**In Silico Study of Entry Inhibitor from Moringa oleifera Bioactive Compounds against SARS-CoV-2 Infection**

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**ABSTRACT**

The aim of this study is to screen the content of bioactive compounds of Moringa oleifera and to identify it as an antiviral against COVID 19 through an entry inhibitor mechanism using bioinformatics tools. The sample was obtained from PubChem database. Amino acids sequences were obtained from the NCBI. Protein modeling is made through the SWISSMODEL site. The target proteins for this study were SARS-CoV-2 Mpro and RdRp. The protein-inhibitory interaction of the drug Moringa oleifera bioactive compounds to SARS-CoV-2 was predicted by molecular docking with PyRx software. The result shows that M. oleifera was a potential antiviral candidate for SARS-CoV-2 with an entry inhibitor mechanism through a compound, especially quercetin. The RFMS value of both interactions between Mpro and quercetin and RdRp with quercetin were not higher than 1.05. This result still needed further research to prove this prediction.

**Keywords:** COVID-19, Mpro, RdRp, Moringa oleifera, Active site.

**INTRODUCTION**

Coronavirus (CoV) also known as COVID 19, has spread worldwide in December 2019 and became an epidemic in January 2020.1,2 WHO declared in March 2020 that this pandemic transmission is a person to person. The case of COVID 19 has enlarged widely to 213 countries and it caused more than 270 million infections over 5 million cases and those numbers still rising.3 Who confirmed the symptoms of COVID 19 were fever, dry cough, respiratory disorders, and olfactory and taste disorders.4-7 The human coronavirus (HCoV) was positive-stranded RNA virus. There were 2 types of protein of HCoV structural and non-structural protein that have different characteristics. The structural protein has characteristics including envelope, matrix, nucleocapsid, and spike. Besides, the non-structural protein has RNA-dependent RNA polymerase (RdRp).8 RdRp has an important role in the HCoV life cycle and also became the main target factor for COVID 19 therapeutics. According to the Genome report, SARS-CoV-2 depends on the viral protein function of the main protease (Mpro).9,10 Mpro has the main role in SARS-CoV-2 transcription and replication.9,11 Hence, Mpro and RdRp were the best candidates for designing antiviral drugs to find therapeutics agents against SARS-CoV-2.

Moringa oleifera is also known as the “miracle tree” because it has abundant benefits.12-14 There were bioactive compounds obtained from M. oleifera including Aurantiamide acid, Anthraquinone, Apigenin, Benzyl isothiocyanate, Chrysin, Dibutyl phthalate, Ellagic acid, Hydroxylorquinone, Isorhamnetin, Kaemferol, Myrcetin, Pterygospermin, Quercetin, Rutin, and β-amyrin which has an antiviral potential compounds against COVID 19 by inhibiting Mpro and RdRp activity.15,16 Besides all the compounds above, Oleic acid was most found at around 84% in M. oleifera.17 M. oleifera was the most appropriate candidate for an antiviral agent against SARS-CoV-2. The aim of this study was to screen bioactive compounds of Moringa oleifera to identify the antiviral potential compounds toward SARS-CoV-2 through an entry inhibitor mechanism.

**METHODS**

**Data mining of sample**

The bioactive compounds of M. oleifera which consist of anthraquinone, apigenin, aurantiamide, benzyli isothiocyanate, chlorogenic acid, chrysins, dibutyl phthalate, Ellagic acid, hesperidin, isorhoifolin, myricetin, pterygospermin, quercetin, rutin, and vitex. The bioactive compounds of M. oleifera were retrieved format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format.18

**Protein modeling**

The structure of Mpro and RdRp as the target proteins which were not available in the RCSB PDB database was modeled based on their amino acid sequence. The NCBI (https://www.ncbi.nlm.nih.gov/) database was used to retrieve sequences of amino acids with fasta format. Furthermore, protein modeling is made through the SWISSMODEL site (https://swissmodel.expasy.org/). The selection of protein models was selected from several parameters such as QME value, QMEAN value, coverage value, local quality value, and comparison plot. In addition, the protein

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structure also reviewed the Ramachandran Plot value according to favored, allowed, and outlier regions. Bioactivity and drug likeness prediction

Bioactivity of active compounds were predicted according to probability values (Pa) through PASS online site (http://way2drug.com/passonline/). To be an effective drug, potential active compounds must be able to reach the target in the body. There were several characteristics that a drug must possess in order to reach the target in the body to be selected as a drug potential. The characteristics reviewed include molecular mass, TPSA value, solubility in lipids, and others. There were several parameters to be reviewed in drug-likeness, named Lipinski, Ghose, Veber, Egan, and Muegge Parameter. Prediction of drug-likeness could be done through the SWISS ADME (http://www.swissadme.ch) website. Active compounds that fulfill five parameters will be selected.

Ligand and protein preparation

The minimization energy process of the ligand was prepared with PyRx software. Ligand preparation aimed to increase flexibility and change the sdf format to pdb. Ligand preparation also to minimize the binding affinity. The target protein in this paper was the Mpro and RdRp. Sterilization of the target protein from water and contaminant ligands was carried out by Discovery Studio software to increase the optimization of binding energy.

Molecular docking and dynamic simulation

Molecular docking with the PyRx software was performed to predict the interaction of protein inhibition on SARS-COV 2 by active compounds from M. oleifera. Molecular docking of the Mpro target was done by blind docking. On the other hand, molecular docking of the RdRp target was done by specific docking at its catalytic sites: Gly-616, Trp-617, Asp-618, Tyr-619, Leu-758, Ser-759, Asp-760, Asp-761, Ala-762, Lys-798, Tys-799, Trp-800, Glu-811, Phe-812, Cys-813, and Ser-814. The validation of the docking results were carried out with a dynamic molecular test by using CABS-flex 2.0. At this stage, the Fluctuation Plot tab on CABS-flex 2.0 showed the residue fluctuation profile due to the RMSF value for protein target.

Docking visualization

The analysis of the docking results was reviewed based on the 2D and 3D forms. Visualization of 2D docking results was done by Discovery Studio software. Moreover, the 3D visualization was carried out with PyMOL. Types of interactions and chemical bonds formed were analyzed using the Discovery Studio software.

RESULT AND DISCUSSION

Bioactivity and drug-like molecule potential of the bioactive compounds in the M. oleifera

The data of bioactive compounds found in Moringa oleifera, such as anthraquinone (CID 6780), apigenin (CID 5280443), aurantiamide acetate (CID 124319), benzyliothiocyanate (CID 2346), chlorogenic acid (CID 1794427), chrysin (CID 5281607), dibutyl phthalate (CID 3026), ellagic acid (CID 5281855), hesperidin (CID 10621),isorhoifolin (CID 9851181), myricetin (CID 5281672), pterygospermin (CID 72201063), quercetin (CID 5280343), rutin (CID 5280805) and vitexin (CID 5280441) were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Biological activity potential of all compounds were evaluated with PASS online site based on their chemical structure. The estimated value of probability was shown by probability activity value (Pa) and probability inactivity (Pi) from 0.000 to 1.000. High value of Pa means higher bioactivity. The result of bioactivity prediction of bioactive compounds of M. oleifera (Table 1).

Drug-likeness analysis aimed to identify molecules considered to be drugs built upon their physicochemical properties. The properties approaches aimed to measure drug likeness consist of octanol–water partition coefficient (ALOGP), number of H-bond acceptors (HBA), number of H-bond donors (HBD), molecular weight (MW), molecular polar surface area (PSA), number of aromatic rings (AROMS), number of structural alerts (ALERTS) and number of rotatable bonds (ROTBs). Based on these properties, there are several relevant drug likeness rules such those proposed by Lipinski, Ghose, Veber, Egan and Muegge. These rules suggest the compound as a drug based on their physicochemical properties. The Lipophilicity (log P_{ow}) is the partition coefficient between water and n-octanol. Water solubility is the value of a drug’s ability for oral targeting. SwissADME provides the number of violations in every rule. The drug likeness prediction of bioactive compounds of M. oleifera (Table 2) and drug likeness parameter of bioactive compounds of M. oleifera (Table 3).

The Binding activity ability and molecules interaction of the bioactive compounds in the M. oleifera and target protein

The bioactive compounds of M. oleifera and target protein generate interactions and binding activity. Based on the result of the study showed two different proteins targets were Mpro and RdRp which interacted with bioactive compounds of M. oleifera. The lowest binding affinity value of Mpro and RdRp interactions were Mpro – Apigenin -7.8 kcal/mol, Mpro – Quercetin -7.3 kcal/mol, RdRp – Quercetin -6.9 kcal/mol, RdRp – Pterygospermin -6.6 kcal/mol (table 4 & 5). Respectively, implying that Mpro more easily binds to Apigenin than Quercetin and RdRp binds strongly to Quercetin than Pterygospermin. There are different amino acid residues of each receptor bound to the ligand based on the visualization by Discovery Studio. The 3D complex of the interactions formed is visualized and can be clearly distinguished between the receptor and its ligand (figure 1 & 2). The types of bonds and variations in the binding positions formed from the complexes were demonstrated in the 2D visualization performed by Discovery Studio (figure 1 & 2). In addition, the results of 2D visualization by Discovery Studio showed different colors in different interactions. The colors indicate the type of bonds formed from the complex. The amino acid residues that bind to the ligand can be seen from the interaction points, distances, chemistry bonds, and type through the discovery studio application (figure 1 & 2, table 4 & 5).

Based on the docking Discovery studio visualization showed that there were several ligands which binds to both Mpro and RdRp active sites. Apigenin (glu-166, cys-145), Chrysin (glu-166, cys-145), and quercetin (cys-145) binds to the active site of Mpro, while Anthraquinone (glu-811, trp-800, lys-798), Apigenin (ser-814, cis-813, asp-760, asp-761), Chrysin (asp-761, lys-798, glu-811), Dibutyl phthalate (asp-761, ser-814, cys-813, trp-800), Pterygospermin (glu-811, lys-798, asp-816), and Quercetin (glu-811, asp-760, asp-761, try-619) which binds to active site of RdRp SARS-CoV-2. The interaction of Mpro and Apigenin generates a conventional hydrogen bond and 2 Pi-sulfur bonds. Mpro and Chrys also generates Pi-Donor hydrogen bond and 2 Pi-sulfur bonds. Mpro and Quercetin generate Pi-alkyl bond. The interaction RdRp and Anthraquinone generates a conventional hydrogen bond, 2 Pi-alkyl bonds, and 2 Pi-anion bonds. RdRp and Apigenin generate 2 conventional hydrogen bonds and 3 Pi-anion bonds. RdRp and Chrysin generate a conventional hydrogen bond, 2 Pi-anion bonds, and a Pi-alkyl bond. RdRp Dibutyl phthalate generates 3 conventional hydrogen bonds, a Pi-anion bond, and a Pi-alkyl bond. RdRp and Pterygospermin generate a conventional hydrogen bond, a Pi-anion bond, and a Pi-alkyl bond. RdRp and Quercetin generate 5 conventional hydrogen bonds (table 4 & 5).

Mpro was a cysteine protease that moderates the maturation cleavage of polyprotein in virus replication and also plays a crucial role in the...
Table 1: Bioactivity prediction result.

| Compound          | Antiviral | Non-Steroidal Anti-Inflammatory Agent | Anti-Inflammatory | Viral Entry Inhibitor | 3C-like protease (Human coronavirus) inhibitor | Viral fusion inhibitor |
|-------------------|-----------|---------------------------------------|-------------------|-----------------------|-----------------------------------------------|------------------------|
| Rutin             | 0.263     | 0.518                                 | 0.728             |                       |                                               |                        |
| Isorhoifolin      | 0.235     | 0.705                                 |                   |                       |                                               |                        |
| Quercetin         | 0.262     |                                       |                   |                       |                                               |                        |
| Hesperidin        | 0.193     |                                       |                   |                       |                                               |                        |
| Ellagic acid      | 0.322     | 0.749                                 | 0.265             | 0.241                 |                                               |                        |
| Aurantiamide acetate | 0.217   | 0.249                                 | 0.209             | 0.341                 |                                               |                        |
| Benzyl isothiocyanate |         |                                       | 0.219             | 0.267                 | 0.011                                         |                        |
| Dibutyl phthalate |           | 0.497                                 | 0.215             | 0.293                 |                                               |                        |
| Pterygospermin    |           |                                       | 0.227             | 0.251                 | 0.010                                         |                        |
| Apigenin          | 0.209     | 0.644                                 | 0.243             | 0.267                 |                                               |                        |
| Chrysins          | 0.212     | 0.637                                 | 0.242             | 0.273                 |                                               |                        |
| Myricetin         | 0.334     | 0.720                                 | 0.272             | 0.197                 |                                               |                        |
| Chlorogenic acid  | 0.303     | 0.598                                 |                   |                       |                                               |                        |
| Vitexin           | 0.360     | 0.606                                 |                   |                       |                                               |                        |
| Anthraquinone     | 0.295     | 0.410                                 | 0.267             | 0.326                 |                                               |                        |

Table 2: Drug likeness prediction result.

| Compound          | MW (g/mol) | MiLogP  | HBD  | HBA  | TPSA (Å²) | Bioavailability |
|-------------------|------------|---------|------|------|-----------|-----------------|
| Rutin             | 610.52     | -3.89   | 16   | 10   | 269.43    | 0.17            |
| Isorhoifolin      | 578.52     | -2.96   | 8    | 14   | 228.97    | 0.17            |
| Quercetin         | 302.24     | -0.56   | 5    | 7    | 131.36    | 0.55            |
| Hesperidin        | 610.56     | -3.04   | 8    | 15   | 234.29    | 0.17            |
| Ellagic acid      | 302.19     | 0.14    | 4    | 8    | 141.34    | 0.55            |
| Aurantiamide acetate | 444.52   | 3.41    | 2    | 4    | 84.50     | 0.55            |
| Benzyl isothiocyanate | 149.21  | 3.28    | 0    | 1    | 44.45     | 0.55            |
| Dibutyl phthalate | 278.34     | 3.43    | 0    | 4    | 52.60     | 0.55            |
| Pterygospermin    | 406.52     | 2.68    | 0    | 2    | 89.12     | 0.55            |
| Apigenin          | 270.24     | 0.52    | 3    | 5    | 90.90     | 0.55            |
| Chrysins          | 254.24     | 1.08    | 2    | 4    | 70.67     | 0.55            |
| Myricetin         | 318.24     | -1.08   | 6    | 8    | 151.59    | 0.55            |
| Chlorogenic acid  | 354.31     | -1.05   | 6    | 9    | 164.75    | 0.11            |
| Vitexin           | 432.38     | -2.02   | 7    | 10   | 181.05    | 0.55            |
| Anthraquinone     | 208.21     | 1.86    | 0    | 2    | 34.14     | 0.55            |

Table 3: Drug likeness parameter.

| Compound          | Lipinski | Ghose | Veber | Egan | Muegge |
|-------------------|----------|-------|-------|------|--------|
| Rutin             | 3        | 4     | 1     | 1    | 4      |
| Isorhoifolin      | 3        | 4     | 1     | 1    | 3      |
| Quercetin         | 0        | 0     | 0     | 0    | 0      |
| Hesperidin        | 3        | 4     | 1     | 1    | 4      |
| Ellagic acid      | 0        | 0     | 1     | 1    | 0      |
| Aurantiamide acetate | 0       | 0     | 1     | 0    | 0      |
| Benzyl isothiocyanate | 0       | 2     | 0     | 0    | 1      |
| Dibutyl phthalate | 0        | 0     | 0     | 0    | 0      |
| Pterygospermin    | 0        | 0     | 0     | 0    | 0      |
| Apigenin          | 0        | 0     | 0     | 0    | 0      |
| Chrysins          | 0        | 0     | 0     | 0    | 0      |
| Myricetin         | 1        | 0     | 1     | 1    | 2      |
| Chlorogenic acid  | 1        | 1     | 1     | 1    | 2      |
| Vitexin           | 1        | 0     | 1     | 1    | 2      |
| Anthraquinone     | 0        | 0     | 0     | 0    | 0      |
| Interaction          | Binding affinity (kcal/mol) | Interaction point                  | Distance (Å) | Chemistry bond       | Types                  |
|---------------------|----------------------------|-----------------------------------|--------------|----------------------|------------------------|
| Mpro – Anthraquinone | -7.0                       | A:GLN110:NE2 - :LIG1:O            | 3.16225      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:SER158:OG - :LIG1:O             | 3.21938      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:ASN151:ND2 - :LIG1              | 4.06044      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:ILE106:CG2 - :LIG1              | 3.90218      | Hydrophobic          | Pi-Sigma               |
|                     |                            | :LIG1 – A:PH294                   | 5.29534      | Hydrophobic          | Pi-Pi T-shaped         |
|                     |                            | :LIG1 – A:VAL104                  | 5.14081      | Hydrophobic          | Pi-Alky                |
|                     |                            | :LIG1 – A:TYR54:OH                | 2.28149      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:ASP187:O                | 2.67618      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – :LIG1:O                   | 2.39102      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:LEU141:O                | 2.0859       | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:SER144:O                | 2.50024      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – :LIG1                    | 4.69304      | Hydrophobic          | Pi-Alky                |
|                     |                            | :LIG1:H – A:LEU141:O              | 2.14346      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1:H – A:SER144:O              | 2.55404      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:GLU166:N - :LIG1                | 4.06547      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:CYS145:SG - :LIG1               | 5.57509      | Other                | Pi-Sulfur              |
|                     |                            | A:CYS145:SG - :LIG1               | 5.14695      | Other                | Pi-Sulfur              |
|                     |                            | :LIG1 – A:MET1:ψ                 | 4.69304      | Hydrophobic          | Pi-Alky                |
|                     |                            | :LIG1:S – A:ASP197:OD2            | 3.77204      | Hydrogen Bond        | Carbon Hydrogen Bond    |
|                     |                            | A:ASP289:OD1 - :LIG1              | 4.12393      | Electrostatic        | Pi-Anion               |
|                     |                            | A:TYR239:OH - :LIG1               | 3.52753      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:TYR237                 | 3.44575      | Hydrogen Bond        | Carbon Hydrogen Bond    |
|                     |                            | A:LYS137:NZ - :LIG1:Σ            | 3.68971      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:CYS145 – A:ASP197:OD2          | 3.77204      | Hydrogen Bond        | Carbon Hydrogen Bond    |
|                     |                            | A:MET1:Σ – :LIG1:O               | 3.84567      | Other                | Pi-Sulfur              |
|                     |                            | :LIG1 – A:LEU287                 | 4.96378      | Hydrophobic          | Pi-Alky                |
|                     |                            | :LIG1 – A:LEU141:O                | 2.17236      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:SER144:O                | 2.29642      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | :LIG1 – A:MET1:65:SD             | 2.67346      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:SER144:O – :LIG1:O             | 3.11122      | Hydrogen Bond        | Conventional Hydrogen Bond |
|                     |                            | A:GLN189:CA - :LIG1               | 3.5174       | Hydrogen Bond        | Carbon Hydrogen Bond    |
|                     |                            | A:MET1:65:SD – :LIG1              | 3.5728       | Other                | Pi-Sulfur              |
|                     |                            | :LIG1 – A:CYS145                | 4.88012      | Hydrophobic          | Pi-Alky                |
Table 5: Molecular docking result of compounds from Moringa oleifera against RdRp.

| Interaction          | Binding affinity (kcal/mol) | Interaction point | Distance (Å) | Chemistry bond                  | Types                        |
|----------------------|----------------------------|-------------------|--------------|---------------------------------|------------------------------|
| RdRp – Anthraquinone | -5.7                       | A:TRP800:NE1 - :LIG1:O | 3.17196      | Hydrogen Bond                   | Conventional Hydrogen Bond   |
|                      |                            | A:GLU811:OE1 - :LIG1 | 4.11437      | Electrostatic                   |                              |
|                      |                            | A:GLU811:OE1 - :LIG1 | 3.6791       | Electrostatic                   |                              |
|                      |                            | LIG1 – A:LYS798    | 5.24834      | Hydrophobic                     |                              |
|                      |                            | :LIG1 – A:LYS798   | 4.93519      | Hydrophobic                     |                              |
|                      |                            | A:CY813:N - :LIG1:O | 3.24885      | Hydrogen Bond                   |                              |
|                      |                            | A:SER814:N - :LIG1:O | 3.02667      | Hydrogen Bond                   |                              |
| RdRp - Apigenin      | -6.4                       | A:ASP760:OD1 - :LIG1 | 4.40583      | Electrostatic                   |                              |
|                      |                            | A:ASP761:OD1 - :LIG1 | 3.90294      | Electrostatic                   |                              |
|                      |                            | A:ASP761:OD1 - :LIG1 | 3.23159      | Electrostatic                   |                              |
|                      |                            | :LIG1:H – A:ASP761:OD2 | 2.80245      | Hydrogen Bond                   |                              |
| RdRp - Chrysin       | -6.4                       | A:GLU811:OE1 - :LIG1 | 4.50699      | Electrostatic                   |                              |
|                      |                            | :LIG1 – A:LYS798   | 3.73501      | Hydrophobic                     |                              |
|                      |                            | A:TRP800:NE1 - :LIG1:O | 3.36979      | Hydrogen Bond                   |                              |
|                      |                            | A:CY813:N - :LIG1:O | 3.17805      | Hydrogen Bond                   |                              |
|                      |                            | A:SER814:N - :LIG1:O | 2.85424      | Hydrogen Bond                   |                              |
| RdRp – Dibutyl Phthalate | -4.4                  | A:ASP761:OD1 - :LIG1 | 3.2988       | Electrostatic                   |                              |
|                      |                            | :LIG1:C – :LIG1    | 3.8863       | Hydrophobic                     |                              |
|                      |                            | :LIG1 – A:LYS798   | 4.3898       | Hydrophobic                     |                              |
|                      |                            | :LIG1:S – A:GLU811:O | 3.68091      | Hydrogen Bond                   |                              |
| RdRp - Pterygospermin| -6.6                       | A:ASP618:OD1 - :LIG1 | 4.34769      | Electrostatic                   |                              |
|                      |                            | :LIG1 – A:LYS798   | 4.34806      | Hydrophobic                     |                              |
|                      |                            | :LIG1:H – A:GLU811:O | 1.93037      | Hydrogen Bond                   |                              |
|                      |                            | :LIG1:H – A:ASP760:OD1 | 2.46817      | Hydrogen Bond                   |                              |
| RdRp – Quercetin     | -6.9                       | :LIG1:H – A:ASP761:OD1 | 2.64803      | Hydrogen Bond                   |                              |
|                      |                            | :LIG1:H – A:ASP760:OD1 | 2.5649       | Hydrogen Bond                   |                              |
|                      |                            | A:TYR619:N - :LIG1:O | 3.19529      | Hydrogen Bond                   |                              |

Figure 1: Protein interaction conserved region amino acid residues of Mpro. (A) 3D structure of protein interactions Mpro with bioactive compounds in Moringa oleifera, (B) Magnification view 3D structure of protein interactions Mpro with bioactive compounds in Moringa oleifera, (C) 2D structure of protein interactions Mpro with bioactive compounds in Moringa oleifera.
Figure 2: Protein interaction conserved region amino acid residues of RdRp. (A) 3D structure of protein interactions RdRp with bioactive compounds in *Moringa oleifera*, (B) Magnification view 3D structure of protein interactions RdRp with bioactive compounds in *Moringa oleifera*, (C) 2D structure of protein interactions RdRp with bioactive compounds in *Moringa oleifera*. 

**Anthroquinone**

**Apigenin**

**Chrysir**

**Dibutyl Phthalate**

**Pterygospermin**

**Quercetin**
Cys-145, Glu-166, and His-163 were the most attractive residue of SARS-CoV-2 Mpro to form hydrogen bonds. The location of Mpro substrate-binding site was between domains I and II, which was Cys-145 and His-41 were catalytic activity site. In line with this study which Mpro binds to apigenin, chrysin, and quercetin in Cys-145. Besides Mpro, RdRp also plays a pivotal role in the viral life cycle. The most reachable and conserved region in viral replication was RdRp, therefore it can be an effective target for antiviral drugs for SARS-CoV-2. RdRp catalytic sites included Ala-762, Asp-618, Asp-761, Gly-616, Leu-758, Lys-798, Phe-812, Ser-760, Ser-814, Trp-617, Trp-800, Tyr-619, Tys-799. In line with this study, RdRp binds to anthraquinone (Glu-811, Trp-800, Lys-798), Apigenin (Ser-814, Cys-813, Asp-760, Asp-761), Chrysin (Asp-761, Lys-798, Gly-616), Dibutyl phthalate (Asp-761, Ser-814, Asp-813, Trp-800), Pterygospermin (Glu-811, Lys-798, Asp-618) and Quercetin (Glu-811, Asp-760, Asp-760, Tyr-619).

A recent report has shown that remdesivir is able to inhibit replication of SARS-CoV-2 in in vitro and in vivo experiment.

Remdesivir is an adenosine analogue that inhibits SARS-CoV-2 RdRp. Therefore, remdesivir can be used as a positive control in this study. According to the result docking simulations. Based on the research showed that remdesivir probably binds to Mpro stronger than to RdRp. The Mpro residues that form a hydrogen bond with remdesivir are His-163, Ser-144, and non-bonded contacts are associated with Glu-166, Cys-145, Met-165, Gln-189, Arg-188, Asp-187, His-41, Met-49, Thr-26, Leu-27, Thr-45, and Thr-25. In line with this study, the result shows that interaction Mpro - Apigenin has the lowest binding affinity value - 7.8 kcal/mol with interaction point active site Glu-166 and Cys-145, and Mpro - Quercetin binding affinity value - 7.3 kcal/mol with interaction point Cys-145. These results indicate that both apigenin, quercetin, and remdesivir bind to Glu-166 and Cys-145 of the active site of Mpro. While the interaction residues between RdRp and remdesivir are 56 residues, 10 of those residues were involved in a catalytic activity such as Ala-558, Asp-684, Asp-760, Asp-761, Cys-813, Gly-559, Ser-682, Ser-759, and Ser-814. In line with this study result shows that interaction RdRp to anthraquinone (Glu-811, Trp-800, Lys-798), Apigenin (Ser-814, Cys-813, Asp-760, Asp-761), Chrysin (Asp-761, Lys-798, Gly-616), Dibutyl phthalate (Asp-761, Ser-814, Asp-813, Trp-800), Pterygospermin (Glu-811, Lys-798, Asp-618) and Quercetin (Glu-811, Asp-760, Asp-760, Tyr-619). These results indicate that catalytic sites Asp-760, Asp-761, and Cys813 were found in the interaction of RdRp and remdesivir, apigenin, chrysin, dibutyl phthalate, pterygospermin, and quercetin.

Molecular dynamics simulation of M. oleifera’s bioactive compounds with SARS-CoV-2 glycoprotein

Simulation parameters and a set of distance restraints used by CABS-flex. Molecular Dynamics (MD) simulations carried out to generate the best convergence between either CABS-flex simulation and protein fluctuation simulation in aqueous solution. MD was carried out by 10 nanoseconds in length. In addition, MD was derived by different force fields for globular protein. MD aims to support molecular docking results. The root-mean-square fluctuation (RMSF) was a measure of the displacement of the position of the protein atom relative to the reference structure. RMSF analyzes the portions of structure that are fluctuating from their mean structure. Figure 3 showed the information of flexibility of Mpro with its interaction with Apigenin and Quercetin, figure 4 showed the information of flexibility of RdRp with its interaction with Pterygospermin and Quercetin. Based on, the RMSFs average value of Mpro and Remdevisir is below 0.4. Meanwhile, based on the result of our molecular dynamics, the average of Mpro-Apigenin’s RMSFs value at the catalytic site is 1.24 nm and the average of Mpro-Quercetin’s RMSFs value at the catalytic site is 1.04 nm. It was
few greater than the RMFS’s value of Remdevisir. Based on, the RMFS value of RdRp and Remdevisir in initial residues which were from first sequence of amino acid to 125th amino acid sequence, showed a relatively higher fluctuation value of 0.6–0.75 nm. Whereas, residues after 125th sequences showed stationary value of 0.4 nm. Meanwhile, according to the result of our molecular dynamics, the average of RdRp-Pterygospermin’s RMFS value at the catalytic site is 0.5 nm and the average of RdRp-Quercetin’s RMFS value at the catalytic site is 0.58 nm. It was a few greater than the RMFS’s value of Remdevisir.

CONCLUSION

Our in silico studies suggest M. oleifera as a potential antiviral candidate for SARS-CoV-2 with an entry inhibitor mechanism through a compound, specifically quercetin. Quercetin shows the activity as antiviral against SARS-CoV-2 by bind to the both active sites of M\(^{\text{f}}\) and RdRp of the SARS-CoV-2 with more negative binding affinity than the other compound, resulting in interactions between hydrogen bonds and hydrophobic bonds. Moreover, the RMFS value of the interaction between M\(^{\text{f}}\) and quercetin and RdRp with quercetin were not higher than 1.05. Furthermore, experimental in vitro and in vivo studies both are necessary to prove this in silico predictions.

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