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In silico Evaluation of Antiviral SARS-Cov-2 from Bioactive Compounds of Bitter Melon (*Momordica charantia L.*) With Papain-Like Protease and Main Protease Enzymes as Targets

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Abstract

The outbreak of coronavirus SARS-CoV-2 in Wuhan, China in December 2019, the cause of Corona Virus Disease of 2019 (COVID-19), represents a pandemic threat to global health. The WHO declared COVID-19 as a pandemic on March 11th 2020. The previous in vivo and in vitro research of crude alkaloid on bitter melon fruit (*Momordica charantia L.)* can prove antivirus activity but the active compound which suitable for this activity is unknown yet especially for anti-SARS-CoV-2. This study aims to evaluate in silico of twenty-five (25) bioactive compounds of bitter melon (*Momordica charantia L.)* as an inhibitor of the Papain-like Protease and Main Protease enzymes. One of the best-characterized drug targets among coronaviruses is the main protease (Mpro, also called 3CLpro), Along with the papain-like protease this enzyme is essential for processing the polyproteins that are translated from the viral RNA. The method used is molecular docking with software PLANTS, YASARA, MarvinSketch, and visualization using PyMOL. As a positive control, klorokuin is used as a Papain-like Protease and Main Protease inhibitor. The results obtained Six (6) candidates for active compounds as Main Protease inhibitors, namely α-elaeostearic acid, erythrodiol, momordicin II, momordicoside C, momordicoside L and momordol and there is one active compound candidates as Papain-like Protease inhibitors namely momordol.

Keywords

Antiviral; Corona Virus Disease of 2019; SARS-CoV-2; Molecular docking; Inhibitor; Bitter melon (Momordica charantia L.); Main Protease, Papain-like Protease; in silico

Introduction

In December 2019, a new coronavirus caused an outbreak of pulmonary disease in the city of Wuhan, the capital of Hubei province in China, and has since spread globally. The virus has been named severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2). Because the RNA genome is about 82% identical to that of the SARS coronavirus (SARS-CoV); both viruses belong to clade b of the genus Betacoronavirus. The disease caused by SARS-CoV-2 is called coronavirus disease 2019 (COVID-19). Whereas at the beginning of the outbreak, cases were connected to the Huanan seafood and animal market in Wuhan, efficient human-to-human transmission led to exponential growth in the number of cases. On 11 March 2020, the World Health Organization (WHO) declared the outbreak a pandemic [1]**.** Repurposed drugs such as hydroxychloroquine, which initially seen as a prospective COVID-19 drug, have been proven to have no clinical benefit in the latest report.

Since ancient times, plants and herbal preparations have been used as medicine. Research carried out in last few decades has certified several such claims of use of several plants of traditional medicine. Popularity of *Momordica charantia* in various systems of traditional medicine for several ailments (antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies) focused the investigator's attention on this plant. Over 100 studies using modern techniques have authenticated its use in diabetes and its complications (nephropathy, cataract, insulin resistance), as antibacterial as well as antiviral agent (including HIV infection) [2].

As a member of the Nidovirus family, coronavirus infection (SARS-CoV-2) can be contracted from animals such as bats, and fellow humans. This virus can enter the human body through its receptors, ACE2 which are found in various organs such as heart, lungs, kidneys, and gastrointestinal tract, thus facilitating viral entry into target cells. The process of CoV entering into the host cell begins through the attachment of the S glycoprotein to the receptor, the ACE2 in the host cells (such as in type II pneumocytes in the lungs). The entry and binding processes are then followed by fusion of the viral membrane and host cell. After fusion occurs, the type II transmembrane serine protease (TMPRSS2) that is present on the surface of the host cell will clear the ACE2 and activate the receptor-attached spike-like, S proteins. Activation of the S proteins leads to conformational changes and allows the virus to enter the cells. Both of these proteins (TMPRSS2 and ACE2) are the main determinants of the entry of this virus [3].

The genomic material released by this virus is mRNA that is ready to be translated into protein. In its genome range, this virus is complemented by about 14 open reading frames (ORF), each of which encodes a variety of proteins, both structural and non-structural that play a role in its survival as well as virulence power. In its phase of transformation, the gene segments that encode nonstructural polyproteins are the ones this process first translates into ORF1a and ORF1b to produce two large overlapping polyproteins, pp1a and pp1ab by contributing a ribosomal frame shifting event. The polyproteins are supplemented by protease enzymes namely papain-like proteases (PLpro) and a serine type Mpro (chymotrypsin-like protease (3CLpro)) protease that are encoded in nsp3 and nsp 5. Subsequently, cleavage occurs between pp1a and pp1ab into nonstructural proteins (nsps) 1–11 and 1–16, respectively. The nsps play an important role in many processes in viruses and host cells [3].

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Because of its broad spectrum of action against viruses, including most coronaviruses and particularly its close relative SARS-CoV-1, and because coronavirus cell entry occurs through the endolysosomal pathway, it made sense in a situation of a public-health emergency and the absence of any known efficient therapy to investigate the possible effect of chloroquine against SARS-CoV-2. A recent paper reported that both chloroquine and the antiviral drug remdesivir inhibited SARS-CoV-2 in vitro and suggested these drugs be assessed in human patients suffering from COVID-19 [4].

Docking is a computational method used to describe interactions between a molecule as a ligand and a receptor or protein. In general, the aim of docking studies is to make accurate structural modeling and predictions of appropriate activities. Docking can be used to select potential specific protein substrates from a large number of chemical databases and to predict the absorption, distribution, metabolism and excretion (ADME) of new drugs and drug candidates. The advantages is inexpensive and it does not leave chemicals which is sent to the environment [5].

Material and Methods

The three dimensional Crystal structure from *Main Protease* and *Papain-like protease* enzymes were downloaded from Protein Data Bank (PDB) at http://www.rscb.org/pdb/. The selected *Main Protease* enzyme with PDB code 5R7Y. It has Root Mean Standard Deviation (RMSD) score: 1,5370. And *Papain-like protease* enzyme has PDB code 3E9S with 0,4730 as score RMSD. Ligand were docked to enzymes using Protein-Ligand Ant System (PLANTS). The active compounds were visualized by Pymol (Figure 1).

Ligand Selection and Preparation

There were twenty five (25) bioactive compounds from bitter melon were screened for *Main Protease* and *Papain-like protease* inhibitors. There were momordicin, momordicin II, cucurbitin, cucurbitacin, cucurbitane, cycloartenol, diosgenin, α-elaeostearic acid, erythrodiol, galacturonic acid, gentisic acid, goyaglycoside, goyasaponin I, goyasaponin II, gypsogenin, karounidiols, lanosterol, momordenol, momordicinin, momordicoside L, momordicoside I, momordicoside C, momordin I, momordin Ic, momordol [6]. Positive controls used were chloroquine as *Main Protease and Papain-like protease* inhibitors [4]. These compounds were downloaded from Pubchem database with .sdf format and then prepared using Marvinskecth from Chemaxon to obtain ligand at pH 7,4 and ten (10) conformation ligand for docking procedure.

Molecular docking and visualization

Running molecular docking using PLANTS (Protein-Ligand ANT system), docking simulation was carried out 10 times. The final result was a docking score that illustrates the total energy of protein-ligand bonds. After the docking process was carried out, the best affinity compound would be selected by comparing the resulting score with the comparison compound score and visualizing it to see the interaction of the compounds at the receptor binding site. Then determined the active and potential representative compounds, and used Pymol applications to display the representative compounds of the compounds at their receptors in 3-dimensional (3D) form. Pymol can determine the distance of hydrogen bonds of active compounds with amino acids from enzymes in angstrom units (Å). Visualization of the test compounds at the binding site was also carried out using Pymol and Yasara applications to be able to see more clearly the amino acids involved and can compare between the results of the visualization of the test compounds at the receptor binding site with different applications.

Toxicity test

All of the bioactive compounds toxicity properties were predicted using pkCSM, a web-based application that can predict small-molecule pharmacokinetic and toxicity properties based on the compounds molecular structure. The structure of each bioactive compound is taken from the Pubchem website and then inserted into the pkCSM application The toxicity tests will result in a

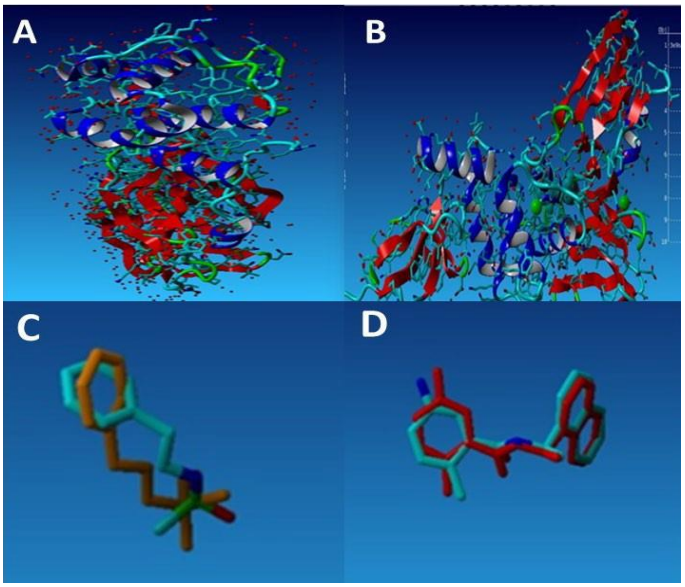

Figure 1: Visualization of Three Dimensional Structure of Reseptor using YASARA A) 5R7Y B) 3E9S C) alligment the ref ligand and redocking ligand of 5R7Y D) alligment the ref ligand and redocking ligand 3E9S

table where a variety of in vitro and in vivo tests were simulated automatically, for the sake of this research we will only be using the Oral Rat Acute Toxicity (LD50) values and the hepatotoxicity results (Figure 2).

Result and Discussion

The docking results of 25 compounds in bitter melon (*Momordica charantia* L.) with the *Main Protease* enzyme receptor with pdb code 5R7Y indicated candidates for active compounds that have same activity or equivalent to the comparison (chloroquine) that is as many as 6 of the 255 compounds while *Papain-like Protease* enzyme receptor with code 3E9S has 1 active compounds from the 255 compounds. Test compounds can be declared active or have an affinity for the receptor if they have a chemPLP score that is more negative (smaller) than the native ligand and can be expressed equal to the comparison (chloroquine) because the chemPLP score produced is more negative or the same than the comparison compound (Figure 3).

The visualization of the compound molecule which is predicted to be active as an inhibitor of Main Protease and Papain-like protease shows that several hydrogen bonds are formed. In the α-elaeostearic acid compound, one hydrogen bond is formed between the –OH group and –HN group from the amino acid SER-46, -OH from the Erythrodiol compound to form two hydrogen bonds with -HN from the amino acids GLN-192 and THR-26. The –OH and C = O groups of the momordicin II compound form three hydrogen bonds with –HN from the amino acids GLY-143, ASN-142, and HIS-163. The –OH, C = O, = O = groups of the momordicose C compound form eight hydrogen bonds with the amino acids ASN-142, THR-24, SER-46, GLN-189, and ARG-188. The –COH, = O and –OH groups in the momordicose L compound form seven hydrogen bonds with = CO and -NH from the amino acids THR-24, THR-26, THR-190, SER-46, GLN-189, ARG-188. While momordol compounds form five hydrogen bonds with amino acids GLU-166, GLN-189, THR-190, ARG-188, GLN-192 at the Main protease receptor (5R7Y) and five hydrogen bonds with amino acids LEU-163, GLY-164 , ASN-268, VAL-166, GLY-270 on the papain-like protease receptor (3E9S). (Figure 4). The hydrogen bond that is formed between the ligand and the receptor amino acid makes the affinity better so that the inhibition of the receptor is better. In the development of medicinal compounds as antivirals with an inhibitory mechanism of Main protease and papain like protease, α-elaeostearic acid, Erythrodiol, momordicin II,

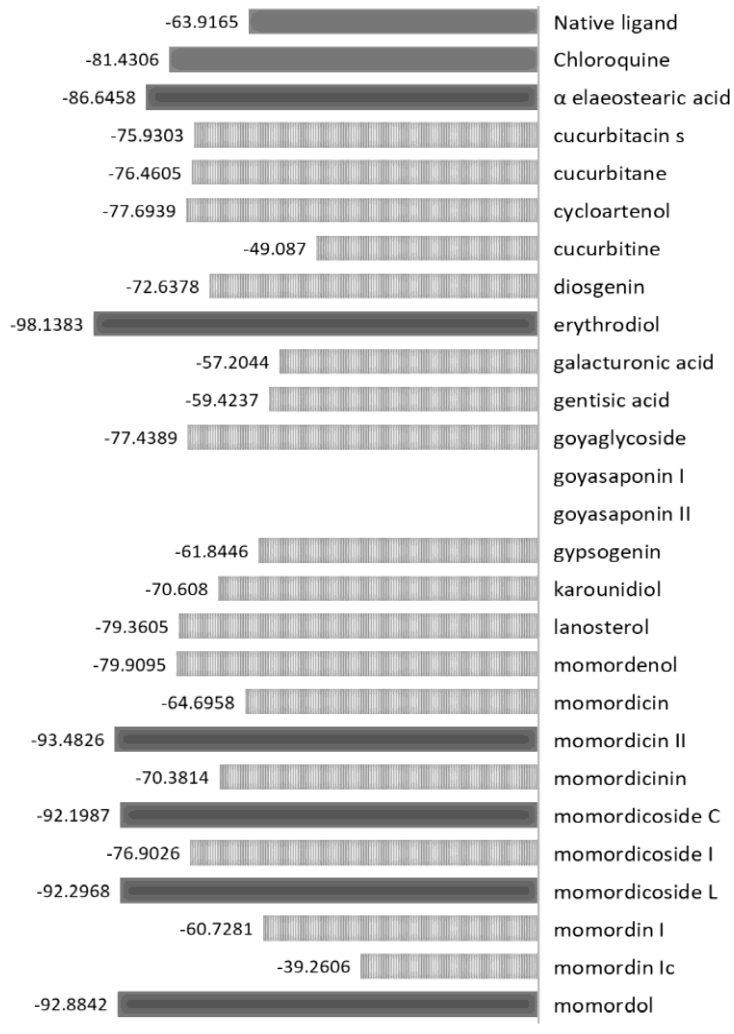

Figure 2: ChemPLP of Test Compounds in 5R7Y Receptor.

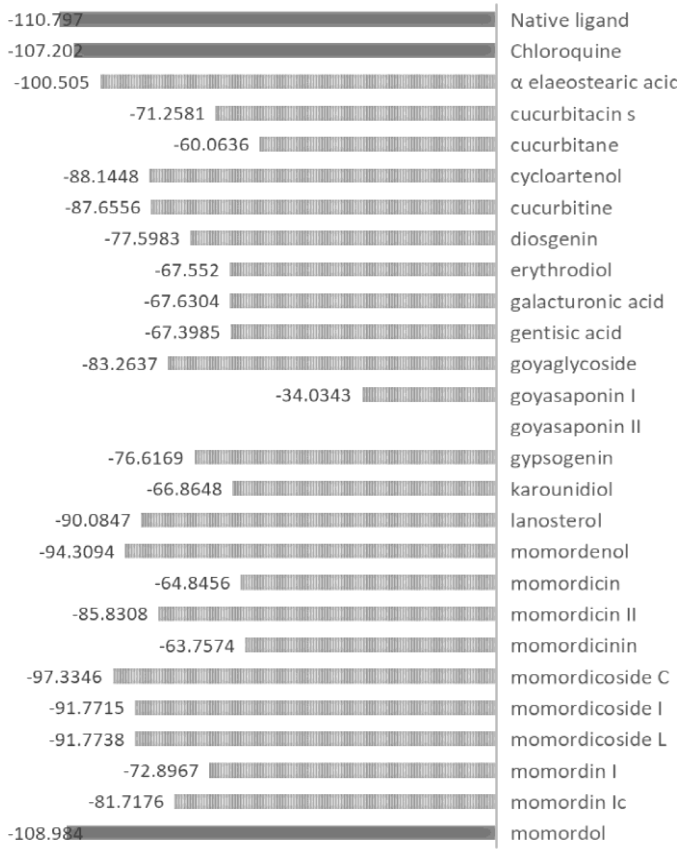

Figure 3: Heat map representing the correlation of features.

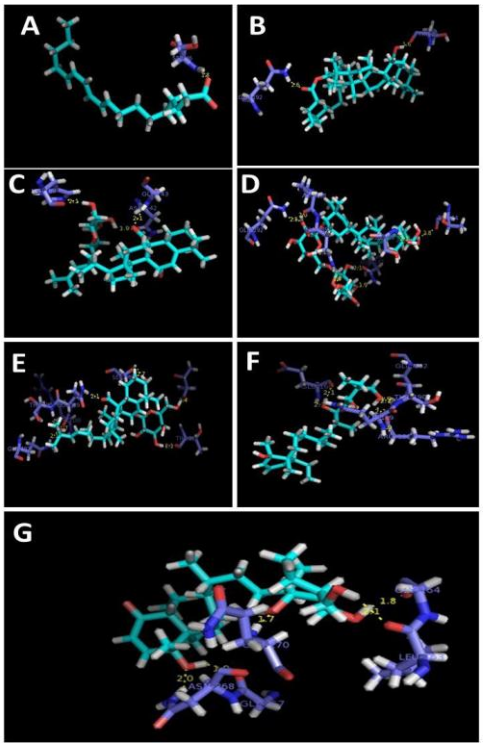

Figure 4: H-Bond Interaction of active compound candidates with amino acids reseptor 5R7Y; A) α-elaeostearic acid, B) Erythrodiol, C) momordicin II, D) momordicose C, E) momordicose L, F) momordol G) momordol in 3E9S.

momordicose C, momordicose L, and momordol in bitter melon fruit (*Momordica charantia L.*) are feasible to be developed because of the score values docking and toxicity test values included in the non-toxic group with a high LD50 value i.e 1.441, 2.659, 4.234, 3.342, 3.179, and 1.629 mol/Kg respectively.

Conclusions

The 6 (Six) potential compounds as Main Protease inhibitor are α-elaeostearic acid, erythrodiol, momordicinin II, momordicose C, momordicose L, and momordol. One active compounds obtained as Papain-like Protease inhibitor is Momordol. It needs other researches in vivo and in vitro to prove antiviral activity as single compound. Computational chemistry helps in the field of design and optimization of new or ongoing processes and products. Computational chemistry can reduce development costs, increase energy efficiency, be environmentally friendly, thereby increasing productivity and profits. It can help to find the new drugs of antiviral SARS-CoV-2 in future.

Competing Interests

Authors have declared that no competing interests exist.

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