchers to analyze and model molecular structures, sequences, and other data. Vina and Autogrid4 are the docking software used by MGLtools. The software provides tools for displaying and editing data as well as performing simple data analysis. Biovia Discovery Studio offers many tools for working with and visualizing data.(Figure 1)

2.2 Retrieval of Three-Dimensional Structure

The Three-dimensional Structure of the SARS coronavirus spike receptor-binding domain with THR304 (Figure 3) was retrieved from the online database RCSB protein data bank and which was later on viewed in PyMol software. The energy minimization and optimization of the target molecule were studied in the Swiss Protein Databank Viewer. In the 3-D structure of THR304, all the water molecules were removed which were not involved in ligand interaction, and all the missing atoms and valences were corrected (Rivas et al 2019).

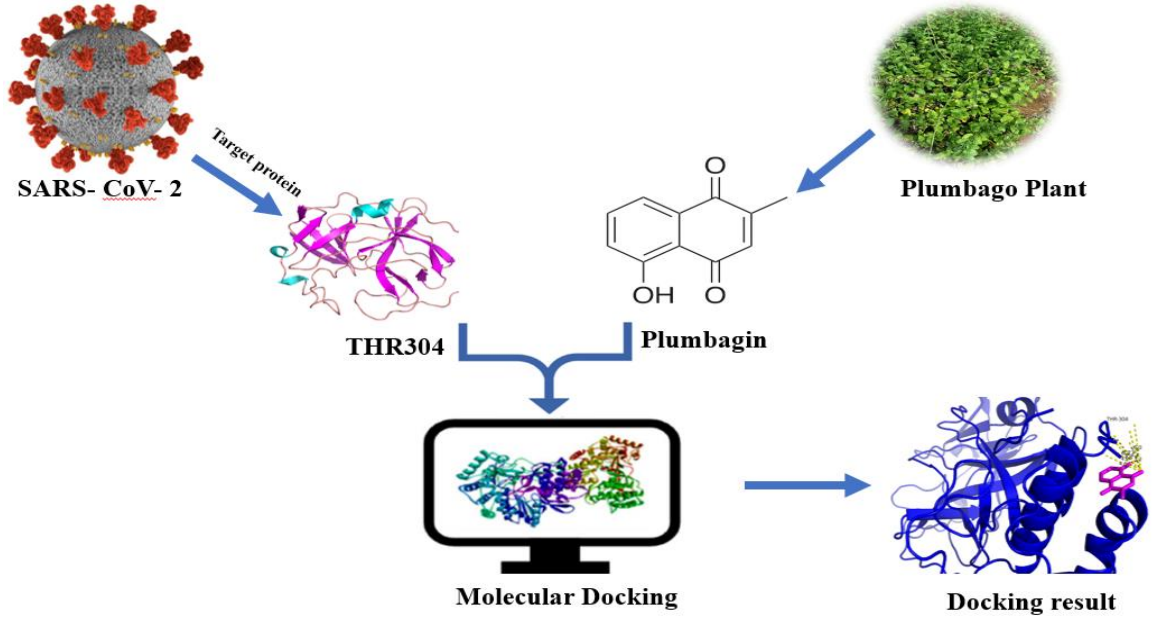


Fig. 1 Graphical abstract

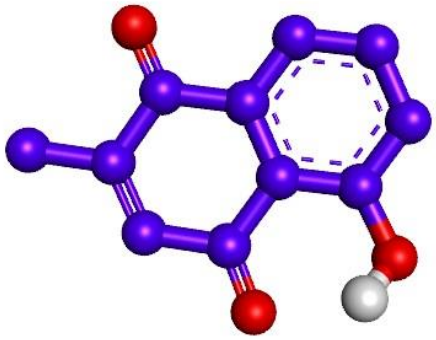


Fig.2 Plumbagin

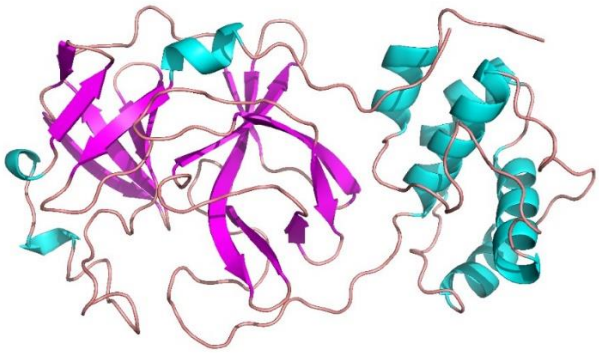


Fig. 3 Three-dimensional structure of SARS CoV2 spike protein

Table 1: Prediction of Molecular properties of Plumbagin by Molinspiration and 'Lipinski's rule of five

Property Name	Property Value	Reference
Molecular weight	188.18 g/mol	Computed by PubChem 2.1(PubChem release 2021.05.07))
XLogP3	**2.3**	Computed by PubChem 3.0 (PubChem release 2021.05.07)
Hydrogen Bond Doner Count	**1**	Computed by PubChem 3.4.8.18 (PubChem release 2021.05.07)
Hydrogen Bond Acceptor Count	**3**	Computed by PubChem 3.4.8.18 (PubChem release 2021.05.07)
Rotatable Bond Count	**0**	Computed by PubChem 3.4.8.18 (PubChem release 2021.05.07)

2.3 Selection and Preparation of Ligands and Prediction of Molecular Properties

For the present study, plumbagin's ligands were selected from sources of plants and herbs. PubChem was used to retrieve the ligands. (Figure 4) and saved in MOL SDF format and energy minimization, hydrogen bonds, and geometrical confirmations were done and modified in the MarvinBean Package. The online tool Lipinski rule of 5 (Lipinski et al 2001). After identified ligands were screened using Molinspiration [www.molinspiration.com], it was discovered that ligands complied with the Lipinski rule of 5. The ligands used in the investigation are listed in Table 1 along with their physiochemical properties and the five criteria of the Lipinski rule.

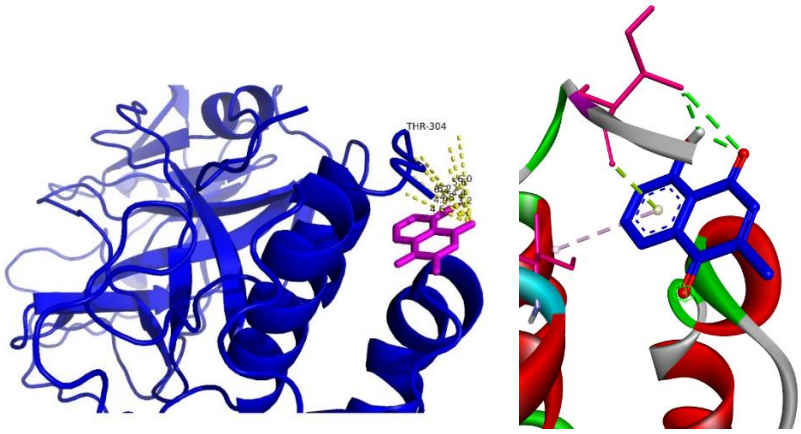
2.4 Molecular docking

Studying the ligand and protein binding affinities as well as the binding of drug targets, protein receptors, or enzymes for interactions is done through virtual screening. We have used a free version of MGL tool

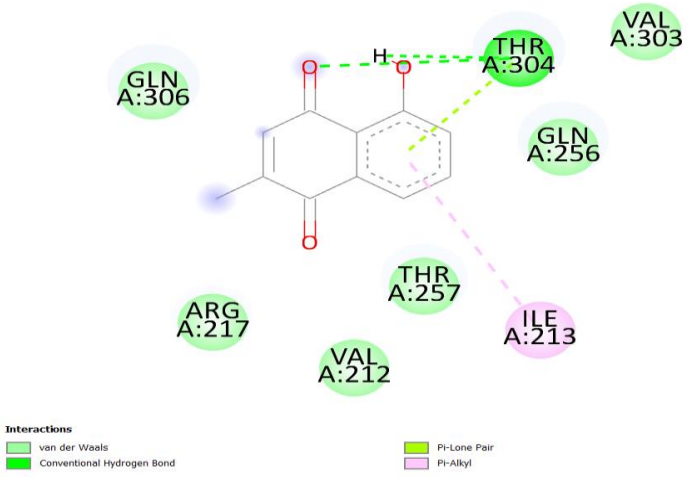
software to study molecular docking. All the default docking algorithms were used, and all the coordinates X, Y, and Z were set in the grids which were placed in the active site pocket center, and the lowest binding energies were the best suitable for interactions (Trott and Olson 2010)

3 Results and discussion

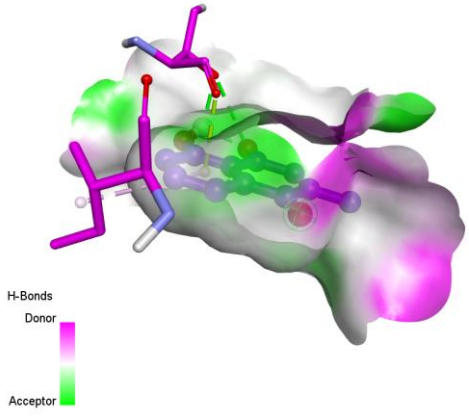
Plumbagin has been found to contain naphthoquinone that may help combat COVID-19. Furthermore, these compounds exhibit potential as therapeutic candidates by hindering viral replication. Furthermore, their antiviral properties have motivated us to find favorable compounds that can impede the initial infection and/or replication of SARS-CoV-2. (Calland et al., 2012; Carneiro et al., 2016; Ismail & Jusoh, 2017). Studies reveal that a number of ligands can interact with different SARS-CoV-2 proteins, and their findings demonstrate a high degree of binding affinity between a particular ligand and the protein that it is associated with (Senger et al., 2020). A well-known tactic in computational drug design, the "lock and key" scheme, is the foundation for binding energy between two molecules. This idea forms the basis of the protein-ligand or even protein-protein perfect fit. H-bonds, van der Waals forces, and other interacting bonds cause specific interactions between two structures and produce a complex between two molecules. In this context, researchers look for the lowest possible energy to generate stability, which takes place when two substances that form a complex come together, as previously described by the “lock and key” scheme (Chen et al., 2020). The root mean square deviation values (RMSD) were used to evaluate the docking results for the tested ligands with the receptor protein in this analysis. These RMSD values were based on the coordinates between the atoms and their conformational changes. The binding energy (kcal/mol) data enables us to investigate and compare the binding affinity of various ligands/compounds with their respective target receptor molecules. Binding energy represents the sum of total internal energy minus the energy which is linked to the unbound system. A greater affinity between the ligand and the receptor is indicated by a lower binding energy. In other words, Lower the binding energy the most favorable is docking results. The ligand with the highest affinity can be selected as a possible drug candidate for further research. The result of docking studies revealed that the ligands had good binding energy with the target molecule PDB ID: 6LU7 (Figure 4) THR304, spike receptor protein of SARS-CoV2, binding energy -5.78 kcal (Table 2). Recently, (Hagar et al. 2020) investigated the anti-covid activities of N-Heterocycles by using computational tools (Hagar et al. 2020). Similar to this, several phytochemicals were examined as potential anti-covid therapies using in silico tools. These included nelfinavir, lopinavir, kaempferol, quercetin, luteolin-7-glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, epicatechin-gallate, zingerol, gingerol, and allicin. (Khaerunnisa et al. 2020). The remarkable energy score values found in our research study involving plumbagin be explain using ligand-protein stabilization through hydrogen bonds observed from in silico poit of view



A B



C



D

Fig. 4 Conformational changes observed due to the binding of plumbagin with the residue THR304 **(A)** 3D surface are interactions of plumbagin with receptor binding domain of SARS-CoV **(B)** is 2D interactions of plumbagin and THR304(**C**) Surface region of Donor and Acceptor of ligand and its interactions with Hydrogen bonds (**D**).

Table 2: Interactions of Plumbagin with receptor protein and values of binding energy and RMSD

Rank	Sub Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	-5.80	0.00	55.36	Ranking
2	1	5	-5.79	0.00	55.18	Ranking
2	2	2	-5.78	0.17	55.15	Ranking
3	1	4	-5.32	0.00	63.28	Ranking
4	1	1	-5.22	0.00	73.84	Ranking
4	2	7	-5.20	0.05	73.84	Ranking
4	3	6	-5.19	0.05	73.82	Ranking
5	1	8	-5.05	0.00	72.80	Ranking
6	1	10	-5.05	0.00	59.90	Ranking
6	2	3	-5.01	1.52	59.53	Ranking

Conclusions

Overall, our molecular docking study has revealed binding interaction profiles that, as stated above, indicate plumbagin have the potential to adversely impact the SARS-CoV-2 infection process. The ability of these compounds to interact with vital proteins of the novel coronavirus confirms that more research must be done on phytochemicals' ability to fend off infections. Therefore, every result from our investigation points to a useful future use of promising plumbagin derived from plants like *Plumbago spp*., in the field of in vivo and in vitro studies that will support our findings against Covid-19.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding this article.

Reference:

1. Bisht, S., Tyagi, R., Madan, S., Saxena, S., Raj, P. M., & Walia, R.(2023). Comparative evaluation of Nephroprotective Potential of Root, Stem And Leaf of Plumbago Zeylanica: An In-Silico And In-Vitro Approach.
2. Dai, W., Zhang, B., Jiang, X. M., Su, H., Li, J., Zhao, Y., ... & Liu, H. (2020). Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science*, *368*(6497), 1331-1335.
3. Hagar, M., Ahmed, H. A., Aljohani, G., & Alhaddad, O. A. (2020). Investigation of some antiviral N-heterocycles as COVID 19 drug: molecular docking and DFT calculations. *International Journal of Molecular Sciences*, *21*(11), 3922.
4. Hall, J.D.C., & Ji, H.F. (2020). A search for medications to treat COVID-19 via in silico molecular docking models of the SARSCoV-2 spike glycoprotein and 3CL protease. Travel Medicine and Infectious Disease, 35, 101646.
5. Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., ... & Yang, H. (2020). Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*, *582*(7811), 289-293.
6. Kent, A. J. (2020). Mapping and Counter-Mapping COVID-19: From Crisis to Cartocracy. *The Cartographic Journal*, *57*(3), 187-195.

7. Khaerunnisa, S., Kurniawan, H., Awaluddin, R., Suhartati, S., Soetjipto, S. (2020). Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study. Preprints, 2020, 2020030226. DOI: 10.20944/preprints202003.0226.v1).
8. Lipinski, C.A., Lombardo, F., Dominy, B.W., & Feeney, P.J. (2001). Experimental and computational approaches to a estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 46, 3-26.
9. Nguyen, A. T., Malonne, H., Duez, P., Vanhaelen-Fastre, R., Vanhaelen, M., & Fontaine, J. (2004). Cytotoxic constituents from Plumbago zeylanica. *Fitoterapia*, *75*(5), 500-504.
10. Rivas, F. (2019). Identification of substituted 5-membered heterocyclic compounds as potential antileukemic agents. European Journal of Medicinal Chemistry, 164, 391-398.
11. Silveira, D., Prieto-Garcia, J.M., Boylan, F., Estrada, O., et al. (2020). COVID-19: is there evidence for the use of herbal medicines as adjuvant symptomatic therapy? Frontiers in Pharmacology, 11, 1-44.
12. Sood, S., Aggarwal, V., Aggarwal, D., Upadhyay, S.K., et al. (2020). COVID-19 pandemic: from molecular biology, pathogenesis, detection, and treatment to global societal impact. Current Pharmacology Reports, 6(5), 212-227.
13. Sood, S., Bhatia, G.K., Seth, P., Kumar, P., et al. (2021). Efficacy and safety of new and emerging drugs for COVID-19: Favipiravir and Dexamethasone. Current Pharmacology Reports, 7(2), 49-54.
14. Talwar, S., Sood, S., Kumar, J., Chauhan, R., Sharma, M., & Tuli, H.S. (2020). Ayurveda and Allopathic Therapeutic Strategies in Coronavirus Pandemic Treatment 2020. Current Pharmacology Reports, 22, 1-10.
15. Trott, O., & Olson, A.J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31, 455–461.
16. Tuli, H.S., Bhatia, G.K., Sood, S., Debnath, P., Aggarwal, D., & Upadhyay, S.K. (2021c). In Silico Analysis and Molecular Docking Studies of Plumbagin and Piperine Ligands as Potential Inhibitors of Alpha-Glucosidase Receptor. Biointerface Research in Applied Chemistry, 11, 9629 – 9637.
17. Tuli, H.S., Bhatia, G.K., Sood, S., Debnath, P., et al. (2020). In Silico evaluation of harmane & palmarin as α Glucosidase inhibitors: Hope for the discovery of antihyperglycemic compounds. International Journal of Pharmaceutical Research, 12, 1331-1336.
18. Tuli, H.S., Sak, K., Aggarwal, P., Iqubal, A., et al. (2021b). Molecular evolution of severe acute respiratory syndrome coronavirus 2: Hazardous and more hazardous strains behind the coronavirus disease 2019 pandemic and their targeting by drugs and vaccines. Frontiers in Cellular and Infection Microbiology, 11, 763687.
19. Tuli, H.S., Sood, S., Kaur, J., Kumar, P., et al. (2021a). Mechanistic insight into anti-COVID-19 drugs: recent trends and advancements. 3 Biotech, 11(2), 1-18.
20. Vardhan, S., & Sahoo, S.K. (2020). Searching inhibitors for three important proteins of COVID-19 through molecular docking studies. Preprint from arXiv, 1-15.
21. Vashishth, A., & Tehri, N. (2020). Novel coronavirus covid-19: characteristics, transmission, pathogenesis and pandemic report. Journal of Biology and Nature, 11(3), 15-21.
22. World Health Organization (2020). Coronavirus disease (covid2019) situation reports.

23. Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., ... & Hilgenfeld, R. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*, *368*(6489), 409-412.
24. Chen, G., Seukep, A.J., Guo, M., 2020. Recent Advances in Molecular Docking for the Research and Discovery of Potential Marine Drugs. Marine Drugs. 18(11), 545.
25. Senger, M.R., Evangelista, T.C.S., Dantas, R.F., Santana, M.V.D.S., Gonçalves, L.C.S., Neto, L.R.D.S., Silva-Junior, .., P, F., 2020. COVID-19: molecular targets, drug repurposing and new avenues for drug discovery. Memórias do Instituto Oswaldo Cruz. 115.
26. Calland, N., Albecka, A., Belouzard, S., Wychowski, C., Duverlie, G., Descamps, V., Hober, D., Dubuisson, J., Rouillé, Y., Séron, K., 2012. Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry. Hepatology. 55(3), 720–729.
27. Carneiro, B.M., Batista, M.N., Braga, A., Nogueira, M.L., Rahal, P., 2016. The green tea molecule EGCG inhibits Zika virus entry. Virology. 496, 215–218.
28. Ismail, N.A., Jusoh, S.A., 2017. Molecular Docking and Molecular Dynamics Simulation Studies to Predict Flavonoid Binding on the Surface of DENV2 E Protein. Interdisciplinary Sciences: Computational Life Sciences. 9, 499–511.
29. Fuzimoto, A.D., Isidoro, C., 2020. The antiviral and coronavirushost protein pathways inhibiting properties of herbs and natural compounds-Additional weapons in the fight against the COVID-19 pandemic? Journal of Traditional and Complementary Medicine. 10(4), 405–419.