SEMARAK ILMU PUBLISHING

Journal of Advanced Research in Applied Sciences and Engineering Technology

Journal homepage:
https://semarakilmu.com.my/journals/index.php/applied_sciences_eng_tech/index
ISSN: 2462-1943

Molecular Docking of Phytochemicals from *M. Charantia* Targeting SARS-CoV-2 Main Protease

Nur Hannani Ahmad Rozani[1], Fatahiya Mohamed Tap[1,*], Fadzilah Adibah Abd Majid[2], Nur 'Ainun Mokhtar[3], Nurul Bahiyah Ahmad Khairudin[4]

[1] School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA Caw Terengganu 23200 Bukit Besi, Terengganu, Malaysia
[2] Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[3] Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Pulau Pinang, Bertam Campus, 13200 Kepala Batas, Pulau Pinang, Malaysia
[4] Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, International Campus, Jalan Sultan Yahaya Petra, 54100 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 6 January 2024
Received in revised form 23 May 2024
Accepted 5 June 2024
Available online 20 June 2024

Keywords:

Molecular docking; COVID-19; Binding affinity; Molecular interactions; Inhibitors

ABSTRACT

Coronavirus Disease 2019 (COVID-19) is a transmittable disease caused by severe acute respiratory coronavirus 2 (SARS-CoV-2). The outbreak of the disease has affected the world in a negative manner. The development and on-going vaccination efforts has reduced the mortality rate in people with existing comorbidities and lowered virus transmission rate. However, there are still the needs for prescribed medicines that can be consumed by COVID-19 infected persons. *Momordica charantia* L. has been used in traditional medicines to treat various diseases including anti-diabetic and cancer. Pharmacological studies on *Momordica charantia* L. revealed that the plant stored many useful phytoconstituents that might be exploited for producing drug compounds to treat COVID-19. Nowadays, computational-aided drug methods are widely used in drug discovery process as it requires minimal compound design and improve the development pipeline. In this study, molecular docking of five phytochemicals from *Momordica charantia* L. were carried out against SARS-CoV-2 main protease (Mpro). The results obtained revealed that some of the phytochemicals showed a better dock score compared to the drug molnupiravir. Based on the dock score and the medicinal properties of each compound, it is suggested that these compounds can be further studied for potential drugs against COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a type of disease that affect the human respiratory system. The transmission of the disease is caused by a new beta coronavirus strain, namely, severe acute respiratory syndrome 2 (SARS-CoV-2). On March 2020, the World Health Organization (WHO) has declared the COVID-19 outbreak a global pandemic, as the number of infected persons increases rapidly within a short time. The symptoms of the disease vary from asymptomatic to severe, which in serious cases, could be fatal and lead to death. The most common symptoms seen in patients with

* *Corresponding author.*
E-mail address: fatahiya@uitm.edu.my

https://doi.org/10.37934/araset.47.1.152165

mild infection include fever, sore throat, cough and shortness of breath [1]. Patients in severe cases often experience organ dysfunction, acute cardiac injury, acute kidney injury and in worst cases, death might occur [1]. According to the evidences from COVID-19 outbreak, people with pre-existing comorbidities such as hypertension, cardiovascular disease and diabetes are at much greater risk of dying from COVID-19 infection [2,3].

The development of vaccines and ongoing vaccination effort have shown drastic reduction in the number of hospitalizations and fatality caused by COVID-19 [4,5]. However, there are still the need for drug compounds that can specifically target and inhibit the viral entry of SARS-CoV-2 into human body. Resultantly, many FDA drugs have been repurposed to alleviate the symptoms and illness associated with COVID-19 infection. Many *in-vitro* studies and clinical trials have been done to assess the efficacy of the repurposed drugs against the deadly SARS-CoV-2. Presently, many therapeutic options are available, such as antiviral therapies, monoclonal antibodies, anti-inflammatory drugs, and immunomodulators agents [6]. Drugs such as hydroxychloroquine, remdesivir, favirapir and molnupiravir have shown a positive result in inhibiting the pathogenicity of the virus [7]. Howbeit, adverse side effects were also observed in the patients.

Researchers have been striving to find safer alternatives to COVID-19 treatments by utilizing phytochemicals as the main compounds produced in the drugs against the disease. *Momordica charantia* L. or commonly known as bitter gourd, is a flowering vine in the *Cucurbitaceae* family and has long been used in food and medicines [8]. It is widely grown in tropical and subtropical regions of the world such as Malaysia, China, Tropical Africa, Middle East, America and Thailand [8,9]. *M. charantia* contains a wide array of novel and biologically active phytochemicals including proteins, flavonoids, triterpenes and steroids that can be used in many disease treatments [8,9]. Medicinally, this plant has been used since ancient times as it possesses various pharmacological function such as antidiabetic, anthelmintic, antimalarial, contraceptive and laxative [9]. Nowadays, the advancement in computational drug methods has permeated all aspects of drug discovery. The techniques in computational modelling have cut down research timeline and cost by reducing wet-lab experiment for designing certain drugs. Molecular docking is one of the computational methods that enable researchers to predict and model the interaction between small molecules and protein at atomic level [10]. It is a method designed to understand drug biomolecular interactions, which is essential for rational drug design and discovery, as well as mechanistic study by inserting small molecule (ligand) into the targeted binding site of the specific region of the DNA/protein (receptor), forming a stable complex of potential efficacy and specificity [11,12]. By observing these interactions, the behaviour of these molecules in the binding site can be characterized, and fundamental biochemical processes can be elucidated [10]. The purpose of docking is to determine the best possible conformational poses of protein-ligand, protein-protein or any other type of interactions with minimal binding affinity [13]. Determination of accurate binding poses of the ligand to the receptor is imperative for deciding its binding affinity, thus qualifying it to be utilized as potential lead compounds.

In the present study, molecular docking method was used to elucidate the ligand-receptor binding mechanism and model the interaction between five phytochemical compounds in *M. charantia* and SARS-CoV-2 main protease.

2. Methodology

2.1 Preparation of Protein and Ligands for Docking

The 3D structure of SARS-CoV-2 main protease in bound with N3 inhibitor (PDB ID: 6LU7) was downloaded from Protein Data Bank. Firstly, all of the water molecules, ions and N3 inhibitor were

removed using Pymol software. Then, the structure of the protein was saved in PDB format for further analyse. The 3D structure of each phytochemical (rutin, kaempferol, quercetin, luteolin and catechin) was retrieved from PubChem in SDF format. Rutin is a compound from the class of flavonols that are widely studied for its use in drug design and formulation. Previous studies on rutin revealed amazing physiological and pharmacological properties of the compound in mammalia systems either in-vitro or in-vivo [14]. Rutin were mainly known for its strong antioxidant property and its role in preventing various pathologies [15]. Other biological activities of rutin such as anti-inflammation [16], [17] anti-microbial [18] anti-tumour [19] and anti-asthma [20] acted as free radical scavenger that contributed to the strong antioxidant property of rutin [14]. Kaempferol is a type of flavonoid compound that were found in glycoside form in most plants [21]. Previous studies found that kaempferol and its derivatives were profuse with biological properties, including cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antimicrobial, antitumor, as well as anticancer properties [21,22].

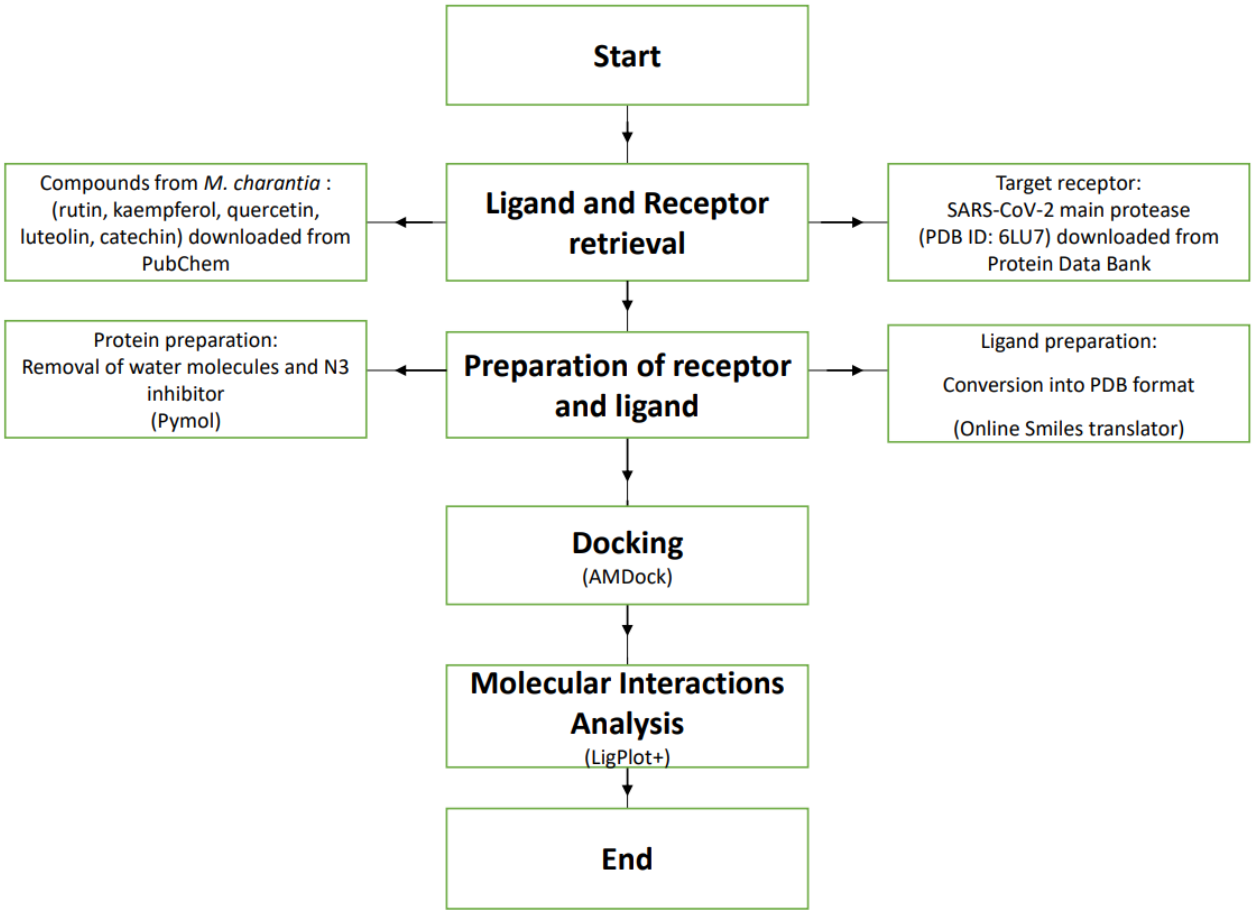


Fig. 1. Flow chart of methodology utilized in present study

Another compound from the subclass flavonol, quercetin, has shown great pharmacological activities in mammals and each of the properties were actively studied by researchers in drug design. Similar as rutin, quercetin shown a strong antioxidant property due to its ability to scavenge free radicals and bind with the transition metal ions [23]. Other pharmacological actions including anticancer, antiviral, anti-allergic, metabolic and inflammatory disorder, eye and cardiovascular disease, and arthritis were also reported [24]. Luteolin, a derivative compound of quercetin is known to possess wide range of pharmacological actions, including anti-inflammatory activity, antioxidant, antimicrobial, anticancer and neuroprotective activities [25]. Catechin is a type of phenolic

compounds that were found concentrated in many dietary products, plants, and fruits. Catechin is known to play important role in molecular mechanisms that involved in angiogenesis, apoptosis, extracellular matrix degradation, and multidrug resistance in cancer and related disorders [26]. Each of the structures were then converted to PDB format using Online Smiles Translator. The 3D structure of reference molecule in SDF format was also downloaded from PubChem and converted into PDB format using Online Smiles Translator.

2.2 Procedures for Molecular Docking

Molecular docking of five phytochemicals from *M. charantia* against main protease of SARS-CoV-2 is performed using AMDock [27]. AMDock (Assisted-Molecular Docking) is a graphical tool that assist users in docking of protein-ligand complexes using AutoDock Vina and AutoDock4 (Valdés-Tresanco *et al.,* [27]). Several programs/scripts are readily integrated into AMDock that minimized the preparation process while keeping control of the docking environment [27]. The grid box was generated using AutoDock Tools to obtain binding position for specific site docking. The grid centre for docking was set at X= -12.2, Y=11.5, Z=68.9. The grid dimensions of the box were set at 40 X 54 X 40. The centre and dimension for the grid box was generated based on the binding site of native inhibitor (N3 inhibitor) on the main protease, to optimize the best substrate-binding site and utilize important amino acid residues involved in the binding. The structure and details about the N3 inhibitor were discussed previously [28] and similar method of retrieving grid box coordinates could also be found in previous study [29-31]. Firstly, docking was performed with reference molecule to validate the docking protocol. Accordingly, the five phytochemicals from *M. charantia* were docked to the target site on SARS-CoV-2 main protease and the binding energy was extracted from the software. The interactions between the ligands and target protein were observed and analysed.

2.3 Visualization of Molecular Interactions

The analysis of molecular interactions between the protein-ligand structure was performed using LigPlot+ [32]. LigPlot+ is a software for the visualization of multiple ligand-protein interactions and generate diagrams that portray all the interactions, including the hydrogen-bond interactions, hydrophobic contacts between the ligand(s) and the main-chain or side-chain residues of the protein [32]. The system is able to plot related sets of ligand-protein interactions, in the same orientation. This feature aids in many research works, especially in drug discovery as it allowed users to analyse a series of small molecules binding to the same protein target, a single ligand binding to homologous proteins, or in general case where both protein and ligand change [32].

3. Results

3.1 Molecular Docking of Phytochemicals from M. Charantia with SARS-CoV-2 Main Protease

The binding energies of five phytochemicals from *M. charantia* and molnupiravir against SARS-CoV-2 main protease are summarized in Table 1. From the result, it is revealed that phytochemicals in *M. charantia* exhibited lower binding energy compared to the reference molecule, molunpiravir. Lower binding energy indicates that the higher binding affinity of the molecules to target receptors [33]. The binding energy of the phytochemicals and reference molecule with SARS-CoV-2 main protease was, -8.9 for rutin, -7.8 for kaempferol, -7.5 for quercetin and luteolin, -7.2 for catechin and -6.7 for drug molnupiravir (Table 1). The range of binding energy with SARS-CoV-2 main protease

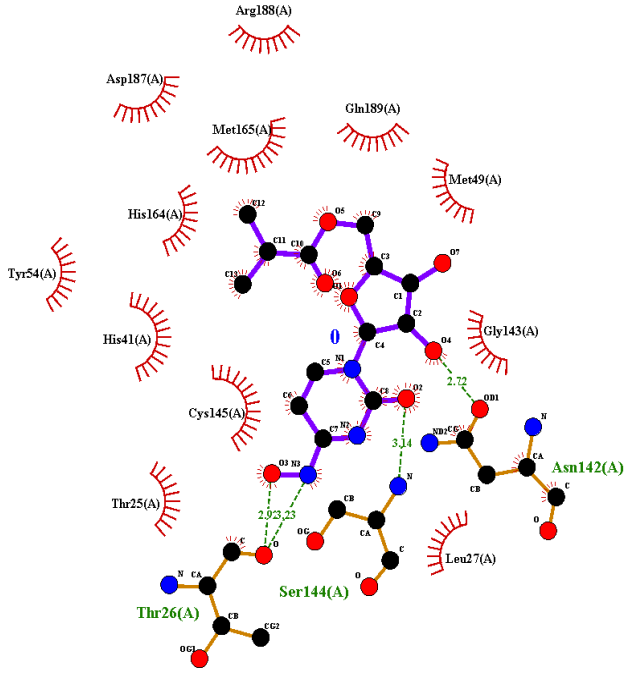
towards phytochemicals were from -8.9 to -7.2 kcal mol-1, which was better and significant than the binding energy of molnupiravir (-6.7 kcal mol-1).

Table 1
Docking score of five phytochemicals from *M. charantia* and molnupiravir with SARS-CoV-2 main protease

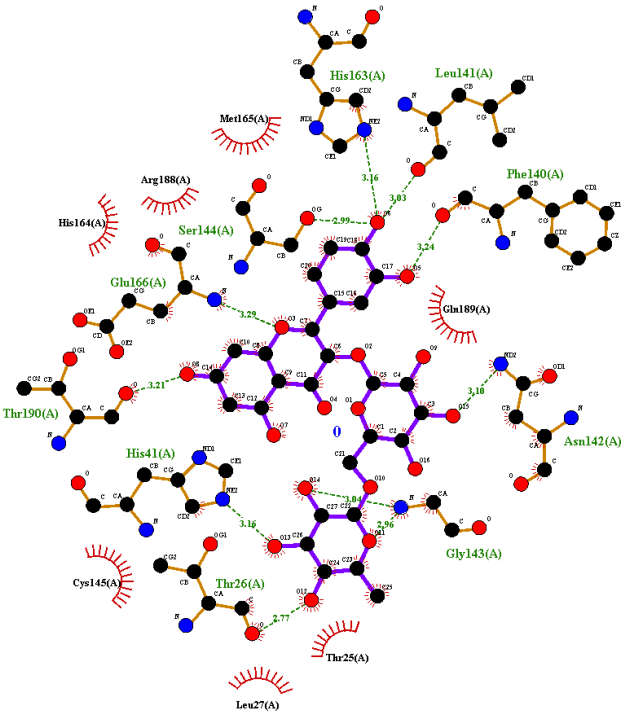
Classifications	Ligands	Molecular Weight (g/mol)	Binding Affinity (kcal/mol)
Flavonoid	Rutin	610.5	-8.9
Flavonoid	Kaempferol	286.40	-7.8
Flavonoid	Quercetin	302.23	-7.5
Flavonoid	Luteolin	286.24	-7.5
Flavonoid	Catechin	290.27	-7.2
Reference molecule	Molnupiravir	329.31	-6.7

3.2 Visualization of Molecular Interactions between Phytochemicals from M. Charantia and Main Protease

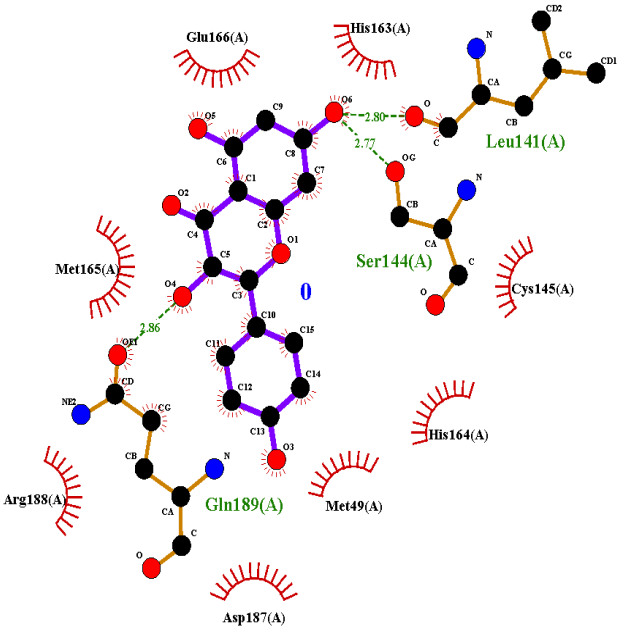
The molecular interactions of phytochemicals from *M. charantia* were analyzed using LigPlot+ [32] and superimposition images of the phytochemicals and molnupiravir on main protease targeted site were generated using Pymol. Rutin showed the most interactions with main protease of SARS-CoV-2. As seen on Figure 2a, rutin interacted with MET165A, ARG188A, HIS164A, CYS145A, LEU27A, THR25A and GLN189A through hydrophobic contacts. Hydrogen bonds with HIS163A, SER144A, GLU166A, THR190A, HIS41A, THR26A, GLY143A, ASN142A, PHE141A and LEU141A were also observed. Superimposition analysis of rutin with reference molecule, molnupiravir revealed that the compound interacted with 12 same residues that matches with the drug (Figure 3a). The visualization of molecular interactions between kaempferol with Mpro through LigPlot+ showed that kaempferol interacted with MET165A, GLU166A, HIS163A, ARG188A, ASP187A, MET49A, HIS164A and CYS145A through hydrophobic contacts (Figure 2b). Kaempferol formed hydrogen bonds with residues GLN189A, SER144A and LEU141A that maintains strong affinity with the target protein. Among 11 residues, interactions with 8 of the residues matches with that to the reference molecule (Figure 3b). Quercetin showed hydrophobic interactions with eight amino acid residues, in which two of the residues are the catalytic dyad, HIS41A and CYS145A (Figure 2c). The compound also forms hydrogen bonds with LEU141A, HIS163A and SER144A. Superimposition analysis of quercetin with molnupiravir showed that the compound interacted with 8 residues that matches to the drug (Figure 3c). Luteolin interacted with GLU166A, GLN189A, HIS164A, MET165A and catalytic residues CYS145A and HIS41A through hydrophobic interactions (Figure 2d). Hydrogen bonds with THR190A, ARG188A, ASN142A and GLN192A residues were also observed during the binding of luteolin with Mpro (Figure 2d). Among interactions with 10 of the residues, 7 of it matches with that to the drug molnupiravir (Figure 3d). As seen in Figure 2e, the compound catechin was observed to have hydrophobic interactions with PRO168A, GLN189A, HIS164A, ARG188A, MET165A, ASP187A and a catalytic residue HIS41A. Catechin also formed hydrogen bonds with GLN192A, GLU166A and THR190A. Superimposition analysis of catechin with reference molecule revealed that the compound interacted with 6 residues that matches with the drug molnupiravir (Figure 3e).



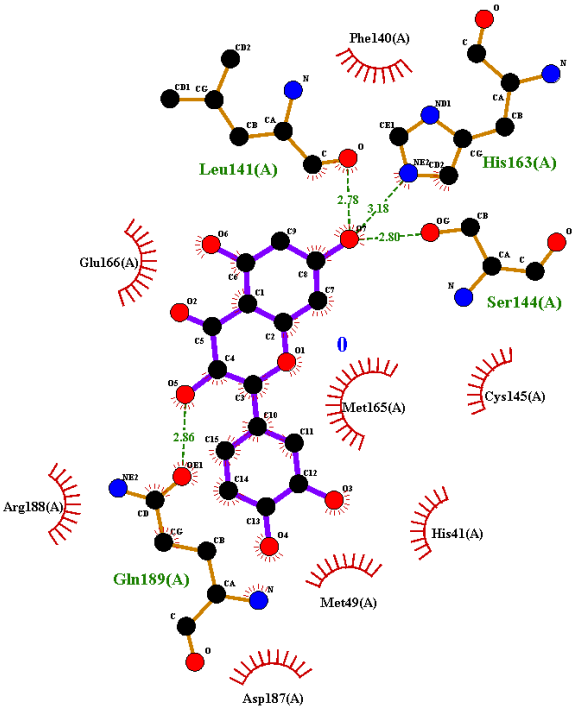
a) Molnupiravir with main protease



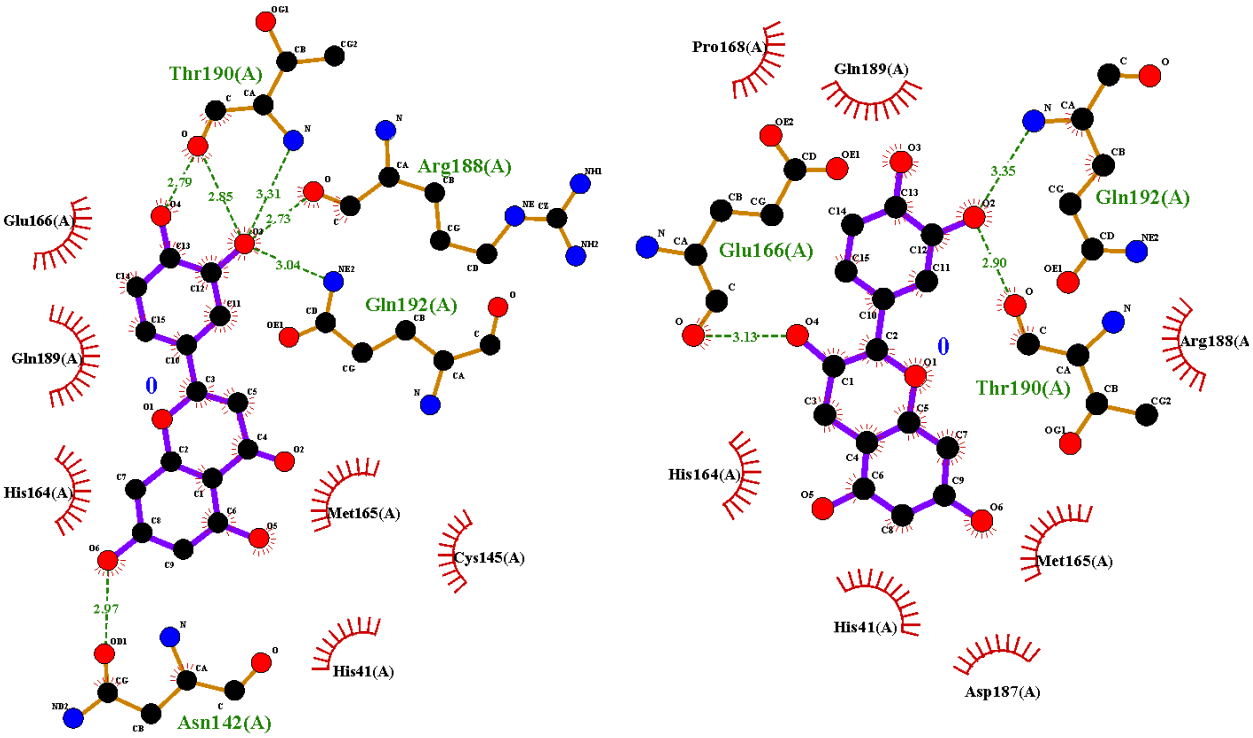
b) Rutin with main protease



c) Kaempferol with main protease



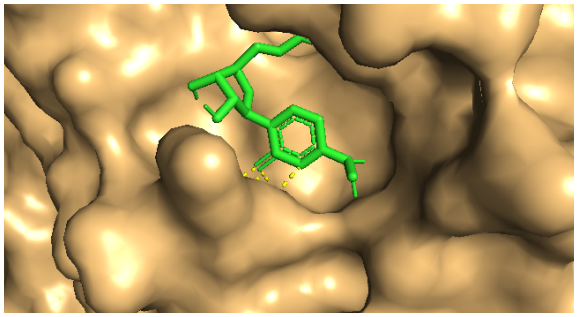
d) Quercetin with main protease



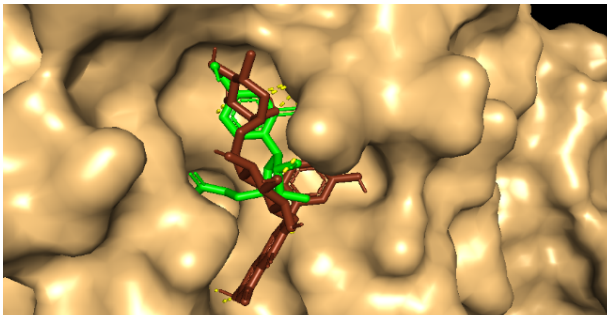
e) Luteolin with main protease

f) Catechin with main protease

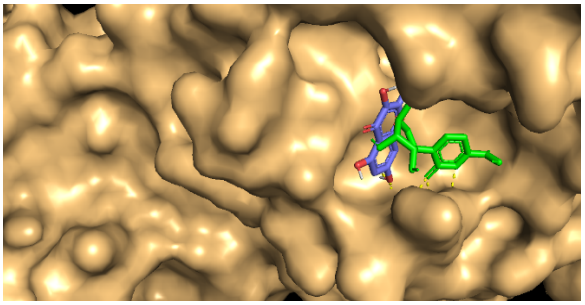
Fig. 2. Visualization of molecular interactions between SARS-CoV-2 main protease and five phytochemicals from *M. charantia* using LigPlot+. (a) rutin, (b) kaempferol, (c) quercetin, (d) luteolin, (e) catechin and (f) reference molecule, molnupiravir. The purple lines denote the ligand structure, the brown lines denote the structure of amino acid residues. The molecular interactions are represented as dashed lines and arcs. The green dashed lines between atoms depict hydrogen bonds, and the numbers above these lines denote the length of the bond. Concurrently, the arc with spokes radiating toward the ligand atoms indicate hydrophobic contacts



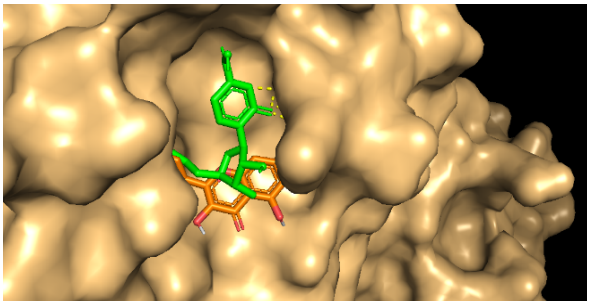
a) Position of molnupiravir on 6LU7 active site



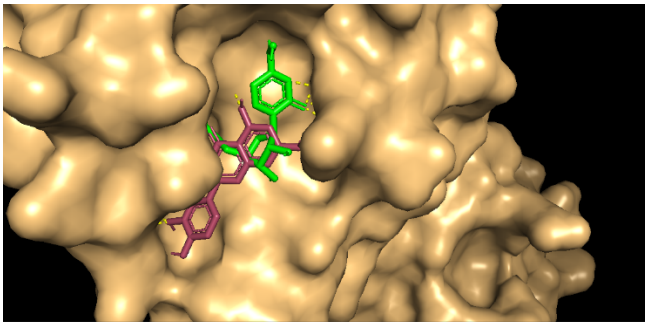
b) Super imposition of molnupiravir and rutin



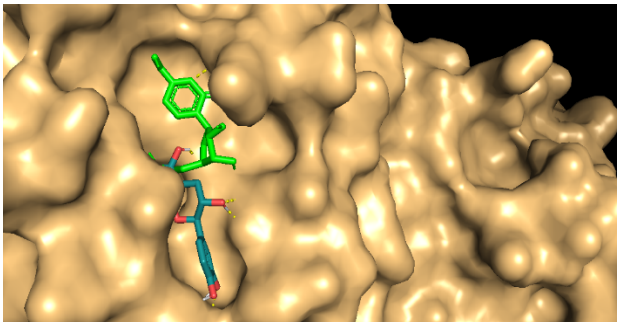
c) Super imposition of molnupiravir and kaempferol



d) Super imposition of molnupiravir and quercetin



e) Super imposition of molnupiravir and luteolin



f) Super imposition of molnupiravir and catechin

Fig. 3. Superimposition images of reference molecule (molnupiravir) and phytochemicals from *M. charantia* on main protease (6LU7) targeted site. a) Position of molnupiravir on 6LU7 active site, b) superimposition of rutin with molnupiravir, c) kaempferol with molnupiravir, d) quercetin with kaempferol, e) luteolin with molnupiravir, f) catechin with molnupiravir. The light-yellow surface-bound structure represent main protease (6LU7), the green stick in the binding site represented the drug molnupiravir and the different coloured sticks represent the phytochemicals. The yellow dashed lines indicate molecular interaction of the ligands with main protease

4. Discussion

SARS-CoV-2 main protease is one of the targeted proteins for the development and design of drugs and vaccines. Main protease is an attractive drug target as it plays pivotal role in post-translational processing of replicase polyproteins [31]. Computational and mechanistic studies of SARS-CoV-2 main protease revealed that the main catalytic residues in the active site of the main protease are CYS145 and HIS41 [31,34]. During main protease catalysis, His41 deprotonates CYS145 thiol, which then reacts with the carbonyl of the scissile amide, giving out an acyl-enzyme intermediate [34]. Previous study revealed that the catalytic dyad involved in the maturation of the virus, thus making it an attractive target site protein [35]. Docking of the five phytochemicals from *M. charantia* and reference drug, molnupiravir, were performed using AMDock. AMDock is a

graphical tool that assisted in molecular docking with AutoDock VINA and AutoDock4. External programs including OpenBabel, PDB2PQR, AutoLigand, and ADT scripts were integrated in AMDock that ease users for the preparation of input structure files, definition of the search space, and it also offers several alternatives and different degrees of user supervision [27]. The pH for docking environment was set as default at 7.4. The pH value can be set for determining the protonation states of the protein and ligand. In present study, the pH value for protonation of both ligand and receptor were set at default, pH 7.4. The particular pH value was selected to emulate the actual pH value in human body. Most biological processes are pH dependent and greatly affected by the pH of the water phase, which includes subcellular translocation [36,37], functional pH dependence [36,38], and structural conformations correlated with its function [36,39,40]. Previous studies reported that changes in pH were observed to exhaustively influenced protein-protein [36,41,42], protein-ligand [36,43-45], protein-membrane [36,46] and peptide-membrane linkage [36,43-45]. The input files needed for docking were prepared automatically using AMDock, which includes protonation of titratable residues, merging of non-polar hydrogens and removal of ion/water [27]. The protein was kept rigid during the docking process, and the ligands were flexible. AMDock provided users with few options to define the box centre and dimensions (search space):

i. "Automatic"
ii. "Centre on Residue(s)"
iii. "Centre on Hetero"
iv. "Box"

The "Box" option was used in this study to define the search space on the protein. This option allows users to define their own desired box centre and dimensions [27]. The "Box" option was used for defining the search space of the SARS-CoV-2 main protease (PDB ID: 6LU7) active site. As mentioned in the procedures for molecular docking, the grid box coordinates were generated based on the binding location of the native inhibitor, N3 [28] on SARS-CoV-2 main protease. Thus, the "Box" option was chosen to set a desired coordinate, that specifically focuses on the active site of the main protease. Current study revealed that the five phytochemicals from *M. charantia*, namely rutin, kaempferol, quercetin, luteolin and catechin have higher binding affinity compared to reference drug, molnupiravir. Binding affinity is the degree of binding conformation between the ligand and protein. Simultaneously, binding energy is referred to the energy released due to the formation, or rather, interaction of the ligand and protein is termed in form of binding energy. The lesser the binding energy, the better binding affinity of the ligand and protein [47,48]. In this study, the protein was kept rigid and the ligands were let flexible, to generate different conformations during the docking process. The ones with the best docking score were chose for study.

Molnupiravir is an oral antiviral drug, which has been reported to exert inhibition against SARS-CoV-2 and other RNA viruses, and showed a high barrier to the development of resistance [49,50]. Antiviral drugs such as molnupiravir usually act as nucleoside analogues that stop RNA chain polymerization, and targets the virus polymerases [49]. Initially, molnupiravir was issued as a possible treatment for influenza viruses, encephalitic alphaviruses, dues to its inhibitory effect in cell cultures [51]. This drug has been seen as a promising drug candidate for the treatment of COVID-19 infection as results from clinical trials shown positive outcomes in the tested patients. Previous studies revealed that molnupiravir impedes the replication of many viruses, including SARS-CoV-2, inhibit the replication of SARS-CoV-22 in human lung tissues, obstruct the transmission of SARS-CoV-2 in ferrets, and reduces SARS-CoV-2 RNA counts in COVID-19 patients [49]. In this study, molnupiravir was used as a reference molecule for docking against SARS-CoV-2 main protease. Molnupiravir with main

protease showed docking score of -6.7 kcal/mol. Five phytochemicals from *M. charantia* namely, rutin, kaempferol, quercetin, luteolin and catechin were used for docking against main protease of SARS-Cov-2. Amongst these phytochemicals, rutin and kaempferol showed the lowest binding energy with main protease, -8.9 and -7.8 kcal/mol, respectively, and bind firmly to the receptor's catalytic residues. The low binding energy indicates that the compounds have high binding affinity with the main protease of SARS-CoV-2. For our phytochemical compounds to be selected as potential inhibitors of SARS-CoV-2 main protease, they must bind to the active site and interact with catalytic residues presented in the active site. Therefore, molecular interactions of the selected five phytochemicals from *M. charantia* were analyzed with LigPlot+ [32].

Rutin is a flavonol, found abundantly in many plants, including *M. charantia*. Previous analysis on this compound revealed that it exhibited various pharmacological activities such as prevention of neuroinflammation on the nerve cells, anticataract and ophthalmic effect, antibacterial activity, antiviral activity and many more [52]. Rutin also been used to treat various chronic diseases including, cancer, diabetes, hypertension, and hypercholesterolemia [53]. In this study, rutin showed the lowest binding energy with main protease, suggesting that it can bind stably and have better affinity with the protein. The high binding affinity of rutin with main protease might be due to the numerous molecular interactions between the protein-ligand complex. Protein-ligand interaction is also an equally crucial parameter that deciding a drug's aptness for further studies [48]. A compound must interact with essential amino acids of the enzyme via hydrogen bonds and hydrophobic interactions in order to inhibit the enzyme [54]. Rutin interacted with both of the catalytic dyad either through hydrophobic contacts or hydrogen bonds. This study also analyzed superimposition of the five phytochemicals with reference molecule, molnupiravir. The result revealed that rutin bind with the most residues that matches with that of the drug molnupiravir, suggesting that it binds with major amino acids responsible for the inhibition of main protease catalysis. Kaempferol is a naturally-occurring compound in many vegetables and fruits [55] including *M. charantia* or bitter gourd. Kaempferol has been demonstrated to exhibit various pharmacological activity against many diseases, including cancer [55,56]. Kaempferol showed better binding affinity compared to the drug molnupiravir at the active site of SARS-CoV-2 main protease. As seen in the diagram, kaempferol interacted with one of the catalytic residues in main protease, CYS145A, suggesting it as potential drug targets to inhibit the viral activity of SARS-CoV-2 Mpro. The other three phytochemical compounds namely, quercetin, luteolin and catechin showed a docking score of -7.5 kcal mol-1, -7.5 kcal mol-1 and -7.2 kcal mol-1, respectively. These three phytochemicals showed comparably better binding affinity to the active site of SARS-CoV-2 main protease than the drug molnupiravir. Visualization of molecular interactions between quercetin, luteolin and catechin with main protease revealed that these compounds interacted with the important residues in the catalytic site, including CYS145A and HIS41A. Current study also included superimposition of molnupiravir with the selected five phytochemicals from *M. charantia*. As seen in Figure 3, the five phytochemicals showed better binding conformation to the main protease active site, compared to reference molecule, molnupiravir. This discovery suggesting that these phytochemicals can be potential drug candidate, targeting SARS-CoV-2 main protease.

5. Conclusions

To summarize, molecular docking of five phytochemicals from *M. charantia* or bitter gourd with SARS-CoV-2 main protease revealed that these compounds have better binding affinity with the protease compared to reference molecule, molnupiravir. The analysis of the molecular interactions between the five phytochemicals with SARS-CoV-2 main protease revealed that the selected

compounds interacted with either both of the catalytic residues (CYS145A and HIS41A) or at least one catalytic residue detected by AutoDock VINA integrated in AMDock. Thus, it is suggested that these compounds can be potential inhibitors and drug targets of the main protease. Further *in-vitro* study should be conducted to evaluate the properties of these phytochemicals for the development of drug target against SARS-CoV-2 main protease.

Acknowledgement
The present work was funded and supported by the Ministry of Higher Education under the Fundamental Research Grants Scheme (FRGS/1/2021/WAB13/UITM/02/3) and Universiti Teknologi MARA Cawangan Terengganu.

References

[1] Wang, Dawei, Bo Hu, Chang Hu, Fangfang Zhu, Xing Liu, Jing Zhang, Binbin Wang *et al.,* "Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China." *jama* 323, no. 11 (2020): 1061-1069. https://doi.org/10.1001/jama.2020.1585

[2] Yang, Jing, Ya Zheng, Xi Gou, Ke Pu, Zhaofeng Chen, Qinghong Guo, Rui Ji, Haojia Wang, Yuping Wang, and Yongning Zhou. "Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis." *Int J Infect Dis* 10, no. 10.1016 (2020).

[3] Bajgain, Kalpana Thapa, Sujan Badal, Bishnu B. Bajgain, and Maria J. Santana. "Prevalence of comorbidities among individuals with COVID-19: A rapid review of current literature." *American journal of infection control* 49, no. 2 (2021): 238-246. https://doi.org/10.1016/j.ajic.2020.06.213

[4] Liang, Li-Lin, Hsu-Sung Kuo, Hsiu J. Ho, and Chun-Ying Wu. "COVID-19 vaccinations are associated with reduced fatality rates: Evidence from cross-county quasi-experiments." *Journal of Global Health* 11 (2021). https://doi.org/10.7189/jogh.11.05019

[5] Moghadas, Seyed M., Thomas N. Vilches, Kevin Zhang, Chad R. Wells, Affan Shoukat, Burton H. Singer, Lauren Ancel Meyers *et al.,* "The impact of vaccination on coronavirus disease 2019 (COVID-19) outbreaks in the United States." *Clinical Infectious Diseases* 73, no. 12 (2021): 2257-2264. https://doi.org/10.1093/cid/ciab079

[6] Sahebnasagh, Adeleh, Razieh Avan, Fatemeh Saghafi, Mojataba Mojtahedzadeh, Afsaneh Sadremomtaz, Omid Arasteh, Asal Tanzifi *et al.,* "Pharmacological treatments of COVID-19." *Pharmacological Reports* 72 (2020): 1446-1478. https://doi.org/10.1007/s43440-020-00152-9

[7] Singh, Thakur Uttam, Subhashree Parida, Madhu Cholenahalli Lingaraju, Manickam Kesavan, Dinesh Kumar, and Raj Kumar Singh. "Drug repurposing approach to fight COVID-19." *Pharmacological Reports* 72 (2020): 1479-1508. https://doi.org/10.1007/s43440-020-00155-6

[8] Anilakumar, K. Raghavan, Garlapati Phani Kumar, and Nallamuthu Ilaiyaraja. "Nutritional, pharmacological and medicinal properties of Momordica charantia." *International Journal of Nutrition and Food Sciences* 4, no. 1 (2015): 75-83. https://doi.org/10.11648/j.ijnfs.20150401.21

[9] Jia, Shuo, Mingyue Shen, Fan Zhang, and Jianhua Xie. "Recent advances in Momordica charantia: functional components and biological activities." *International journal of molecular sciences* 18, no. 12 (2017): 2555. https://doi.org/10.3390/ijms18122555

[10] Meng, Xuan-Yu, Hong-Xing Zhang, Mihaly Mezei, and Meng Cui. "Molecular docking: a powerful approach for structure-based drug discovery." *Current computer-aided drug design* 7, no. 2 (2011): 146-157. https://doi.org/10.2174/157340911795677602

[11] Rohs, Remo, Itai Bloch, Heinz Sklenar, and Zippora Shakked. "Molecular flexibility in ab initio drug docking to DNA: binding-site and binding-mode transitions in all-atom Monte Carlo simulations." *Nucleic acids research* 33, no. 22 (2005): 7048-7057. https://doi.org/10.1093/nar/gki1008

[12] Dar, Ayaz Mahmood, and Shafia Mir. "Molecular docking: approaches, types, applications and basic challenges." *J Anal Bioanal Tech* 8, no. 2 (2017): 1-3. https://doi.org/10.4172/2155-9872.1000356

[13] Anthony, Josephine, Vijaya Raghavan Rangamaran, Kumar T. Shivasankarasubbiah, Dharani Gopal, and Kirubagaran Ramalingam. "Applications of Molecular Docking: Its Impact and Importance outside the Purview of Drug Discovery." In *Applied Case Studies and Solutions in Molecular Docking-Based Drug Design*, pp. 278-306. IGI Global, 2016. https://doi.org/10.4018/978-1-5225-0362-0.ch011

[14] Chua, Lee Suan. "A review on plant-based rutin extraction methods and its pharmacological activities." *Journal of ethnopharmacology* 150, no. 3 (2013): 805-817. https://doi.org/10.1016/j.jep.2013.10.036

[15] Middleton, Gordon W., and John H. Williams. "Significant gastric reflux of technetium-99m-MIBI in SPECT myocardial imaging." *Journal of Nuclear Medicine* 35, no. 4 (1994): 619-620.
[16] Han, Yongmoon. "Rutin has therapeutic effect on septic arthritis caused by Candida albicans." *International immunopharmacology* 9, no. 2 (2009): 207-211. https://doi.org/10.1016/j.intimp.2008.11.002
[17] Guardia, Teresita, Alejandra Ester Rotelli, Americo Osvaldo Juarez, and Lilian Eugenia Pelzer. "Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat." *Il farmaco* 56, no. 9 (2001): 683-687. https://doi.org/10.1016/S0014-827X(01)01111-9
[18] Dall'Agnol, R., A. Ferraz, A. P. Bernardi, D. Albring, C. Nör, L. Sarmento, L. Lamb, M. Hass, G. Von Poser, and E. E. S. Schapoval. "Antimicrobial activity of some Hypericum species." *Phytomedicine* 10, no. 6-7 (2003): 511-516. https://doi.org/10.1078/094471103322331476
[19] Ramanathan, R., N. P. Das, and C. H. Tan. "Inhibitory effects of 2-hydroxy chalcone and other flavonoids on human cancer cell-proliferation." *International Journal of Oncology* 3, no. 1 (1993): 115-119. https://doi.org/10.3892/ijo.3.1.115
[20] Jung, Chan Hun, Ji Yun Lee, Chul Hyung Cho, and Chang Jong Kim. "Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin." *Archives of pharmacal research* 30 (2007): 1599-1607. https://doi.org/10.1007/BF02977330
[21] Imran, Muhammad, Bahare Salehi, Javad Sharifi-Rad, Tanweer Aslam Gondal, Farhan Saeed, Ali Imran, Muhammad Shahbaz *et al.,* "Kaempferol: A key emphasis to its anticancer potential." *Molecules* 24, no. 12 (2019): 2277. https://doi.org/10.3390/molecules24122277
[22] M Calderon-Montano, J., E. Burgos-Morón, C. Pérez-Guerrero, and M. López-Lázaro. "A review on the dietary flavonoid kaempferol." *Mini reviews in medicinal chemistry* 11, no. 4 (2011): 298-344. https://doi.org/10.2174/138955711795305335
[23] Bentz, Alexandra B. "A Review of quercetin: chemistry, antioxident properties, and bioavailability." *Journal of young investigators* (2017).
[24] Batiha, Gaber El-Saber, Amany Magdy Beshbishy, Muhammad Ikram, Zohair S. Mulla, Mohamed E. Abd El-Hack, Ayman E. Taha, Abdelazeem M. Algammal, and Yaser Hosny Ali Elewa. "The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin." *Foods* 9, no. 3 (2020): 374. https://doi.org/10.3390/foods9030374
[25] Sathya, Sethuraman, and Kasi Pandima Devi. "The use of polyphenols for the treatment of Alzheimer's disease." In *Role of the mediterranean diet in the brain and neurodegenerative diseases*, pp. 239-252. Academic Press, 2018. https://doi.org/10.1016/B978-0-12-811959-4.00015-8
[26] Zanwar, Anand A., Sachin L. Badole, Pankaj S. Shende, Mahabaleshwar V. Hegde, and Subhash L. Bodhankar. "Antioxidant role of catechin in health and disease." In *Polyphenols in human health and disease*, pp. 267-271. Academic Press, 2014. https://doi.org/10.1016/B978-0-12-398456-2.00021-9
[27] Valdés-Tresanco, Mario S., Mario E. Valdés-Tresanco, Pedro A. Valiente, and Ernesto Moreno. "AMDock: a versatile graphical tool for assisting molecular docking with Autodock Vina and Autodock4." *Biology direct* 15, no. 1 (2020): 1-12. https://doi.org/10.1186/s13062-020-00267-2
[28] Jin, Zhenming, Xiaoyu Du, Yechun Xu, Yongqiang Deng, Meiqin Liu, Yao Zhao, Bing Zhang *et al.,* "Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors." *Nature* 582, no. 7811 (2020): 289-293. https://doi.org/10.1038/s41586-020-2223-y
[29] Ahmed, Shimaa A., Doaa A. Abdelrheem, HR Abd El-Mageed, Hussein S. Mohamed, Aziz A. Rahman, Khaled NM Elsayed, and Sayed A. Ahmed. "Destabilizing the structural integrity of COVID-19 by caulerpin and its derivatives along with some antiviral drugs: an in silico approaches for a combination therapy." *Structural Chemistry* 31 (2020): 2391-2412. https://doi.org/10.1007/s11224-020-01586-w
[30] Das, Pratik, Ranabir Majumder, Mahitosh Mandal, and Piyali Basak. "In-Silico approach for identification of effective and stable inhibitors for COVID-19 main protease (Mpro) from flavonoid based phytochemical constituents of Calendula officinalis." *Journal of Biomolecular Structure and Dynamics* 39, no. 16 (2021): 6265-6280. https://doi.org/10.1080/07391102.2020.1796799
[31] Islam, Rajib, Md Rimon Parves, Archi Sundar Paul, Nizam Uddin, Md Sajjadur Rahman, Abdulla Al Mamun, Md Nayeem Hossain, Md Ackas Ali, and Mohammad A. Halim. "A molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2." *Journal of Biomolecular Structure and Dynamics* 39, no. 9 (2021): 3213-3224. https://doi.org/10.1080/07391102.2020.1761883
[32] Laskowski, Roman A., and Mark B. Swindells. "LigPlot+: multiple ligand–protein interaction diagrams for drug discovery." (2011): 2778-2786. https://doi.org/10.1021/ci200227u
[33] Pandey, Preeti, Jitendra Subhash Rane, Aroni Chatterjee, Abhijeet Kumar, Rajni Khan, Amresh Prakash, and Shashikant Ray. "Targeting SARS-CoV-2 spike protein of COVID-19 with naturally occurring phytochemicals: an in

silico study for drug development." *Journal of Biomolecular Structure and Dynamics* 39, no. 16 (2021): 6306-6316. https://doi.org/10.1080/07391102.2020.1796811

[34] Chan, HT Henry, Marc A. Moesser, Rebecca K. Walters, Tika R. Malla, Rebecca M. Twidale, Tobias John, Helen M. Deeks *et al.,* "Discovery of SARS-CoV-2 M pro peptide inhibitors from modelling substrate and ligand binding." *Chemical science* 12, no. 41 (2021): 13686-13703. https://doi.org/10.1039/D1SC03628A

[35] Chang, Gu-Gang. "Quaternary structure of the SARS coronavirus main protease." *Molecular biology of the SARS-Coronavirus* (2010): 115-128. https://doi.org/10.1007/978-3-642-03683-5_8

[36] Talley, Kemper, and Emil Alexov. "On the pH-optimum of activity and stability of proteins." *Proteins: Structure, Function, and Bioinformatics* 78, no. 12 (2010): 2699-2706. https://doi.org/10.1002/prot.22786

[37] Horiguchi, Masakuni, Makoto Arita, Daisy E. Kaempf-Rotzoll, Masafumi Tsujimoto, Keizo Inoue, and Hiroyuki Arai. "pH-dependent translocation of α-tocopherol transfer protein (α-TTP) between hepatic cytosol and late endosomes." *Genes to Cells* 8, no. 10 (2003): 789-800. https://doi.org/10.1046/j.1365-2443.2003.00676.x

[38] D. L. B. S. Designed Research; J and M. J. S. K. S. Performed Research; J, "contributed new reagents/analytic tools," 2008.

[39] Imamoto, Yasushi, Miki Harigai, and Mikio Kataoka. "Direct observation of the pH-dependent equilibrium between L-like and M intermediates of photoactive yellow protein." *FEBS letters* 577, no. 1-2 (2004): 75-80. https://doi.org/10.1016/j.febslet.2004.09.065

[40] Smedley III, James G., Joshua S. Sharp, Jeffrey F. Kuhn, and Kenneth B. Tomer. "Probing the pH-dependent prepore to pore transition of Bacillus anthracis protective antigen with differential oxidative protein footprinting." *Biochemistry* 47, no. 40 (2008): 10694-10704. https://doi.org/10.1021/bi800533t

[41] Schreiber, Gideon, and Alan R. Fersht. "Energetics of protein-protein interactions: Analysis ofthe Barnase-Barstar interface by single mutations and double mutant cycles." *Journal of molecular biology* 248, no. 2 (1995): 478-486. https://doi.org/10.1016/S0022-2836(95)80064-6

[42] Schreiber, Gideon, and Alan R. Fersht. "Interaction of barnase with its polypeptide inhibitor barstar studied by protein engineering." *Biochemistry* 32, no. 19 (1993): 5145-5150. https://doi.org/10.1021/bi00070a025

[43] Chaves, Francisco A., Katherine A. Richards, Andrew Torelli, Joseph Wedekind, and Andrea J. Sant. "Peptide-binding motifs for the I-Ad MHC class II molecule: alternate pH-dependent binding behavior." *Biochemistry* 45, no. 20 (2006): 6426-6433. https://doi.org/10.1021/bi060194g

[44] Olson, Linda J., Ole Hindsgaul, Nancy M. Dahms, and Jung-Ja P. Kim. "Structural insights into the mechanism of pH-dependent ligand binding and release by the cation-dependent mannose 6-phosphate receptor." *Journal of Biological Chemistry* 283, no. 15 (2008): 10124-10134. https://doi.org/10.1074/jbc.M708994200

[45] Hom, Robert A., Mohsin Vora, Maryann Regner, Oksana M. Subach, Wonhwa Cho, Vladislav V. Verkhusha, Robert V. Stahelin, and Tatiana G. Kutateladze. "pH-dependent binding of the Epsin ENTH domain and the AP180 ANTH domain to PI (4, 5) P2-containing bilayers." *Journal of molecular biology* 373, no. 2 (2007): 412-423. https://doi.org/10.1016/j.jmb.2007.08.016

[46] Re, Francesca, Silvia Sesana, Alberto Barbiroli, Francesco Bonomi, Emanuela Cazzaniga, Elena Lonati, Alessandra Bulbarelli, and Massimo Masserini. "Prion protein structure is affected by pH-dependent interaction with membranes: a study in a model system." *FEBS letters* 582, no. 2 (2008): 215-220. https://doi.org/10.1016/j.febslet.2007.12.003

[47] Joshi, Tushar, P. Sharma, S. Mathpal, H. Pundir, V. Bhatt, and S. Chandra. "In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking." *European Review for Medical & Pharmacological Sciences* 24, no. 8 (2020).

[48] Umar, Abd Kakhar, James H. Zothantluanga, Keerthic Aswin, Saipul Maulana, Muhammad Sulaiman Zubair, H. Lalhlenmawia, Mithun Rudrapal, and Dipak Chetia. "Antiviral phytocompounds "ellagic acid" and "(+)-sesamin" of Bridelia retusa identified as potential inhibitors of SARS-CoV-2 3CL pro using extensive molecular docking, molecular dynamics simulation studies, binding free energy calculations, and bioactivity prediction." *Structural Chemistry* 33, no. 5 (2022): 1445-1465. https://doi.org/10.1007/s11224-022-01959-3

[49] Kabinger, Florian, Carina Stiller, Jana Schmitzová, Christian Dienemann, Goran Kokic, Hauke S. Hillen, Claudia Höbartner, and Patrick Cramer. "Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis." *Nature structural & molecular biology* 28, no. 9 (2021): 740-746. https://doi.org/10.1038/s41594-021-00651-0

[50] Jayk Bernal, Angélica, Monica M. Gomes da Silva, Dany B. Musungaie, Evgeniy Kovalchuk, Antonio Gonzalez, Virginia Delos Reyes, Alejandro Martín-Quirós *et al.,* "Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients." *New England Journal of Medicine* 386, no. 6 (2022): 509-520. https://doi.org/10.1056/NEJMoa2116044

[51] Singh, Awadhesh Kumar, Akriti Singh, Ritu Singh, and Anoop Misra. "Molnupiravir in COVID-19: A systematic review of literature." *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 15, no. 6 (2021): 102329. https://doi.org/10.1016/j.dsx.2021.102329

[52] Ganeshpurkar, Aditya, and Ajay K. Saluja. "The pharmacological potential of rutin." *Saudi pharmaceutical journal* 25, no. 2 (2017): 149-164. https://doi.org/10.1016/j.jsps.2016.04.025

[53] Al-Dhabi, Naif Abdullah, Mariadhas Valan Arasu, Chang Ha Park, and Sang Un Park. "An up-to-date review of rutin and its biological and pharmacological activities." (2015).

[54] Rudrapal, Mithun, Ismail Celik, Johra Khan, Mohammad Azam Ansari, Mohammad N. Alomary, Fuad Abdullah Alatawi, Rohitash Yadav *et al.,* "Identification of bioactive molecules from Triphala (Ayurvedic herbal formulation) as potential inhibitors of SARS-CoV-2 main protease (Mpro) through computational investigations." *Journal of King Saud University-Science* 34, no. 3 (2022): 101826. https://doi.org/10.1016/j.jksus.2022.101826

[55] Ren, Jie, Yifei Lu, Yanhong Qian, Bozhou Chen, Tao Wu, and Guang Ji. "Recent progress regarding kaempferol for the treatment of various diseases." *Experimental and therapeutic medicine* 18, no. 4 (2019): 2759-2776. https://doi.org/10.3892/etm.2019.7886

[56] Chen, Allen Y., and Yi Charlie Chen. "A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention." *Food chemistry* 138, no. 4 (2013): 2099-2107. https://doi.org/10.1016/j.foodchem.2012.11.139