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Potential Phytochemical Inhibitor from *Allium cepa* for the Medication of COVID-19 using In-Silico Approach

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Abstract. Infection of extreme acute respiratory syndrome coronavirus 2 triggers Coronavirus disease 2019 (COVID-19). COVID-19 has adverse consequences on persons and is getting worse in all nations. The aim of this research is to investigate the development of in-silico approach of phytochemical inhibitor used to fight COVID-19 pathway inhibition. In medicinal plants, there are many phytochemicals, however the bioactive mechanism remains uncertain. In-silico experiments offer additional evidence to confirm the inhibition of medicinal plants. Molecular docking was used to evaluate phytoconstituents from *Allium cepa* as COVID-19 M-pro inhibitor, compared to remdesivir (standard drug). STITCH database used to predict the interaction network process of the most potential compound. The most potential compound was oleanolic acid. Oleanolic acid with a docking score of -9.20 kcal/mol was reported as anti-COVID-19 activity. This docking score was higher than remdesivir. Oleanolic acid interacted with GLU166, CYS44, HIS41, and THR25 via the hydrogen bond. From STITCH Database, oleanolic acid interact with CASP-9, XIAP, CASP-3 signalling pathway. Oleanolic acid from *Allium cepa* has been reported as a possible COVID-19 M-pro inhibitor and should be studied in future studies. The experiment indicates that phytochemical inhibitor can be helpful in the medication of COVID-19.

Keywords: *Allium cepa*, covid-19, in-silico, molecular docking, STITCH.

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Introduction

COVID-19 was first found in Wuhan, China and became a global pandemic announced by WHO on 11th March 2020 [1]. COVID-19 was induced by poisoning of SARS-CoV-2. In all nations, COVID-19 has devastating consequences on persons. Since 2019, SARS-CoV-2 has been destroying the human immune, triggering differing degree of organ failure [2]. SARS-CoV-2 was identic to MERS-CoV and SARS-CoV by constranting coronavirus host infection patterns in other vertebrates. The transmitting of SARS-CoV-2 amongst human could also be feasible. By taking angiotensin-converting enzyme 2 (ACE-2) in human as a receptor, it can infect human cells [3]. According to the WHO, the amount of confirmed COVID-19 death 567.734 and 12.847.288 deaths world on 12 July 2020 04:59 GMT, while worldwide death is calculated at 7% and getting worse. The present pandemic there is no clear antiviral medication yet. There is an immediate need, when the things are getting worse. Unfortunately, with the current pandemic, there is no particular antiviral medication. There is an immediate need for an untreated patient to be handled and mortality reduced [4]. To find new medicines, vaccine expert and physician are working hard toward COVID-19. Remdesivir has been approved in the United States to enhance the health status of patients with COVID-19 and has been licensed for emergency use in the treatment of COVID-19. Therefore, to manage this disorder, possible chemotherapic agents are urgently needed [5]. However, there are great deals of clinical testing in China, but no medication for the treatment of COVID-19 has been approved [6].

Due to the essential function in health treatment, medicinal plants have been as pharmaceutical. Medicinal plant has antiviral efficacy, but their mechanism for COVID-19 has not yet been clarified [7]. The basis of therapeutic potential is bioactive compounds from plant chemicals. There are several chemical substances with distinct roles in phytochemical spectra. It is known that plant contain healing activities and have greater affinity in the human than drugs [8]. Herbal medicine will become new medicine with and pharmaceutical and chemical screening [7]. Recently, research of conventional medicinal plants had increased since the natural resources and diversity of phytochemical constituents make rapid development in modern pharmacology. In-silico methods is frequently applied in attempts to elucidate the bioactive compounds of medicinal plants as computer technology has grown up. Applying simulated drug development screening will indicate the effect of medicinal plants, minimize expenses, and increase the efficacy of the whole process. Several FDA approved medicines were produced from phytochemicals or animals utilizing in-silico methods [9]. Molecular docking experiments may be an easy key to discovering effective nature medicine [10]. Quercetin, which has antiviral effects, is found in onions (*Allium cepa*) [11].

Computational tools used to design medicines. Low time requirements for computational methods allow it possible to identify potential medication and foresee the adverse effects of experimental drug through high throughput screening of current medicines. Clinical testing is needed. In this work, computational chemistry plays an important role in researching novel medicines through molecular docking experiment. With the usage of computer software to develop experimental drugs, the recognition of specific goals has now evolved. The molecular docking research was performed between phytochemical inhibitors from *Allium cepa* and receptor for SARS-CoV-2. The design of experimental research can be told by computational methods. Recent study has shown the utility of computational work for finding possible SARS-CoV-2 antiviral medicines. The binding score between the ligand and the binding site in receptor calculated by molecular docking. The aim of this work is to perform in-silico approach from *Allium cepa* to fight COVID-19 pathway inhibition, hoping to be a better-activating potential medicine.

Experimental

Molecular Docking Method

The binding energy and binding mode of the phytochemical constituents to the key protease target was examined by molecular docking. The study was conducted using AutoDock Tools 4. The binding energy of the compound to the receptor was validated using Auto Dock 4.2 (Scripps Research Institute, La Jolla, CA, USA) was used to validate the

binding energy of ligand-receptor complex. The 2D interactions were obtained from Discovery Studio 4.5 [12]. The bioactive compounds from *Allium cepa* were obtained from Pubchem database in the .sdf format. Remdesivir was chosen from the the database in Pubchem sdf format. Docking and binding energy is used to evaluate the antiviral efficacy of the *Allium cepa* phytoconstituents [13].

We conducted a docking simulation into Covid-19 main protease-X77 complex as protein receptor from several phytoconstituents. The structure of Covid-19 main protease-X77 was retrieved in the Protein Data Bank (PDB ID 6W63; www.rscb.org/pdb) [14]. Redocking of the co-crystal X77 at the active site of SARS-CoV-2 Mpro was conducted to accurately refine the molecular docking. Chimera version 1.13.1 was used to prepare the protein receptor and the ligand [15]. All the water molecules were withdrawn, and atoms of hydrogen were introduced. The evaluation number and maximum generation were set at 250000 and 27000 respectively [16]. A ligand has been docked at the SARS-CoV-2 main protease active site.

Autodock includes pre-calculated grid maps. Using ADT, the parameters were set for the Grid box. The Lamarckian Genetic can be validated with 50 docking runs by the best conformation of the ligand. The default parameter was set. After the full execution of AutoDock, ten ligand conformations in the receptor complex were obtained, which were evaluated by binding energy [17]. The lowest docking energy was determined. Molecular interactions such as hydrogen bonding, hydrophobic interaction, and electrostatic were examined [18]. The Dock Score feature was used for scoring all the ligands. Analyses have been identified for the best pose. The affinity between the ligand and the candidate target for COVID-19 inhibitor is shown by the docking score in the current work [19].

STITCH Database

The network interaction in oleanolic acid was identified and created using Search Tool for Interacting Chemicals (STITCH) database 5.0 (http://stitch.embl.de/) [20]. The STITCH is a database of predicted protein-organism interactions to investigate ligand-receptor interactions [21]. Oleanolic acid was inserted and arranged as default [22]. The species was set to Homo sapiens, and description of network interaction could be obtained.

Results and Discussion

Bioactive compound from phytochemical inhibitor has been identified to demonstrate antiviral activity. Phytochemical inhibitors of *Allium cepa* have been studied as possible treatment for COVID-19 inhibitor. The phytochemical constituens compared to remdesivir as standard medicine. The presence of plentiful medinal plants has been examined by previous research. The binding energy derived from6W63 protein docking with native ligand was -9.24 kcal/mol can be seen in Table 1. The main protease was used to construct a model for the SARS-CoV-2 M-pro structure. The model 6W63 calculated from X-Ray crystallographic. The X77 inhibitor was bonded to protein receptor make a complex structure. AutoDock Tools has measured the binding mode and binding energy of X77 compound as native ligand [23].

The docking results between SARS-CoV-2 M-pro and *Allium cepa* phytochemical compounds from: quercitrin, progesterone, peonidin 3-arabinoside, methyl ferulate, kaempferol 4'-glucoside, (-)-jasmonic acid, oleanolic acid, cyanidin 3-O-glucoside, and 9 cyanidin 4'-glucoside was -7.19 kcal/mol,-8.38 kcal/mol, -7.08 kcal/mol, -5.19 kcal/mol, -6.27 kcal/mol, -5.43 kcal/mol, -9.20 kcal/mol, -7.03 kcal/mol, and -5.78 kcal/mol respectively. The rank was as follows: methyl ferulate > cyanidin 4'-glucoside > kaempferol 4'-glucoside > cyanidin 3-O-glucoside > arabinoside > peonidin 3-arabinoside > quercitrin > progesterone > oleanolic acid. The docking results can be seen in Table 1. The lower binding energy means affinity getting higher. The binding energy displays the enzyme-ligand interaction affinity via an optimized algorithm that function as an inhibitor. It can also control their antiviral behaviors in comparison to phenolic hydroxyl. Different hydrophobic groups can contribute significantly to the binding of the target proteins to the hydrophobic cavity.

Table 1. Binding energy of *Allium cepa* with 6W63

Compound name	Binding Energy (kcal/mol)	Pubchem ID
quercitrin	-7.19	5280459
progesterone	-8.38	5994
peonidin 3-arabinoside	-7.08	91810651
methyl ferulate	-5.19	5357283
kaempferol 4'-glucoside	-6.27	5491693
(-)-jasmonic acid	-5.43	5281166
oleanolic acid	-9.20	10494
cyanidin 3-O-glucoside	-7.03	441667
cyanidin 4'-glucoside	-5.78	73981555
remdesivir	-7.63	121304016

Table 1. Residue interaction *Allium cepa* with 6W63

Compound name	Hydrogen Bond	Electrostatic	Hydrophobic
native ligand		HIS 41, MET 49	MET165, LEU141, LEU27
cyanidin 4'-glucoside	**GLY143, GLU166,**	-	-
cyanidin 3-O-glucoside	ASN142, THR26, GLN192, THR25, **GLU166**, **GLY143**,SER144, THR26, HIS41, HIS163,	-	CYS145
oleanolic acid	**GLU166,** HIS41, CYS44, THR25	-	CYS145, MET165, MET49, LEU167, PRO168
(-)-jasmonic acid	ARG188	-	MET49, PRO52, CYS44,
kaempferol 4'-glucoside	**GLU166**, THR25, PHE140, HIS41, ASN142	-	HIS41, THR25
methyl ferulate	THR190, GLN192	-	LEU167, MET165 PRO168
peonidin 3-arabinoside	**GLU166, GLY143,** THR26	-	**MET165, LEU141**
progesterone	**GLY143**, THR190, GLN192	-	**MET165**
quercitrin	**GLU166**, GLY143, PHE140, THR26, GLN189	-	MET49, MET145
remdesivir	**GLU166**, **CYS145**, ASN142, GLN189	-	MET49, HIS41

Molecular docking methods showed details and the visualization of the ligand-inhibitor binding role. The same inhibition process can have a common binding mode and binding position. Remdesivir has hydrogen bonds and hydrophobic bonds with 6W63 protein in Table 2. Hydrogen bonds in ligand cyanidin 3-O-glucoside, cyanidin 4'-glucoside, oleanolic acid, kaempferol 4'-glucoside, peonidin 3- arabinoside, quercitrin, and progesteron identical with native ligand. Oleanolic acid has the highest affinity of all substances. The docking study revealed that some compounds ranked by binding energy (ΔG). In *Allium cepa*, the most suggested compound was oleanolic acid. The affinity of drug candidate depends on the residue interaction and bonfing occurring in active protein site. Figure 1 showed the comparison of the most recommended compound with native ligand.

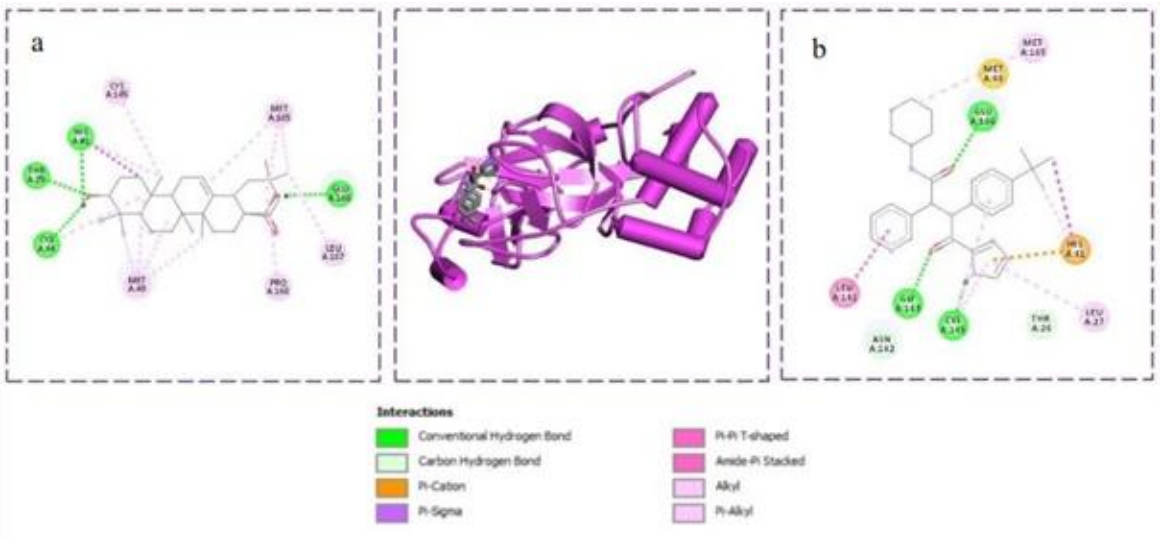

Figure 1. Comparison of 3D structure of the most recommended compound with native ligand (a) oleanolic acid (b) ligand X77

In this research, the most potential phytochemical constituents was selected compared to ligand X77 as native ligand and the standard medicine to propose potential treatment of antiviral based on molecular docking. The docking results demonstrated the phytochemical binding energy with the SARS- CoV-2 Mpro receptor using Auto Dock Software. Docking methods are used to explore phytochemical- receptor interaction. Complex stability is derived from amino acid residue visualization and binding energy. The phytochemical inhibitors were analyzed using AutoDock Software.

Most countries depend on herbal products due to their protection and medicinal capabilities [24]. A variety of some bioactive compounds recorded have antiviral effect. We explored phytochemical inhibitors as a possible candidate for COVID-19 main protease inhibitor. The results from this analysis will provide chances to create the appropriate medication for COVID-19 M-pro with 6W63 receptor.

Viruses required to the host protein to reproduce until hijacking the host cell. Usage of the host protein inhibitor may block viral replication. Antiviral medicine would be the host protein inhibitor [25]. Several medicines are expected to be used in the treatment of COVID-19 but are still under debate [26]. However, molecular target of secondary metabolites has not been established in experiment in vivo and in vitro, and the pathways remain unknown. The pharmacophore modelling is valid for predicting and simulating the binding state of ligands and proteins [27].

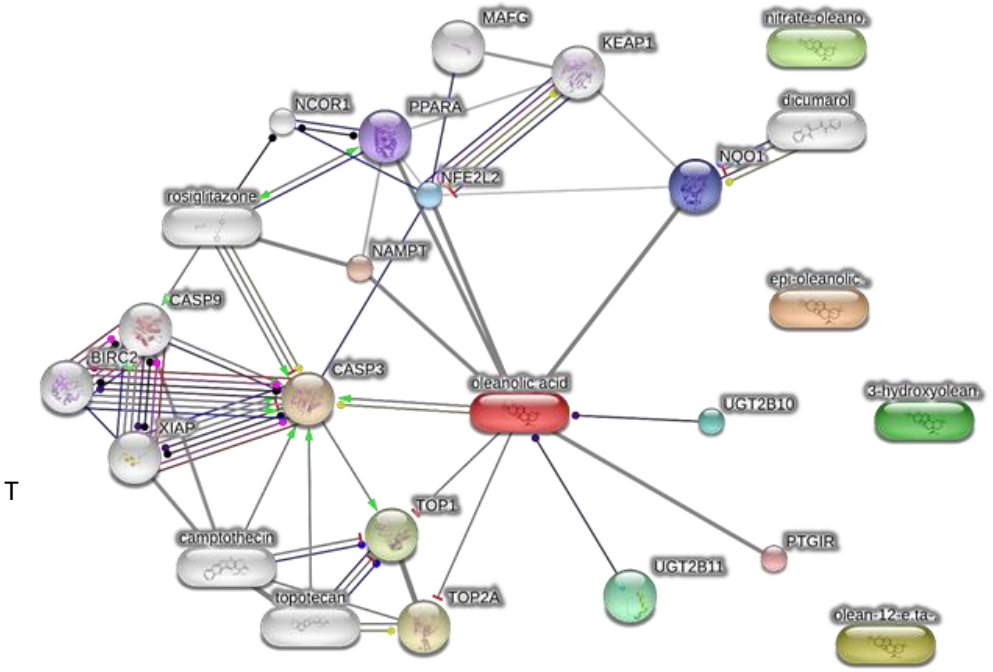

Figure 2. Interaction network of oleanolic acid based on STITCH database

STITCH index showed the objective of oleanolic acid interface network. There were ten nodes in the first shell: UGT2B11, NFE2L2, UGT2B10, PPARA, NQO1, CASP3, PTGIR, TOP2A, NAMPYT, and TOP1. Ten nodes in the second were topotecan, XIAP, camptothecin, CASP9, BIRC2, rosiglitazone, MAFG, NCOR1, dicumarol, and KEAP1. The CASP9, CASP3, and XIAP signaling pathways were the significant anti-COVID19 linked with oleanolic-acid-target genes via KEGG pathways. Inhibition, catalysis, and activation were indicator for this indicator.

The binding capacity of oleanolic acid is higher than remdesivir to SARS-COV-2. These coupled with ACE2 binding to the signaling pathway of CASP-3 to control apoptosis, which is worth further analysis and aims to give analytical guide. A strong affinity of oleanolic acid with receptor was discovered by molecular docking approach. Different biological activity and mechanisms for the medication of COVID-19 were involved in the phytochemical constituents by combining the critical target proteins XIAP, CASP-9, and CASP-3. Figure 2 showed the interaction network.

There are antiviral effects in oleanolic acid. XIAP (X-linked apoptosis inhibitor) is a multifunctional protein that manages apoptosis and caspase, and manages inflammatory signals and tollerance, cell invasion, cell proliferation, and metastasis, based on STITCH. XIAP acts as a shortest inhibitor caspase. Caspase 3, cycteine peptidase, apoptosis-related, involved in activating caspases conduct for apoptosis enforcement. Caspase 9, peptidase cysteine-related apoptosis. Oleanolic acid has the viral myocarditis KEGG pathway (pathway ID 05416). Based on the previous analysis, oleanolic acid induced apoptosis involving the release of cytochrome C mitochondria into the cytosol and enabled caspase-3 and caspase-9 activation with polymerase (ADP-ribose and PARP cleavage) [28].

Oleanolic acid was the most suggested phytochemicals in *Allium cepa,* which could be possible inhibitor of COVID-19. The expected drug binding and docking score ranking would be helpful in explaing the findings COVID-19 clinical trials. This work rationalizes restricted drug efficacy evidence for treatment of COVID-19 and offers details on the selection of drug candidates for in-vivo and in-vitro tri

Conclusion or Closing Remarks

In silico method of *Allium cepa* for medication of COVID-19 reported as well. *Allium cepa* is potent phytochemical for creating new COVID-19 medical. The most potential phytochemical in *Allium cepa* was oleanolic acid, which can be a possible inhibitor of COVID-19. The most potential compound was oleanolic acid with a docking score of -9.20 kcal/mol was reported as anti-COVID-19 activity. This docking score was higher than remdesivir. Oleanolic acid interacted with GLU166, CYS44, HIS41, and THR25 via the hydrogen bond. From STITCH Database, oleanolic acid interact with CASP-9, XIAP, CASP-3 signalling pathway. More study and research are needed to explore the possible uses of phytochemicals. These findings show that anti-viral and anti-inflammatory properties from oleanolic acid could be beneficial in treatment of COVID-19.

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