In silico screening of potential compounds from begonia genus as 3CL protease (3Cl pro) SARS-CoV-2 inhibitors

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Abstract

Background: The emergence of Coronavirus disease (COVID-19) has been declared a pandemic and made a medical emergency worldwide. Various attempts have been made, including optimizing effective treatments against the disease or developing a vaccine. Since the SARS-CoV-2 protease crystal structure has been discovered, searching for its inhibitors by *in silico* technique becomes possible.

Objective: This study aims to virtually screen the potential of phytoconstituents from the Begonia genus as 3Cl pro-SARS-CoV-2 inhibitors, based on its crucial role in viral replication, hence making these proteases "promising" for the anti-SARS-CoV-2 target.

Methods: *In silico* screening was carried out by molecular docking on the web-based program DockThor and validated by a retrospective method. Predictive binding affinity (Dock Score) was used for scoring the compounds. Further molecular dynamics on Desmond was performed to assess the complex stability.

Results: Virtual screening protocol was valid with the area under curve value 0.913. Molecular docking revealed only β-sitosterol-3-O-β-D-glucopyranoside with a lower docking score of -9.712 kcal/mol than positive control of indinavir. The molecular dynamic study showed that the compound was stable for the first 30 ns simulations time with Root Mean Square Deviation <3 Å, despite minor fluctuations observed at the end of simulation times. Root Mean Square Fluctuation of catalytic sites HIS41 and CYS145 was 0.756 Å and 0.773 Å, respectively.

Conclusions: This result suggests that β-sitosterol-3-O-β-D-glucopyranoside might be a prospective metabolite compound that can be developed as anti-SARS-CoV-2.

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Introduction

The Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) is an acute respiratory tract disease that was first identified in Wuhan at the end of 2019.[1] World Health Organization (WHO) declared the disease into global pandemic according to the increasing transmission rate and incidence of cases in 2020.[2] As of December 2021, more than 264 million people were positively confirmed with this disease, and 5.2 million have passed away.[3] The common symptoms in COVID-19 patients include cough, fever, myalgia, dyspnea, and smell blindness.[4] The lack of standard drugs has made several synthetic drugs to be repurposed in order to suppress viral replication, such as oseltamivir, which inhibit neuraminidase, azithromycin and hydroxychloroquine increases the pH of the cell and interferes with the interaction of spike protein (S) to angiotensin-converting enzyme 2 (ACE2) receptor.[5,6] Despite the usage of repurposed drugs being found to possess side effects,[7,8] it encourages researchers globally to find drugs that safe, effective and selective for anti-COVID-19.[9] Recently, several targets such as spike glycoprotein (S), RNA-dependent RNA polymerase (RdRp), papain-like-protease (Pl-Protease) and main protease/3 chymotrypsin-like protease (3CLpro) have been identified as drug target of SARS-CoV-2.[10] Plpro merely cleavage of 3 sites in polyprotein chain resulted in 3 non-structural proteins (NSP 1-3).[11] Otherwise, 3Clpro cleavage 11 sites generated 13 nsp that essentially required in replication of the virus; therefore, this protein is promising as a target to be COVID-19.[12]

The Begonia is one of the largest genera of flowering plants with leaves and flowers of beautiful shapes and various colours.[13]

Literature studies reveal 1.870 plant species belonging to the Begonia genera.[14,15] Begonia commonly used to be decorative plant and some of them are empirically used to treat various diseases*, e.g., Begonia barbata* C.B. from Bangladesh was used to treat snakebite,[16] *Begonia hatacoa*, *Begonia megaptera*, *Begonia picta* from Nepal has anthelmintic activity, *Begonia panchtharensis* to relieve stomachache and *Begonia rubella* used for wound healing.[17] On the other hand, Indonesia has two species of begonia that are used in traditional medicine, *i.e.*, *Begonia baliensis* with the local name Bacem kebo from Bali that was used to treat cough,[18] and *Begonia medicinalis* or Polohi Wasu from North Morowali, Central Sulawesi Province that was used to treat numerous diseases like cancer, tumour and asthma.[19] A preliminary study by *in silico* screening on molecular docking and molecular dynamics approach prompted us to identify the potential metabolites in begonia genera as anti-COVID-19 through 3Clpro inhibitor.

Materials and Methods

Preparation of Begonia's metabolite structures

Begonia metabolite structures were obtained from a literature study. The 2D structure of metabolites was optimized using LigPrep integrated in Schrodinger 2020-3 software by converting it to 3D structure and protonated at pH 7.4 with Epik and OPLS_2005 forcefield. These processes might restore improper or missing bonds and assign protonation, possible ionization, and tautomeric states.

Receptor preparation

The crystal structure of 3 Chymotrypsin-like protease (3Clpro) was retrieved from Protein Data Bank (http://www.rcsb.org/pdb) (PDB ID: 6M2N) used as a receptor model of the SARS-CoV-2. The crystal structure of 3Clpro protein (6M2N) is a homo-tetramer with 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (Baicalein) as a co-crystallized ligand. It has a 2.20 Å resolution, 1223 total amino acid residues, and a weight of 136.38 kDa. In this study, we only use one monomer (Chain A) according to the quality structure based on the percentage of favourable regions of amino acids checked by Molprobity (molprobity.biochem.duke.edu/index.php). The quality structure reported that 98% of amino acids lie in a favourable region, indicating the protein is high quality and meets our expectations.[20,21] The protein was prepared following our previous study[22] by removing water and residual solvent; furthermore, the protein also protonated and optimized hydrogen bonds using ProtAssign and PROPKA. On the other hand, the partial charge was also added using the OPLS_2005 forcefield by the Protein Preparation Wizard panel in Maestro Schrodinger 2020-3.

Molecular docking

Before Begonia metabolites docked to the receptor, the docking protocol was validated by calculating the enrichment factor and plotting Receiver Operating Characteristic (ROC) curves. These processes are carried out by comparing active set ligands and decoy docking scores. Active set ligands were obtained from bindingdb (https://www.bindingdb.org/bind/index.jsp) for COVID-19 data with requirement activity IC_{50}<50 μM (97 compounds). Meanwhile, decoys were generated from the DUDE-Z server (tldr.docking.org) based on similarity properties of active sets, *i.e.*, molecular weight, hydrophobicity (LogP), charge, number of rotatable bonds, and the number of hydrogen bond donors and acceptors (1400 compounds).[23] The Molecular docking study was conducted using the web-based program for protein-ligand docking called DockThor (https://dockthor.lncc.br/v2/) with docking region at the centre of the co-crystallized ligand binding site. Begonia compounds were then docked to a similar region of the protocol, and the docking score was calculated to assess which compounds have potential activity as an inhibitor of 3CL protease SARS-CoV-2.[24]

Molecular dynamics

Molecular dynamics (MD) studies were performed with Desmond in Schrodinger 2020-3 refer to our previous protocol.[22] MD process began by immersing the ligand-protein complex in the simple point charge at a 10 Å water box. Moreover, to simulate under physiological conditions, the system added salt, which consists of Sodium and chloride ions at 0.15 M. These counter ions (33Na^+ and 29 Cl^-) were also helpful in neutralizing the charges of the system. The MD process runs in an OPLS_2005 force field at NPT conditions with 300 K temperature over 50 ns with recording intervals of 1.2 ps for energy and 20 ps for trajectory.

Results

The retrospective method successfully validated the docking protocol by comparing the dock score of a decoy set or inactive as a false positive and the compound with known activity as a true positive against 3Clpro SARS-CoV-2 receptor. The dock score was plotted on the Receiver Operating Characteristic (ROC) curves with Area under Curve (AUC) value is 0.913 (Figure 1). The docking result showed that only one, namely β-sitosterol-3-O-β-D-glucopyranoside,[1] with dock score -9.721 kcal/mol, had slightly higher affinity than co-crystallized ligand (baicalein) and even with protease inhibitor standard drugs, *i.e.*, Indinavir[2] (dock score=-9.715 kcal/mol) (Table 1). Molecular interactions at 4 Å cut off showed the ligand corresponding to the numerous binding mode of 1 to the receptor, followed by polar interactions with THR24; THR25; THR26; HIS41; SER46; HIS164; ASN142; GLN189; THR190; and GLN192, hydrophobic interactions with LEU27; CYS44; MET49; CYS145; MET165; LEU167; PRO168; ALA191, positive charge with GLU166, and negative charge with ARG188 (Figure

Table 1. Top 10 docking score of Begonia's metabolites and reported antiviral.

No.	Metabolite_Pubchem ID	Docking Score
1	β-sitosterol-3-O-β-D-glucopyranoside_12309057	-9.721
2	Cyanidin 3-(6''-(Z)-caffeylsambubioside)_44256820	-9.684
3	Cyanidin 3-(6''-p-coumarylsambubioside)_131753089	-9.623
4	Physcion-10,10'-bianthrone_179377	-9.575
5	Pelargonidin 3-sambubioside_44256622	-9.522
6	(-)-Auranamide_173952	-9.475
7	Kaempferol 3-O-rutinoside_5318767	-9.472
8	β-Sitosterol_222284	-9.432
9	Cyanidin 3-(6''-(E)-caffeylsambubioside)_44256747	-9.29
10	Isoquercetin_5280804	-9.287
11	Indinavir	-9.715
12	Saquinavir	-9.415
13	Lopinavir	-9.285
14	Ritonavir	-9.25
15	Carfilzomib	-9.098
16	Remdesivir	-8.472
17	Co-crystallized ligand (Baicalein)	-8.189

2A). Similar to 1, 2 exhibit various interactions of binding mode to the 3Clpro receptor, *i.e.*, polar interactions with THR24; THR25; THR26; HIS41; THR45, SER46, ASN142; HIS164; and GLN189, hydrophobic interactions with LEU27, VAL42, CYS44; MET49; CYS145; and MET165, positive charge with GLU166 (Figure 2B). Hydrogen bond was also observed among -NH group of 2 structures that act as donor and GLU166 as acceptor proton. Besides, hydrophobic interactions support the ligand among the protein cavities. In this study, both compounds have contacts with catalytic sites of 3Clpro SARS-CoV-2 (CYS145 and HIS41), which reveal that the compounds might inhibit proteolysis activity.

Compound 1 was selected for further analysis by molecular dynamics simulation, according to the best docking score and binding interactions, compared with 2 as the positive control. The stability interactions, including non-bonding interactions within essential amino acid residues in the binding site on the 3Clpro receptor, were studied for 50 ns simulation time. The Root Mean Squared Deviation (RMSD) and Root Mean Squared Fluctuation (RMSF) plot of complex protein-ligand (Figure 3) helped determine the stability interactions between protein-ligand system. It showed that 1 was stably bound to the receptor for the first 30 ns according to the lower RMSD value (<3 Å), suggesting the compound was stably bound to the binding site for the time. Meanwhile, after 30 ns, the escalation RMSD value of 1 reached 5 Å suggesting minor conformational changes that occurred in the last 20 ns simulation times. However, 2 exhibited higher RMSD (>4 Å) for equilibration states in the first 20 ns, even after the simulation. This result explained that 2 has significant conformational changes, which means it was unable to stabilize its conformations implicating to diffuse away of the ligand from the binding site of the 3Clpro receptor and verified by the docking score of this compound slightly higher than 1, indicating 2 had a lower affinity to bound with 3Clpro than begonia phytoconstituents. Furthermore, the RMSF value was used to assess the fluctuations of specific amino acids that interact with the ligand during simulation time.

Table 2. MMGBSA energy calculations after 50 ns simulation times.

No.	Compounds	MMGBSA binding free energy (kcal/mol)
1	β-sitosterol-3-O-β-D-glucopyranoside	-39.97340
2	Indinavir	-32.02555

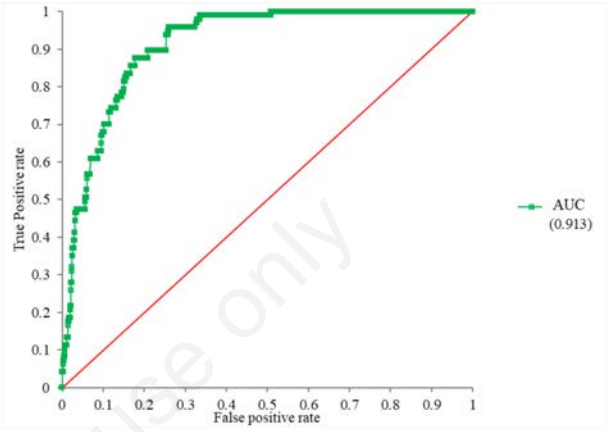

Figure 1. Retrospective validation of our docking protocol has excellent performance according to the ROC curves (AUC=0.913), which can be used for further virtual screening. The ROC curves were plotted as true positive rate or sensitivity (x-axis) versus false positive rate or specificity (1-sp). The diagonal red line (baseline) represents the results expected from the random selection of ligands.

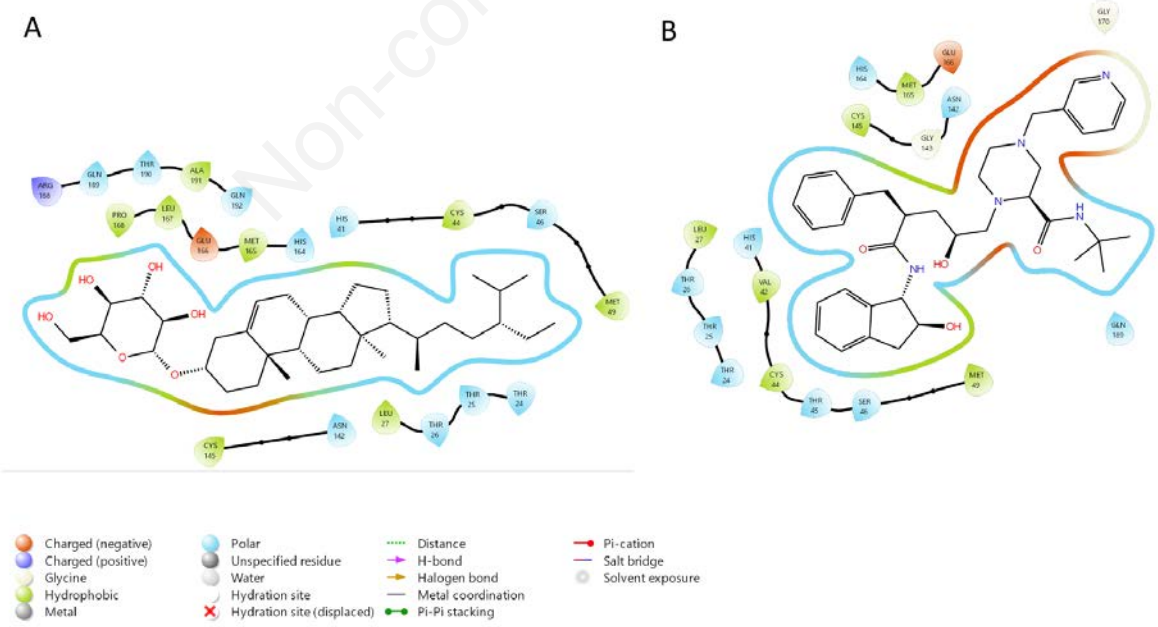

Figure 2. Molecular interactions of β-sitosterol-3-O-β-D-glucopyranoside (A) and Indinavir (B) against binding sites of 3Clpro SARS-CoV-2. Both compounds interacted with catalytic sites suggesting being active as 3Clpro inhibitors.

This study focused on the interactions with a catalytic site that correspond to proteolytic activity (HIS41 and CYS145). Compound 1 has an RMS value of 0.756 and 0.773 which is higher than 2 (0.606 and 0.539) for HIS41 and CYS145. These results showed that 1 has a stable bound to 3Clpro for 50 ns according to RMSD and RMSF plots. The MMGBSA calculations exhibited that compound 1 exhibited lower binding free energy than compound 2 as the positive control (Table 2); which means the compound 1 has more stable for 50 ns simulation times than 2 and potentially inhibit 3Clpro SARS-CoV-2.

Discussion

Molecular docking is a computational method broadly used to predict interactions of the ligand with protein, which is helpful to understand their conformational binding that leads to optimization and determination activity of the hit.[25,26] Even though the docking method has been used extensively in drug discovery, the protocol needs to be validated by internal or retrospective validation. The advantages of retrospective validation methods are capable of an independently distinguished number of actives in the decoy set and offer specific information and sensitivity of the target related to docking performance.[27] The docking method in this study has excellent performance according to the AUC value >0.9;[28] which suggests that our docking protocol could be feasible to screen the begonia's compounds that potentially have activity as 3Clpro SARS-CoV-2 inhibitor. The parameter of molecular docking result was the docking score used to evaluate which compounds possess potential activity as an inhibitor of the viral protease. Dock score represents estimated free energies reflecting the complex ligand-receptor's binding affinity with a more negative value implicating stronger binding, and the best conformation ligand-bound was formed.[24] However, molecular interactions also play crucial roles in protein-ligand binding. Hydrogen bonds contribute to the stability of protein structure and folding. This interaction occurred when the proton covalently attached to one electronegative donor atom that shares with another electronegative acceptor atom and was first recognized at Pauling's proposal for secondary structure elements of the protein.[29] Meanwhile, hydrophobic interaction contributes to the stability of protein and hydrogen bonds.[30] It involves contacts between non-polar groups of the compound and binding pockets of receptors that implicate tighter binding of protein-ligand complex.[29] Meanwhile, polar and charge interactions depend on the local environment and are attributed due to electrostatic interaction energy among electron clouds involved in various drug–receptor interactions and play a significant role in ligand–binding affinity.[31]

Since molecular docking has limited capabilities to evaluate the interaction stability between protein-ligand complexes, molecular dynamics was able to calculate the atom movements based on Newton's classical motion equation to determine the stability interactions of protein-ligand in dynamics conditions using RMSD, RMSF, and MMGBSA as parameters.[32] The RMSD and RMSF plots helped determine the stability interactions protein-ligand system, which is higher values implicate more fluctuation or displacement interaction of the compound to the protein.[33] Our study revealed that compound 1 has a more stable bound to the 3Clpro SARS-CoV-2 receptor than 2 according to the lower RMSD value; Although 1 has a higher RMS value which means it is more fluctuative interact with the specific amino acid, the values are still in acceptable range (1-3 Å).[33,34] One of the popular methods to estimate the binding affinity of protein-ligand complexes in dynamic conditions is MMGBSA calculations. Our study revealed that 1 has a lower MMGBSA value than 2, indicating that the compounds are more stable for 50 ns. It is based on thermodynamic energy calculation that represents more negative values, indicating stronger binding.[35,36]

Begonia (*Begoniaceae*) is one of the largest genera in *Angiospermae*. The begonia genus consists of 1870 species widely distributed in the tropic or subtropic of Africa, America, and even Asia.[37] *Begonia genus* was estimated to have 450 species in Asia, especially in the Malesia region, and half of them are found in Indonesia.[18] Some species of *Begoniaceae* are known not only as ornamental plants but have been used as vegetables and medicinal herbs, *i.e.*, *Begonia glabra* was used to treat a fresh wound. In contrast, *Begonia fibristipula* was used to relieve fever, cough, and pain and as a bitter tea beverage. On the other hand, *Begonia grandis* is used to clean wounds and treat various diseases.[38] In Indonesia, some Begonia species that are empirically used as traditional medicine are *Begonia baliensis* from Bali, *Begonia lombokensis*, and *Begonia lempuyangensis* to treat coughs and various diseases. At the same time, *Begonia medicinalis* from Morowali is

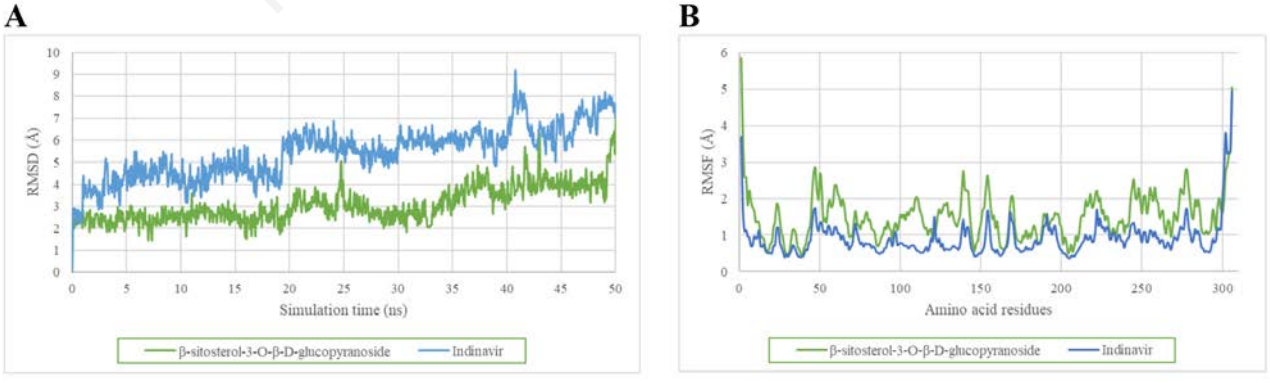

Figure 3. Root Mean Square Deviation (RMSD) plot exhibits that β-sitosterol-3-O-β-D-glucopyranoside has a lower RMSD value, meaning the complex ligand-receptor has more stable than Indinavir (A). However, β-sitosterol-3-O-β-D-glucopyranoside has a higher Root Mean Square Fluctuation (RMSF) that reflects the amino acids are fluctuative quitely value than Indinavir but is still on the acceptable threshold (<3 Å) (B). It can be known that β-sitosterol-3-O-β-D-glucopyranoside is stably bound to the 3Clpro SARS-CoV-2 receptor for 50 ns simulation times.

used for the palliative treatment of Diabetes, gout, laxative, and TBC.[18,19] Various medicinal potencies of the *Begonia genus* are related to the numerous phytoconstituents in these plants.

The bioactive compounds from the Begonia genus reported so far are phenol, flavonoid, steroid, terpenoid, alkaloid, saponin, and tannin.[19,39-42] Our research strategy by *in silico* screening successfully identifies potential compounds that actively inhibit 3Clpro from the *Begonia genus* and understand their inhibitory mechanism with fast and less time-consuming to discover anti-SARS-CoV-2 drugs. As for to date, the best we know is that this is the first report of terpenoid including steroid from begonia that actively not only as an anticancer but also active against 3Clpro SARS-CoV-2 activity suggesting this approach can be used for discovering potential drug compounds from nature.[41] The potential activity from the begonia plant still needs to be assayed further to support the application as medicine to treat the COVID-19 pandemic.

Conclusions

Molecular docking and molecular dynamics analysis successfully identified steroidal glycosides of β-sitosterol-3-O-β-D-glucopyranoside as inhibitor 3Clpro SARS-CoV-2. This compound was reported on several plants; *B. malabarica, B. nantoensis,* and *B. medicinalis*. These plant extracts could be selected for in vitro study and further development of the steroidal glycosides compound against SARS-CoV-2.

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