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IN SILICO STUDY REVEALED THE ANTI-VIRAL POTENCY OF *Allium cepa* BIOACTIVE COMPOUNDS INHIBIT M-PRO SARS-COV-2 PROTEIN

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Abstract

A zoonotic disease called COVID-19 was initially spread from animals to people. SARS-CoV-2, as opposed to SARS, was a pandemic that resulted in about 274,676,729 cases globally. Numerous studies have looked into potential novel treatments for COVID-19 infection. In this study, 16 phytochemicals from *Allium cepa* L. were examined in silico for their potential to bind to the primary protease of COVID-19 (PDB ID: 6LU7). Lipinski's rules are used to choose the ligands. Protein protease's (Mpro) Cys-145 and His-41 are its active sites. The chosen ligands are examined using molecular docking with PyRx and Vina Wizard and 2D visualization with LigPlot+. Selected ligands' binding energy value will be contrasted with hydroxychloroquine as a control. According to the results, the compounds luteolin (CID: 5280445), isorhamnetin (CID: 5281654), and apigenin (CID: 5280443), which had binding energies of 7.4, 7.2, and -7 kcal/mol, respectively, become potential COVID-19 inhibitors. These substances have higher binding energies than the control, hydroxychloroquine (CID: 3652), which has a lower binding energy (-6 kcal/mol). Due to its important pharmacokinetic features, luteolin demonstrated the best binding efficacy to Mpro, enabling the development of new drugs.

INTRODUCTION

According to the World Health Organization (WHO), around 274,676,729 cases of COVID-19 have been reported worldwide until December 2021. COVID-19 is caused by SARS-CoV-2, which is classified in the coronavirus family. This strain has widely attacked the human immune system and triggered various organ failures [1,2]. Unfortunately, no particular anti-viral drug exists to treat COVID-19 infection [3]. Wang *et al*. from the Institute of Virology in Wuhan have discovered some anti-viral drugs, including remdesivir and chloroquine [4]. Currently, the research has focused on targeting protease, helicase, polymerase, and immunomodulators such as interferon by repositioning drugs for anti-viral treatment [5, 6].

Various mathematical models have been established as technology develops to help researchers develop drugs. Bioinformatics has been one of the most important and innovative approaches to the design of new drugs. Various bioinformatics techniques can easily reduce the trial time,

possible errors, and high costs of clinical and laboratory trials [7]. Molecular docking is one bioinformatic technique focusing on energy bonding and chemical stability among atoms or molecules, which has become the most important method for drug design [5].

The current therapeutic strategies focus on inhibiting viral infections using enzyme inhibitors in natural compounds due to their minimal side effects [5]. *Allium cepa*, known as shallots, has been observed to contain phytochemicals that can block the necessary genetic materials and proteins of SARS-CoV-2 [8]. Many studies have also shown that shallots can be used to treat viral infections and prevent severe diseases [9]. Therefoe, this study aimed to find effective natural compounds from shallots that can exhibit effective inhibitory efficacy against the main protease of COVID-19 by using a molecular docking approach. The main protease, Mpro, is the main enzyme of SARS-CoV-2 that mediates viral replication and transcription [10]. This protease has been successfully deposited in the PDB (PDB ID: 6LU7). In addition, it has been considered a possible target protein to inhibit viral replication [11, 5, 12]. The result of this study is expected to provide novel knowledge about the potential of *Allium cepa*'s active compounds for inhibiting the SARS-CoV-2's main protease.

MATERIALS AND METHODS

The main protease M^{pro} structure model was obtained from the Protein Data Bank (PDB-ID: 6LU7). The ligands were downloaded from PubChem, including 2-propenyl propyl disulfide (CID: 16591), apigenin (CID: 5280443), bis(1-methylethyl) disulfide (CID: 77932), cis-1 propenyl propyl trisulfide (CID: 5352694), cycloallin (CID: 12305353), dimethyl disulfide (CID: 12232), dimethyl trisulphide (CID: 19310), dipropyl disulfide (CID: 12377), isopropyl propyl disulfide (CID: 118529), isorhamnetin (CID: 5281654), isovanilin (CID: 12127), luteolin (CID: 5280445), methiin (CID: 9578071), methyl propyl disulfide (CID: 16592), S-propyl L-cysteine (CID: 101975) and S-propylcysteine (CID: 125198).

Those ligands were examined based on Lipinski's five rules, which include (a) molecular mass, (b) degree of lipophilicity (LogP), (c) hydrogen bond donors, (d) hydrogen bond acceptors, and (e) molar refractivity. Molecular docking is carried out to check binding affinity, amino acid residues, and bonding interactions using Pyrx integrated into the Vina Wizard program [15,16]. The docking approach in this present study was based on the blind docking which target whole protein structure. Binding affinity determines the strength of ligand-receptor interaction, with more negative binding affinity leading to stronger ligand-receptor interaction and better molecular docking prediction [17]. Molecular docking will show up in 3D visualization. Meanwhile, 2D visualization was carried out using the LigPlot+ program. The main protease and ligands were converted into.pdbqt format for molecular docking, including 3D visualization, using the Pyrx program. Then, it was run using the Vina Wizard program. For 2D visualization, the data must be converted into the pdb format using PyMol and visualized using the LigPlot^{+} program.

RESULTS AND DISCUSSION

Lipinski's five rules for 16 phytochemical compounds and their molecular docking results are shown in Table 1. Lipinski's rule of five is one of the methods commonly used to predict aspects of the ADME (absorption, distribution, metabolism, and excretion) of drugs, especially for oral drugs [18]. Results show that Lipinski's five rules are qualified, where all phytochemical compounds have (a) a molecular mass lower than 500 daltons, (b) a degree of lipophilicity expressed as LogP lower than 5, (c) hydrogen bond donors less than 5, (d) hydrogen bond acceptors less than 10, and (e) a molar refractivity between 40 and 130 [5, 23]. If the results are not under the rules, the compound will be difficult to absorb, and the permeability will be low [14]. Therefore, an *in silico* analysis of physicochemical properties and pharmacokinetic profiles is needed as an initial reference in developing drug compounds [24]. We check all potential compounds' binding affinity with the main protease SARS-CoV-2 as a target receptor protein [19].

Based on molecular docking results, the 16 compounds have the potential to become drugs (Figure 1). Luteolin, isorhamnetin, and apigenin interact with the MPRO protein with a stronger binding affinity (-7.0 to -7.4 kcal/mol) than the control (hydrochloroquine; -6.0 kcal/mol). In general, the affinity values of this phytochemical compound ranged from 16–23% lower than the control. The lower affinity value of docking means higher bonding energy [23]. Meanwhile, the binding affinity value of the other phytochemical compounds is higher than the control. It was assumed that those were not effective for drug development. Luteolin has a binding affinity of -7.4 kcal/mol. It has hydrophobic contacts with the previously mentioned receptor active site, Cys-145[A], as well as His164[A], His41[A], Met165[A], Glu166[A], and Gln189[A] residues. Other interactions include Asn142[A], Thr190[A], Gln192[A], and Arg188[A], linked via hydrogen bonds. Isorhamnetin has a binding affinity of -7.2 kcal/mol and interacts hydrophobically with Cys145[A], Leu141[A], Met165[A], Leu167[A], Gln192[A], and Gln189[A], as well as Phe140[A], Thr190[A], and Glu166[A] linked by hydrogen bonds. Additionally, the apigenin interacts with the residues Phe140 [A], His164 [A], Met165 [A], Cys145 [A], Gln189 [A], Met49 [A], His41 [A], Arg188 [A], and Glu166 [A], where Cys-145 and His-41 are active sites. It also forms hydrogen bonds with Ser144[A], Leu141[A], His163[A], and Asp187[A]. As a control, hydroxychloroquine has a binding affinity of -6.0 kcal/mol with nine hydrophobic interactions, including with Thr190[A], Arg188[A], Met165[A], His164[A], His163[A], Asn142[A], Phel40 [A], Glu166[A],

Pro168[A], and five hydrogen bonds interaction, namely with Gln189[A], Cys145[A], Ser144[A], Gly143[A], Leu141[A]. The difference between phytochemical compounds (luteolin, isorhamnetin, and apigenin) and hydroxychloroquine is that when bound to luteolin, isorhamnetin, or apigenin, Cys145[A] forms hydrophobic bonds, whereas when bound to hydroxychloroquine, it forms a hydrogen bond. Tahir ul Qamar *et al*. mentioned that the active sites of Cys-145 and His-41 are Mpro-binding sites, where ligands that bind to the active site of this receptor can significantly inhibit the receptor's performance [21].

Table 1. Phytochemical properties based on Lipinski's five rules of *Allium cepa* bioactive compounds and their result of molecular docking toward M^{pro} Target Protein (6LU7)

Compounds	Mass Weight (Dalton)	LogP	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Luteolin CID: 5280445	286.24	2.13	4	6	72.48
luteIsorhamnetin CID: 5281654	316	2.31	4	7	78.94
Apigenin CID: 5280443	270.24	2.42	3	5	70.81
Hydroxychloroquine (Drug) CID: 3652	335.5	3.78	2	4	98.27
Cycloallin CID: 12305353	177	0.045	2	4	42.03
Isovanilin CID: 121227	152	1.21	1	3	40.05
S-propyl L-cysteine CID: 101975	163	0.54	3	3	43.23
S-propylcysteine CID: 125198	163	0.54	3	3	43.23
Methiin CID: 9578071	151	-0.36	3	4	34.68
Isopropyl propyl disulphide CID: 118529	150	3.19	0	0	45.48
Bis (1-methylethyl) disulphide CID: 77932	150	3.19	0	0	45.48
2-Propenyl propyl disulphide CID: 16591	148	2.96	0	0	45.40
Dipropyl disulfide CID: 12377	150	3.19	0	0	45.50
Cis-1-propenyl propyl trisulfide CID: 5352694	180	3.96	0	0	52.74
Methyl propyl disulphide CID: 16592	122	2.41	0	0	36.26
Dimethyl trisulphide CID: 19310	126	2.27	0	0	34.62
Dimethyl disulphide CID: 12232	94	1.63	0	0	27.03

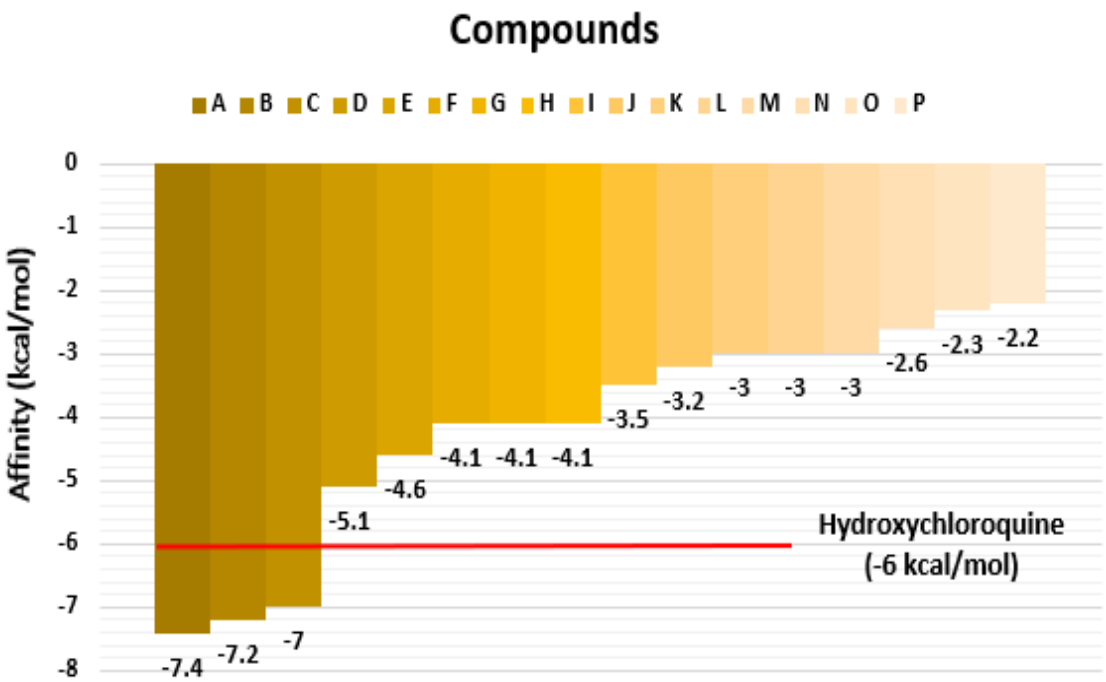

Figure 1. Comparison of the affinity values of sixteen compounds with hydroxychloroquine based on the results of docking the target protein M^{pro} 6LU7. [A] Luteolin, [B] Isorhamnetin, [C] Apigenin, [D] Cycloallin, [E] Isovanillin, [F] S-propyl L-cysteine, [G] S-propylcysteine, [H] Methiin, [I] Isopropyl propyl disulfide, [J] Bis (1-methylethyl) disulfide, [K] 2-Propenyl propyl disulfide, [L] Dipropyl disulfide, [M] Cis-1-propenyl propyl trisulfide, [N] Methyl propyl disulfide, [O] Dimethyl trisulphide, [P] Dimethyl disulfide

Based on research conducted by Tallei et al., it is known that hydroxychloroquine has proven to be an anti-viral agent for the treatment of the coronavirus and has been recognized by the Food and Drug Administration (FDA) [16]. However, a recent study by Singh et al. explained that hydroxychloroquine is ineffective and may even increase the risk of side effects. The review was conducted by observing 14 studies, including 12 evaluating hydroxychloroquine treatment for 8,569 COVID-19 patients with mild-to-severe symptoms and two studies investigating hydroxychloroquine for preventing COVID-19 in 3,346 asymptomatic individuals. The review found that hydroxychloroquine did not reduce all-cause mortality in COVID-19 patients [15].

Based on the results of docking with binding affinity and molecular interactions, visualization results, and the Lipinski rule of five, three compounds, including luteolin, isorhamnetin, and apigenin, are predicted to inhibit SARS-CoV-2 penetration into human cells. Based on the results of literature studies, luteolin is the most promising as a drug development agent of the three compounds that have a binding affinity greater than hydroxychloroquine. Although some compounds also have anti-viral potential, they are thought to have the possibility of drug-induced liver injury (DILI), cytotoxicity, and mutagenicity (AMES). For example, apigenin is considered non-toxic but can be an inhibitor of various isoforms of drug-metabolizing enzymes. Meanwhile, 3-acetyl alanine, cubebene, kaempferol-3-O-rutinside, meliacinin, and azadirachtin were suggested to cause DILI. On the other hand, isorhamnetin is predicted to be mutagenic [13]. Thus, only luteolin possessing significant pharmacokinetic properties makes it possible to develop appropriate COVID-19 drugs. It is also supported by the research conducted by Daniel et al.; the results from RCS, classical MD simulation, and metadynamics simulations suggest luteolin as a blocker of SARSCoV-2 cell entry [21]. Molecular docking has the ability to predict with a high degree of accuracy the conformation of ligands at the appropriate target binding sites. It will help to understand how ligands recognize and interact with macromolecules. In addition, it will explain how ligands have certain electrostatic and stereochemical properties to achieve high receptor binding affinity [24]. In this case, it can be concluded that the bond energy is inversely proportional to the bond affinity. Another thing that also becomes an indicator of an MD's success is looking at the RMSD value. The RMSD value shows the accuracy or validity of the MD results [5, 24].

The 3D visualization has been successfully described, where several ligands are bound to the binding site (Figure 2). Apigenin, dimethyl disulfide, dimethyl trisulfide, isorhamnetin, isovanillin, hydroxychloroquine, luteolin, methiin, and S-propylcysteine are the ligands. The 3D

visualization of macromolecular structures can verify the binding sites, including crevices, cavities, and sub-pockets. The dock score function was used to score all the dock ligands. The analysis from the best pose can then be identified. A docking score indicates the binding efficiency between the phytochemical constituents and the corresponding candidate targets for the treatment of COVID-19 inhibitors [20]. Meanwhile, the 2D visualization was shown (Figure 3). It can be done by using LigPlot+ to check the protein-ligand interaction based on hydrophobic groups and hydrogen bonds in 2D [22].

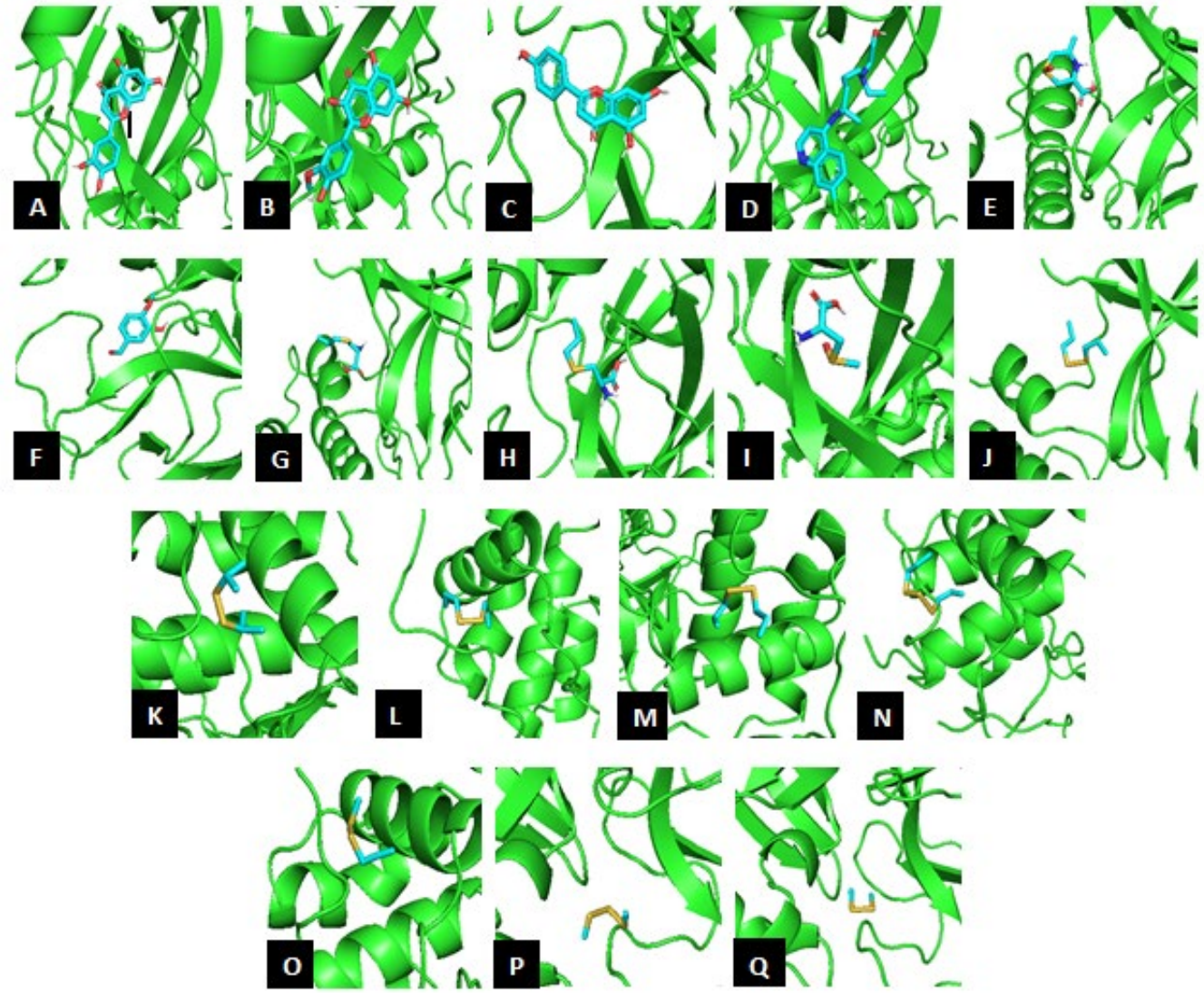

Figure 2. Results of 3D visualization of compounds bound to the target protein M^{pro} 6LU7. [A] Luteolin, [B] Isorhamnetin, [C] Apigenin, [D] Hydroxychloroquine (positive control), [E] Cycloallin, [F] Isovanillin, [G] S-propyl L-cysteine, [H] S-propylcysteine, [I] Methiin, [J] Isopropyl propyl disulfide, [K] Bis (1-methylethyl) disulfide, [L] 2-Propenyl propyl disulfide, [M] Dipropyl disulfide, [N] Cis-1-propenyl propyl trisulfide, [O] Methyl propyl disulfide, [P] Dimethyl trisulphide, [Q] Dimethyl disulfide

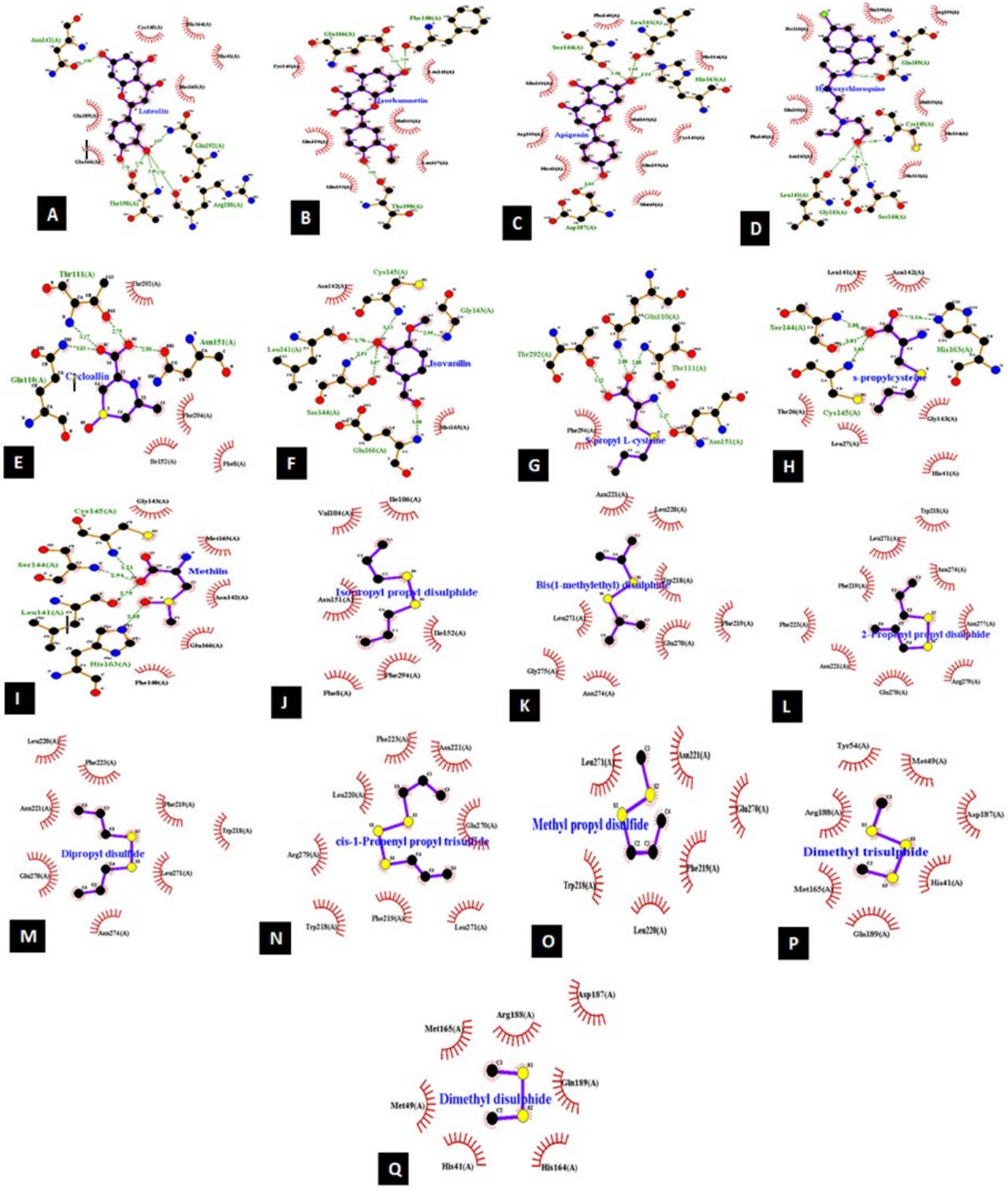

Figure 3. The results of the interaction between the compound molecules that bound to the target protein M^{pro} 6LU7 and visualized in 2D. [A] Luteolin, [B] Isorhamnetin, [C] Apigenin, [D] Hydroxychloroquine (positive control), [E] Cycloallin, [F] Isovanillin, [G] S-propyl L-cysteine, [H] S-propylcysteine, [I] Methiin, [J] Isopropyl propyl disulfide, [K] Bis (1-methylethyl) disulfide, [L] 2-Propenyl propyl disulfide, [M] Dipropyl disulfide, [N] Cis-1-propenyl propyl trisulfide, [O] Methyl propyl disulfide, [P] Dimethyl trisulphide, [Q] Dimethyl disulfide

CONCLUSION

The phytochemical compounds luteolin (CID: 5280445), isorhamnetin (CID: 5281654), and apigenin (CID: 5280443), found in *Allium cepa* L., have been shown to have the highest bonding energy towards the Mpro protein of SARS-CoV-2 in a recent study. These results indicate that these compounds may have the potential as COVID-19 treatments. However, only luteolin has pharmacokinetic properties that make it a viable candidate for further development. It is important to note that these findings are just an initial screening and more research, including in vitro and in vivo testing in animal models or clinical trials in humans, is needed to confirm their effectiveness and safety.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this study.

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