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Original Article

Bioinformatics Analysis of Bioactive Compounds of Four *Capsicum* Species against SARS-CoV-2 Infection

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ABSTRACT

Background: Plant-based remedies against Covid-19 and their research to discover antiviral compounds have been growing rapidly. However, there are little interest to explore and collect information of bioactive compounds of pepper (*Capsicum* sp.) to fight off the disease. This study aimed to bioinformatically explore and identify bioactive compounds in chili pepper fruits from four *Capsicum* species (*C. annuum, C. baccatum, C. chinense, C. frutescens*) which were compatible to fight off SARS-CoV-2 and provide indirect and direct virus inhibition from previous studies.

Methods: Protein-ligand interactions were obtained from protein data bank (PDB), PubChem, and SwissModel for homology modeling. Docking was performed using PyRx and visualized using BIOVIA Discovery Studio Visualizer 2016.

Results: Four chili pepper species were rich in organic acid compounds (100 times higher than carotenoids concentration; 0.2-156 mg/kg F.W). A type of fatty acids composition in seeds was slightly different from flesh and peels by the small amount of pharmaceutically valuable palmitoleic acids in seeds (approximately 30 mg/kg F.W). Composition of flavonoid relatively varied among the species but luteolin was found in all chili peppers (0.5-18 mg/kg F.W). Most of the compounds were actively interacting with 3CLPro rather than ACE-2 and TMPRSS2 which were well covered up by only 10 and 17 molecules respectively.

Conclusion: Four chili pepper species contained bioactive compounds that are medicinally important to fight against SARS-CoV-2 infection.

Keywords: binding energy, bioactive compounds, Covid-19, *Capsicum*, carotenoid.

1. Introduction

Corona virus disease 19 or so called Covid-19 is currently the most infectious disease caused by Severe Acute Respiratory Syndrome Corona Virus type 2 (SARS-CoV-2)[1]. The origin of the virus still remains unclear despite little evidence of sequence similarity from other strains being discovered. First statements of bats as the source of covid-19 outbreaks came from Zhou who reported 96.2% similarity from corona virus isolated from Rhinolophus affinis [2]. Then, another study claimed most of pangolins (70%) contains the virus which the sequence shared 99% similarity with the current human SARS-CoV-2 [3]. Therefore, pangolin was likely to be the host of intermediate (human-to-animal or vice versa) transmission. However, human-to-human transmission occurs mainly through droplets of respiratory systems which spread by short-distance airborne and close contact that environment humidity favoring the virus survival is at 40-50% [4,5]. Epidemiology research estimated the infection rate is ranging from 1.4-3.9 and according to the data updated by Worldometer Coronavirus on October 4th 2020 at 22.27 GMT, more than 35 million people from 214 countries and territories have been infected with 98.7 deaths/1 million [6]. This number has been dominated by India and several american countries (USA, Mexico, Canada, Brazil) where the cases outnumber those in China as the epicenter.

SARS-CoV-2 virus,.`11111111 consists of a positive single stranded RNA (ssRNA) encapsulated by an envelope and enters human and animal respiratory tract cell by binding a spike glycoprotein to the receptor angiostenin converting enzyme 2 (ACE-2). Subsequently, the spike glycoprotein is cleaved by transmembrane protease serine 2 (TMPRSS2) belonging to human cells in order to release viral ssRNA into a host cell [7]. TMPRSS2 also plays a role in spike protein priming process, and the inhibition of the protease causes a robust blockade of viral entry [7, 8]. Upon the virus assemblies, 3-chymotrypsin-like protease (3CLPro) was expressed by virus genome to cleave a viral polyprotein into 11 distinct sites that are critically important for viral replication [10]. Besides, successful infection depends on the virulence factors acting as antagonists of interferon (IFN) signaling pathway. Thus, cellular response and defense which are notably suppressed cause an enormous number of cell death in the corresponding organ that mimics to another disease (e.g. pneumonia) and subsequently leads to organ failures [11].

In response to Covid-19 pandemic, plant-based drugs are very promising industries besides chemical drugs and vaccine manufacturing [12]. Popular compounds which scientifically and statistically have been proven to cure SARS-CoV infection are hesperidin and cannabinoids (spike protein inhibitor) from citrus and marijuana, respectively [13, 14], chloroquine and hydroxychloroquine (Coronavirus proteinase inhibitor) [15] from sunflower [16], as well as luteolin and benzoic acid [17, 18]. However, food preferences, restriction of a plantation, low reproduction, and well-described side effect of those compounds cause limitation in public consumption [19]. Therefore, it is crucial to find other sources of the compounds which is permitted to grow and safe for human.

Besides the attribute of spiciness and flavor, herb-based crop, such as chili pepper (*Capsicum* sp.) is a promising source for the supply of drugs production due to the highly cultivated in numerous country around the world such as China (12.4 million tons), Mexico (2 million

tons), Turkey (1.8 million tons), and Indonesia (1.3 million tons) [20-21]. There are four major *Capsicum* species cultivated, including bell peppers (*C. annuum*), South American peppers (*C. baccatum*), carolina reaper (*C. chinense*), and cayenne pepper (*C. frutescens*) [22]. Historically, chili peppers are also known to possess high medicinal values such as being used as ancient pain relievers by Maya and Astec physicians to treat coughs, sore throats, asthma, and toothaches, as well as stomachaches [23, 24]. Two major compounds in chili peppers have been known to have a curative effect, including predominantly capsaicin which possesses antibacterial, antifungal activities, and cytotoxicity-linked-to-antivirus activity [25], as well as carotenoid carrying antioxidant activity [26, 27]. Meanwhile, no previous studies have investigated chili pepper's other compounds which have antiviral effects both *in vitro* and *in silico* analysis.

At present, computational strategy or bioinformatics analysis to study the potential bioactive compounds in the plant provides pinpoint screening involving all aspects of the compounds. The detail of bioactive compounds includes structures, binding affinities, polarity, and type of interaction of the compounds with the help of mathematic's algorithm. Bioinformatics analysis is also simpler, safer, and lower in cost that can be performed before putting any strategies in vitro and in vivo assay. The bioinformatic analysis can be extremely useful in drug discovery and its potential clinical benefit [28]. Therefore, based on the background aforementioned, this study aimed to explore and identify bioactive compounds in pepper fruit from four species of *Capsicum* which is bioinformatically compatible to fight off SARS-CoV-2. The possibility of medicinal value from previous studies which associates indirectly with the virus inhibition action is also discussed.

2. Materials and Methods

2.1. Research design and data collection of bioactive compounds

This research used four commonly cultivated types of pepper, namely *C. annuum, C. baccatum, C. chinense,* and *C. frutescens* which were used as the subjects of this study. Bioactive compounds exploration was focused on fruit. However, if available, other parts of the plants were also be used. Bioactive compounds data of four peppers in details were obtained from previous scientific studies and other credible sources (Appendix 1; Table A.1-5) [29-41]. The compounds were described for common groups (flavonoids, carotenoid, organic acids, capsaicinoid, and fatty acids) and concentration range. The contemporary medicinal value which provided both direct and indirect links to the antiviral activities were also discussed according to the previous studies [43-56].

2.2. Bioinformatics analysis

Two ARS-CoV-2 protein, ACE-2 (2AJF), and 3CLPro (6LU7) (Details in Table 1) were obtained from RCSB PDB (https://www.rcsb.org/). Human TMPRSS-2 protein was modeled using homology modeling method using SwissModel web server (https://swissmodel.expasy.org/) based on protein sequence (UniProt ID: O15393) with Serin Protease Hepsin (5CE1) as a template. The proteins prepared under Discovery Studio 2016 protocols were described by Baskaran [57] to remove ligands and water molecules. Meanwhile, *Capscium* spp. bioactive compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and those energies were minimized before

SDF to PDB format conversion using PyRx software ver 0.9.7.

Flexible ligand docking of prepared Capsicum spp. bioactive compounds against the proteins were carried out using Autodock Vina integrated into PyRx to predict binding energy and possible protein-ligand interaction. The active site of each protein was determined by analyzing the interaction between the protein and its native ligand (Table 1). Visualization of protein-ligand interaction was performed using BIOVIA Discovery Studio Visualizer 2016 Client v16.1.0 x64 64/32 Bit (Biovia Corporate Americas; San Diego, CA).

Table 1. Active sites of four protein docked with *Capsicum* spp. secondary metabolites.

No.	Protein	Protein Data Bank (PDB) ID	Amino acids of active sites
1	ACE-2	2AJF	Ser19; Thr31; Tir34; Glu35; Glu38; Tir41; Met82; Tir83; Glu329; Lys353; Gly354
2	TMPRSS2	-	His296; Ile346; Ser441
3	3CLPro	6LU7	His41; Met49; Leu141; Phe141; Asn142; Gly143; Cys145; His163; His164; Glu166; Met165; Leu167; Pro168; Gln189; Thr190; Ala191

2.3. Data analysis

All physiological data are expressed as mean ± error standard. Medicinal values of chili peppers' secondary metabolites from previous researches were presented in description table (Table 2).

3. Results

3.1. Secondary metabolites from four *Capsicum* species: A literature exploration

3.1.1. Flavonoids content

Bioactive compounds of four chili pepper species (*C. annuum, C. frutescens, C. baccatum,* and *C. chinense*) were recorded from secondary sources and classified into five major groups, including flavonoid, carotenoid, organic acids, capsaicinoids, and fatty acids. Several compounds in some plant species were detected in extremely high content while others were undetected or no previous study conducted (N/A; not available). Among those four compounds, flavonoids mostly are present in the large amount. There were 13 types of flavonoid compounds explored and seven of them show an extremely low concentration (less than 0.1 mg/kg F.W). Furthermore, among the four species of Capsicum, *C. chinense* demonstrated the highest flavonoid types followed by *C. annuum* (Fig. 1).

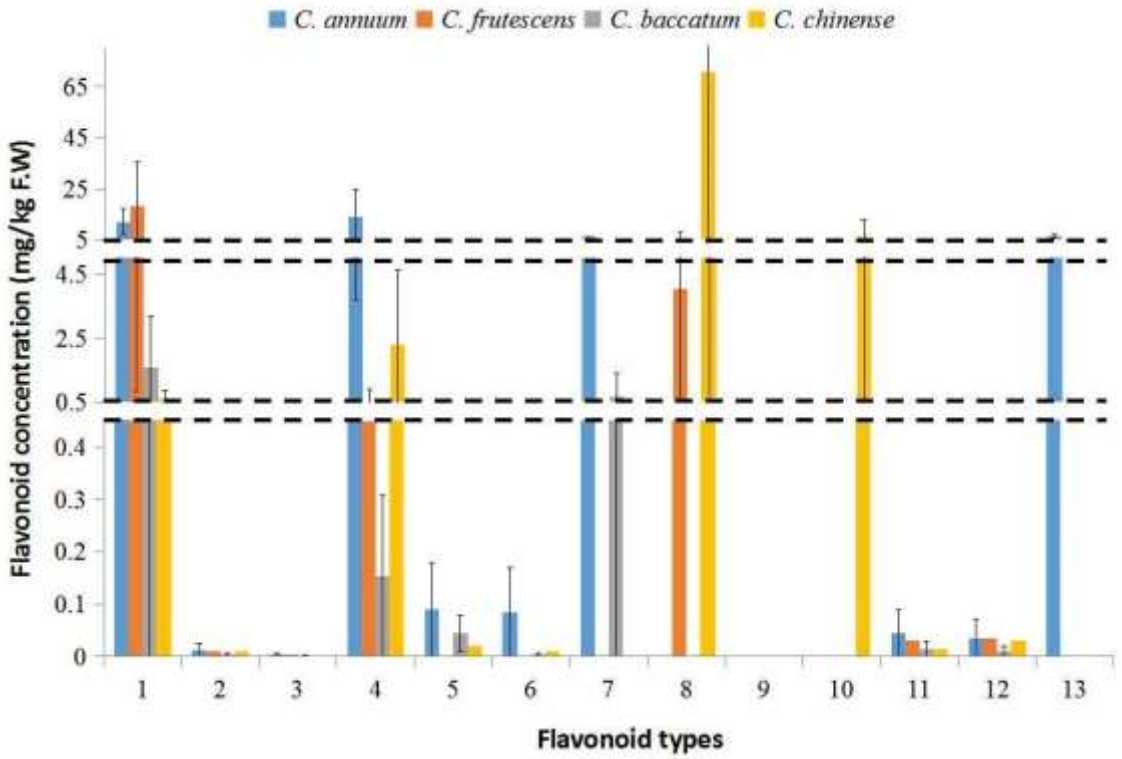

Figure 1. Average flavonoid compounds from *Capsicum* species. All compounds are denoted by arabic numeral: 1: luteolin (overall); 2: luteolin (apiosyl-acetyl)-glucoside; 3: luteolin 7-O-apiosyl-glucoside; 4: quercetin (general); 5: quercetin 3-O-rhamnoside-7-O-glucoside; 6: quercetin 3-O-rhamnoside; 7: kaempferol; 8: catechin; 9: epicatechin; 10: rutin; 11: apigenin 6-C-pentoside-8-C-hexoside; 12: apigenin 6,8-di-C-hexoside; 13: myricetin. The caps show maximum and minimum concentration. Note: Details of reference can be seen in Table A.1 (Appendix 1) [29-33].

3.1.2. Carotenoids content

In general, the carotenoid content of the four *Capsicum* species was detected above 5 mg/kg F.W, except for phytoene which was present in a low concentration. Furthermore, almost all carotenoid in *C. annuum*, except neoxanthin, was present in the highest amount compared with the three other species. Seven types of carotenoid (beta carotene; beta-cryptoxanthin; violaxanthin; zeaxanthin; antheraxanthin; capsorubin; capsanthin) were present in all species and capsanthin had the highest concentration (80-145mg/kg F.W) (Fig. 2).

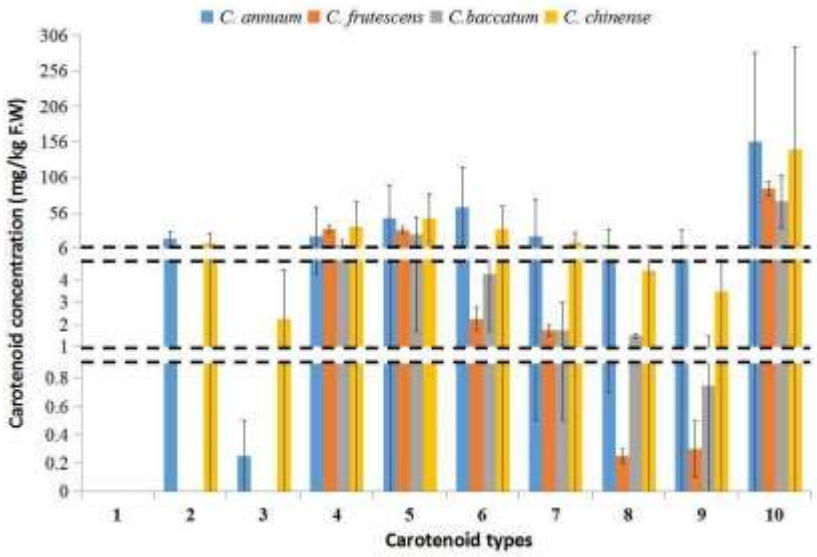

Figure 2. Carotenoid compounds from four Capsicum species. All compounds are denoted by Arabic numeral: 1: phytoene; 2: lutein; 3: neoxanthin; 4: beta carotene; 5: beta-cryptoxanthin; 6: violaxanthin; 7: zeaxanthin; 8: antheraxanthin; 9: capsorubin; 10: capsanthin. The caps show maximum and minimum concentration. Note: Details of reference can be seen in Table A.2 (Appendix 1) [33-35].

3.1.3. Organic acids content

Figure 3 explains organic compounds accumulated by four species. Among those compounds, only five compounds were present completely in all species. Both sugars generally outnumbered other organic compound in which glucose and sucrose were the highest in *C. frutescens*. Each non-sugar organic acid predominantly accumulated in each species, such as citric and isocitric acid in *C. baccatum*, malic acid in *C. frutescens* while fumaric, succinic, and ascorbic acids were found high substantially in *C. chinense* and folate in *C. annum* with the value above 100 mg/100 g F.W (Figure 3).

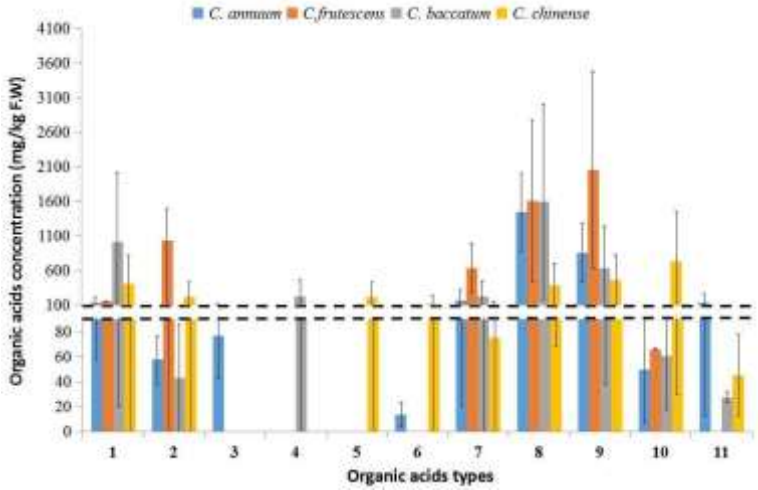

Figure 3. Organic acids from four *Capsicum* species. All compounds are denoted by Arabic numerals: 1: citric acids; 2: malic acids; 3: quinic acids; 4: isocitric acids; 5: fumaric acids; 6: succinic acids; 7: sucrose; 8: glucose; 9: fructose; 10: ascorbic acids (Vitamin C); 11: folate. The caps show maximum and minimum concentration. Note: Details of reference can be seen in Table A.3 (Appendix 1) [36-42].

3.1.4. Capsaicinoid content

Capisaicin content in all chili peppers was highest in capsaicin. Average capsaicin content was accounted for 88.7-228 mg/kg F.W. *C. annuum* showed a slight concentration value difference between capsaicin and dihydrocapsaicin (106 and 99 mg/kg F.W). Capsaicin and dihydrocapsaicin content were highest in *C. frutescens* (228 and 123 mg/kg F.W, respectively) while in the same species, nordihydrocapsaicin was recorded the lowest, accounted for 3.35 mg/kg F.W (Fig. 4).

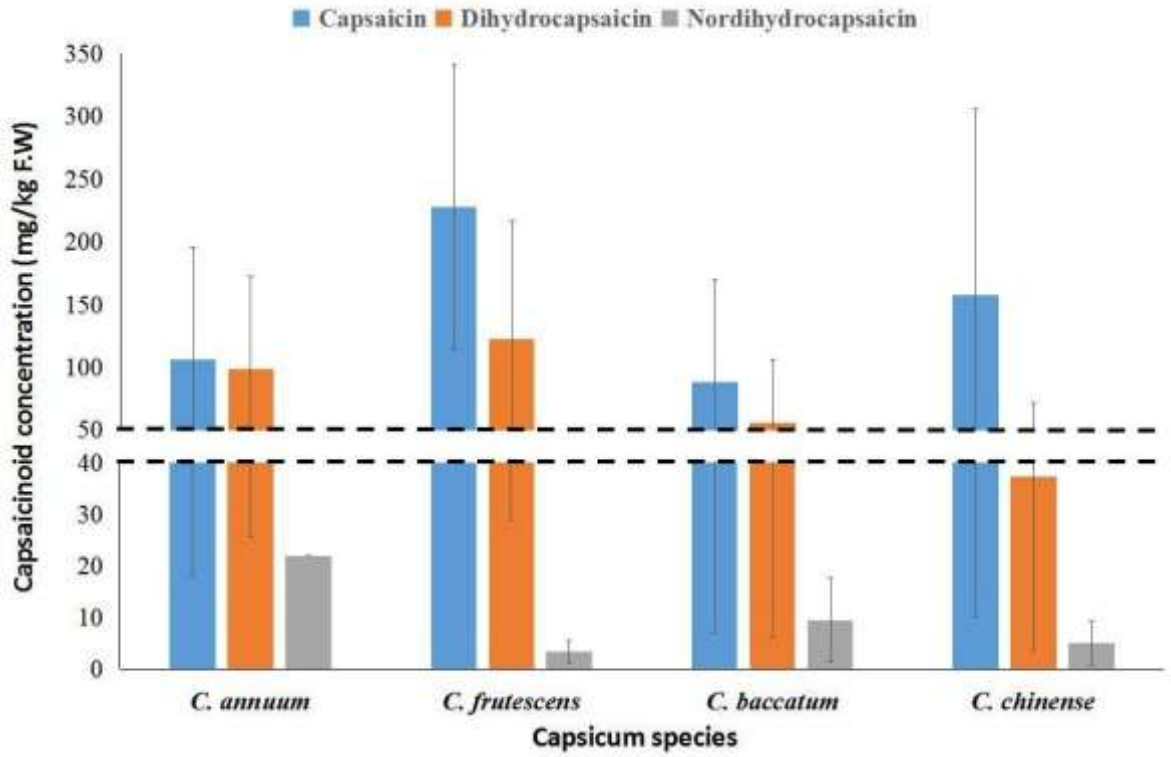

Figure 4. Capsaicinoid content present in four *Capsicum* species. The caps show maximum and minimum concentration. Note: Details of reference can be seen in Table A.4 (Appendix 1) [35, 38, 39].

3.1.5. Fatty acids content

Generally, fruit flesh contains more abundant fatty acids than seeds. Fatty acids content, both in seeds and flesh, were highest in *Capsicum annuum*. There were 10 compounds detected from fruit flesh which four of them were also found in the seed, i.e. palmitic (16:00); stearic (18:00); Oleic (18:1n-9); elaidic (18:1n-7); linoleic (18:2n-6). The highest fatty acid was reported in seed (Linoleic acid) with the value of approximately 670 mg/100 mg total lipids, while palmitoleic acid was only found in the seeds with a low amount (less than 3 mg/100 mg total lipids). The similar results were also consistently recorded in the all species flesh, where linoleic acid had the highest share of total lipids (240-302 mg/100 mg total lipids). Meanwhile, almost remaining compounds were detected less than 10 mg/100 mg total lipids (Fig. 5).

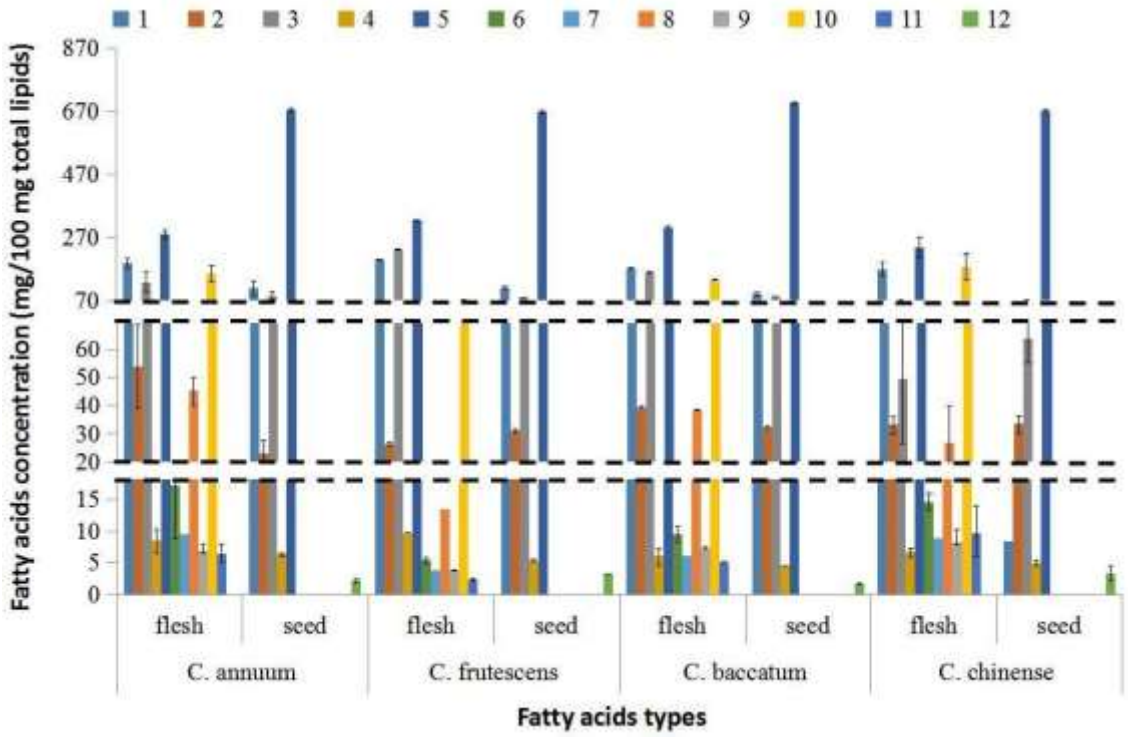

Figure 5. Fatty acids present in four *Capsicum* species, on either flesh or seeds of mature fruits. Each bar color represents each fatty acids labelled by Arabic numerals: 1: palmitic (16:00); 2: stearic (18:00); 3: oleic (18:1n-9); 4: elaidic (18:1n-7); 5: linoleic (18:2n-6); 6: capric (10:00); 7: tridecanoic (13:00); 8: 5-cis-tetradecenoic (14:1n-9); 9: arachidic (20:00); 10: alpha linoleic acid (18:3n-3); 11: behenic (22:00); 12: palmitoleic (16:1n-7). The caps show maximum and minimum concentration. Note: Details of reference can be seen in Table A.5 (Appendix 1) [42].

3.2. Medicinal properties of chili peppers bioactive compounds

Several bioactive compounds discovered in chili pepper according to previous studies have been proved to have medicinal and antiviral activities. Flavonoid was the most recognizable compounds because all compounds mentioned had direct action for coronavirus inhibition (see Table 1 with */asterisk label). This compound group consists of two general compounds (luteolin and quercetin) and seven remaining specific compounds that bind and inhibit viral protease and spike protein, whereas, only myricetin has proved to interfere with viral helicase. Other metabolites that involve a direct viral susceptibility include capsaicin and folate in which changes the structure of another protease (V-3CL-Protease) and binds active site of furin protein, respectively. Fatty acids have a direct ability to dissolve and break viral envelope integrity while on the other side stimulating neutrophils proliferation. Virus entry blockage by pH alteration mediated by ascorbic and citric acids has been mentioned as well (Table 2).

Most organic acid and fewer carotenoids involve in adaptive immunity modulation and oxidative stress suppression. Adaptive immunity, such as expression of CD4, CD5, MHCII, are facilitated by carotenoids in which protects from UV damage. Those functions can be a single compound mechanism or need other carotenoids, such as lutein with zeaxanthin for a humoral immunity modulation.

Table 2. Biomedical and antiviral function of pepper compounds against SARS-CoV-2 invasion

Groups	Compounds	Function	Reference
Flavonoid	Luteolin (overall)* Quercetin (general)*	Inhibiting the activity of 6LU7 main protease; binds C1-terminal of the spike S2 domain	[43-44]
	Luteolin 7-O-apiosyl-glucoside* Catechin* Epicatechin*	Inhibiting the activity of 6LU7 main protease	[43]
	Apigenin 6-C-pentoside-8-C-hexoside* Apigenin 6,8-di-C-hexoside*	Binding C1-terminal of the spike S2 domain	[44]
	Kaempferol*	Inhibiting the activity of the C-terminal of S1 domain.	
	Myricetin*	potently inhibiting the SARS-CoV helicase protein in vitro by affecting the ATPase activity, but not the unwinding activity of nsP13	[45]
Carotenoids	Lutein	increasing expression of CD5, CD4, CD8 and MHC II.	[46]
	β-carotene	Immunomodulator, protection against UV which suppresses immune system, improving T-helper (CD4+)	[47]
	Zeaxanthin	Modulating humoral immune along with lutein combination.	[48]
Organic acids	Citric acids**	Inducing a low pH transition of viral protein for disabling virus entry	[49]
	Malic acids	Having an unclear direct function	[50]
	Fumaric acids	Activating the Nrf2 antioxidant response pathway (suppressing cytotoxic effects of oxidative stress) in multiple sclerosis (enhancing infection risk).	[51]
	Succinic acids	Enhancing adaptive immunity in T-cells; increases TNF alpha and interleukin-1beta; affects SUCNR1 in macrophages for anti-inflammatory responses.	[52]
	Ascorbic acids**	Reducing pH value which inhibits virus-cell fusion; improves lymphocyte proliferation; reducing cytokine storm.	[53]
	Folate*	Interacting with furin protein relating to virus entry.	[54]
Capsaicinoid	Capsaicin*	Promoting structural changes in the viral protease (V-3CL-Protease) by inducing folding of the enzyme.	[55]
Fatty acids	General**	Completely disintegrating the viral envelope causing leakage at higher concentrations; increases immunity through neutrophils production at low intakes while high intake causes leukocyte production inhibition.	[56]

* direct actions towards viral protein
** direct actions towards non-protein compounds of virus

3.3. Chili Peppers' bioactive compounds-targeted proteins interaction

Binding energy all bioactive compounds was ranging from -3.9 to -8.9 kcal/mol with different levels for each protein tested. Three out of four compounds group were potentially classified to fight off SARS CoV-2 infection according to bioinformatics analysis (see Supplementary Table 2 for active sites and binding energy details). Meanwhile, other compounds that did not exhibit binds on the active site were immediately eliminated. In general, two major groups, carotenoids and flavonoids tend to bind the proteins compared to the capsaicin group and organic acids. Most of the compounds were actively interacting with 3CLPro rather than ACE-2 and TMPRSS2 which were well covered up by only 10 and 17 molecules, respectively. Hydrogen bond interaction was prevalent in all groups except for carotenoid. Flavonoid occasionally was only dominating on 3CLPro in the first place while ACE2 and TMPRSS2 were mostly bound tightly by the carotenoid group. Although most of the organic acids were the weakest group among the three bioactive groups, folate was considerably strong while being interacted with TMPRSS2 and 3CLPro with the binding energy -7.8 and -8.1 kcal/mol, respectively. Furthermore, the abundant capsaicin group, consisting of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, also contributed to TMPRSS2 and 3CLPro blockage with the BE value of 5.5-6.0 kcal/mol (Fig. 6).

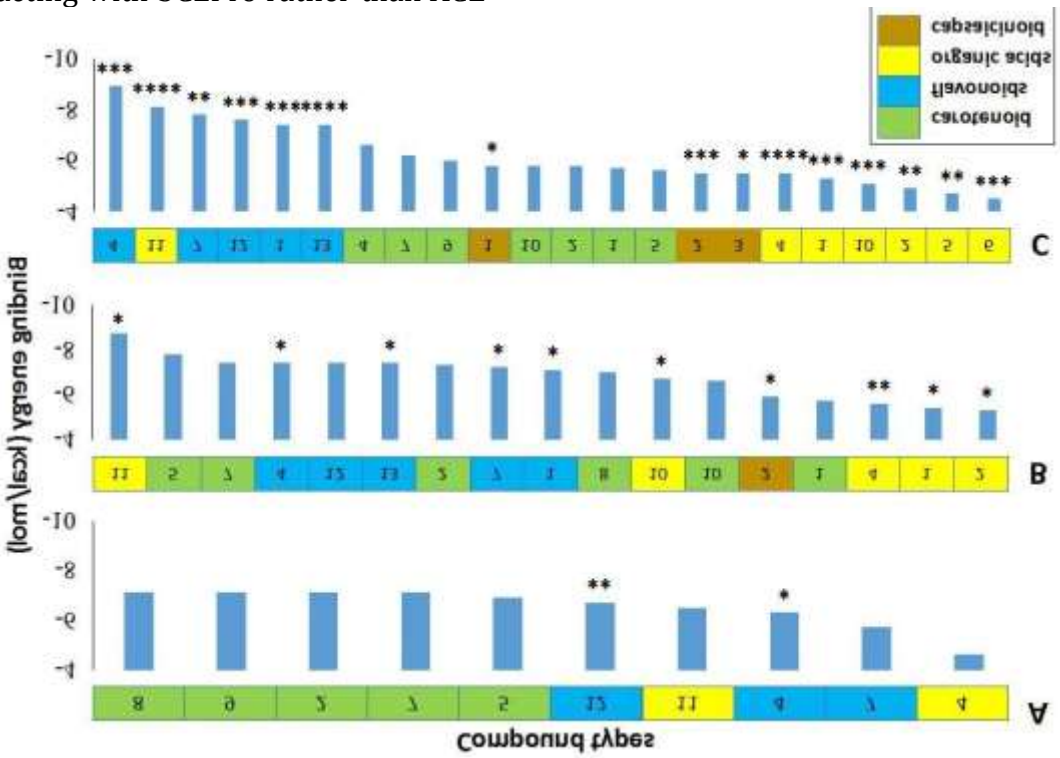

Figure 6. Binding energy (BE) of peppers bioactive compounds against (a) ACE-2; (b) TMPRSS2; and (c) 3CLPro. Shade colors show the compound groups and each number label corresponds to compound type according to the previous figures (Fig. 1-5): green: carotenoid (1, 2, 4, 5, 7, 8, 9, 10); blue: flavonoids (1, 4, 7, 12, 13); brown: capsaicin group (1, 2, 3); yellow: organic acids (1, 2, 4, 5, 6, 10, 11). Asterisks (*) below the bar show the number of hydrogen bonds of each compound. Note: Details of binding energy and the active site can be seen in Table A.6 (Appendix 2).

4. Discussion

Chili pepper (*Capsicum* spp.) has been known not only as a food enhancer, spice, and vegetable but also as a component in herbal medicines performed by ancient physicians. Having abundant phytochemicals, such as flavonoids, carotenoids, capsaicinoids, organic acids, and fatty acids, chili pepper possesses antibacterial, anti-inflammatory, and antioxidant activities which revealed comprehensively medicinal values of *Capsicum* genera [58-59]. Furthermore, antiviral properties have been discovered by several previous studies, such as by high content of phenylpropanoid and flavonoids for herpes simplex virus (HSV) I treatments [25] and type II [60], powerful inhibition of Lassa virus entry by capsaicin [61]. No recent papers have mentioned chili pepper capability to counterattack SARS-CoV-2. However, bioactive compounds from other species were proved to have antiviral activity against SARS-CoV-2 spread, such as luteolin, folate, and others according to the previous bioinformatics studies (see Table 1).

Two general actions involved in the antiviral properties against SARS-CoV-2 infection, namely disruptions of virus entry and virus assembly. The entry of this virus into respiratory organ cells is mediated by the interaction between the spike protein of the virus and ACE2 (Angiotensin-converting enzyme 2) attached to the outer surface of the cell membrane of the human lung cells, then membrane fusion occurs to inject viral RNA. Viral RNA that enters the cell will be translated to produce a polyprotein. This polyprotein then undergoes auto cleave and releases 3C-like main protein protease (3CLpro). The 3CLpro then cleaves the polyprotein to produce other proteins that are important in viral replication [62]. Therefore, ACE2, TMPRSS2, and 3CLpro have a crucial role in coronavirus infection so that proteins are often a target for discovering coronal antiviral drug candidates. Inhibition of those protein activities will automatically inhibit the recognition, fusion, and replication of the virus to the target cell. So, we need compounds that can block the activity of the proteins.

All bioactive compounds which were available online as protein data bank had interacted with both human-based cell protein (TMPRSS2) and viral-based proteins (ACE-2 and 3CLPro). TMPRSS2 (Transmembrane Protease Serine 2) is a transmembrane enzyme that has an important role in Sars-Cov2 infection. Previous research has stated that the Spike protein from SARS-Cov 2 is a substrate for TMPRSS2. After the Spike protein binds to ACE2, TMPRSS2 will cut the Spike so that it creates the viral and cellular membrane in close proximity for fusion. Therefore, the activity of TMPRSS2 is to initiate membrane fusion of the virus [63]. TMPRSS has a triad of catalytically active site residues consisting of His296, Ile346, and Ser441 which play a role in cutting the junction amino acids Arg685/Ser686 and Arg815/Ser816 from Spike proteins [64]. Therefore, the strategy to inhibit TMPRSS2 in cutting Spike protein is to block the active site residues (His296, Ile346, dan Ser441) so it can't interact with the spike protein. Thus, TMPRSS2 cannot cut the spike protein. The results of virtual screening showed that folate and quercetin were bound at the active site of TMPRSS specifically His296 and Ser441 with low binding energy, -8.7 kcal/mol for folate and -7.4 kcal/mol for quercetin. Blocking the active site residue by folate and quercetin will prevent TMPRSS2 from cutting protein spikes so that the virus fusion process does not occur (Fig. 7A and B).

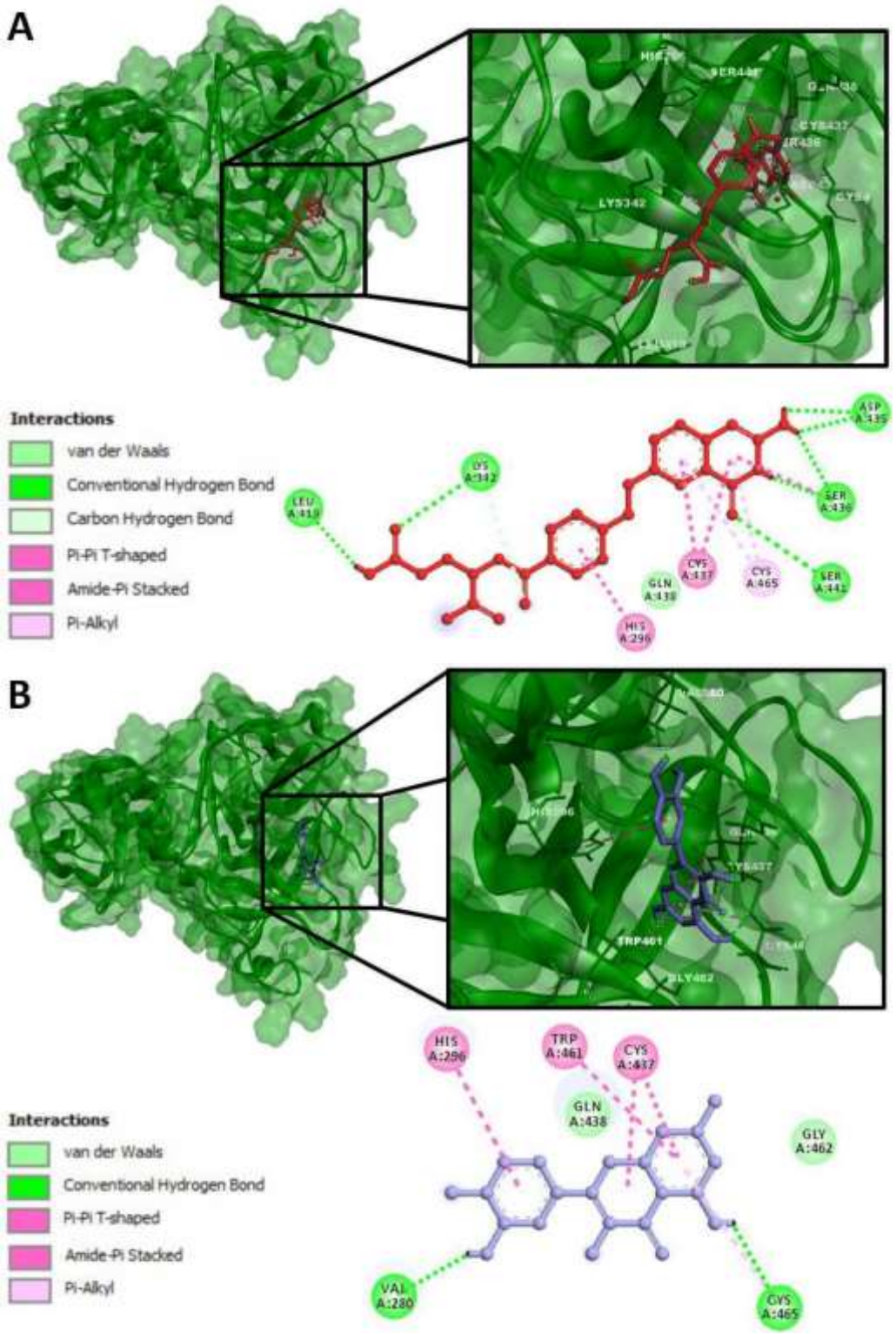

Figure 7. TMPRSS2 was docked with folate (A) and quercetin (B). Each figure is shown in 3D (above) and 2D view (below) with the type of interactions explained.

ACE-2 (Angiotensin-Converting Enzyme 2) is a protein found on the lung cell membrane surface. This protein has been proven by many studies to be the specific receptor of the SARS-Cov2 Spike RBD (Receptor Binding Domain). In recent years, ACE-2 is considered a target host for a SARS-Cov 2 infection treatment to prevent the virus from binding to its target cells [62]. One of the strategies is

to block the bond between ACE2 and Spike RBD with potential compounds. This compound must bind to ACE-2 in the region where ACE-2 binds to the spike RBD, thus preventing the spike RBD binding to ACE-2. The virtual screening results showed that several compounds in chili bind to the Spike RBD binding site of ACE-2 with low binding energy. lutein and zeaxanthin were the best results. Lutein with a binding energy of -7.1 kcal/mol formed hydrophobic interactions with His34, Tyr41, and Lys353 residues which had an important role in binding with spikes RBD. Zeaxanthin with the same binding energy formed an interaction with Tyr83 and Met82 which were also where ACE2 binds to Spike RBD. The interaction of lutein and zeaxanthin in these residues will prevent the Spike RBD from binding to ACE2. So that SARS Cov2 cannot bind to its target cells (Fig. 8A and B).

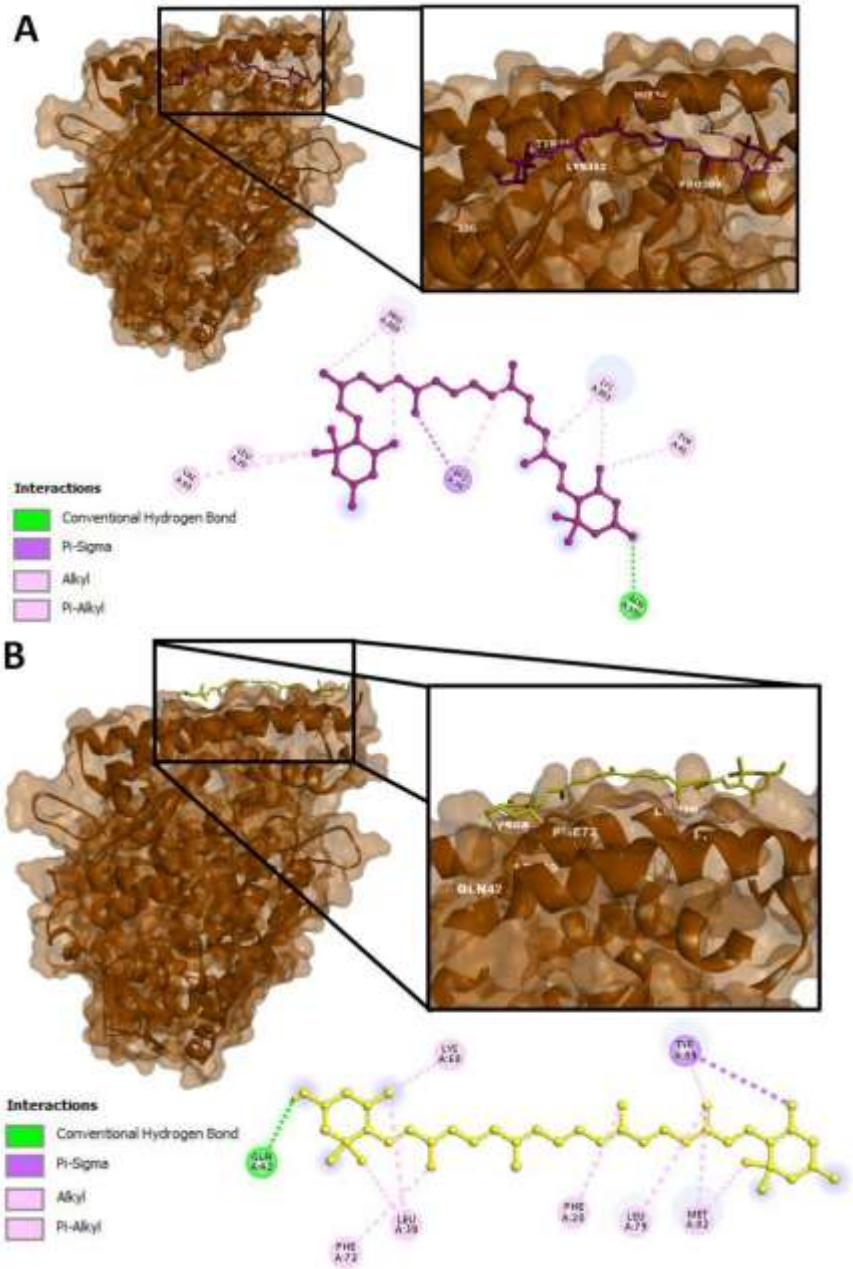

Figure 8. ACE-2 was docked with lutein (A) and zeaxanthin (B). Each figure is shown in 3D (above) and 2D view (below) with the type of interactions explained.

3CLpro (3C-like Protease) protein consists of three domains, domain I (8-101 residues), II (102-184 residues), and III (201-306 residues). The active site of this protein is between domains I and II, which is marked by the presence of Cys-His catalytic dyad [51]. Cys-His catalytic dyad plays a role in hydrolyzing the substrate through the mechanism of deprotonation of thiol, nucleophilic attack, release of N-terminus substrate, hydrolysis of thioester, and release of carboxylic acid [66]. Therefore, the hydrolysis activity of 3CLpro can be inhibited by blocking the active site of this protein. The docking result showed that the compounds in chili were selected which were bound on the active site and the lowest binding affinity. Among the selected compounds, folate gives the best result. Folate interacted with 3CLpro on its active site, namely in Thr45, Gly143, Ser144, His163, His164, Glu166, and Cys145 with binding affinity values of -8.1 kcal/mol, the lowest compared with other compounds. Moreover, folate also bond to the amino acid Cys145 which is an important amino acid in substrate hydrolysis. Interaction between folate and Cys145 would inhibit the hydrolysis ability of the 3CLpro protein so that the replication of the virus could be stopped. Therefore, folate had high potency to inhibit the activity of 3CLpro protein through a competitive inhibitor mechanism (Fig. 9A and B).

Chili has a lot of bioactive compounds including flavonoids and carotenoids. Flavonoids found in chili pepper are quercetin and kaempferol. Quercetin has a higher content and based on the results of previous research quercetin has a function to inhibit the cellular entry of SARS-CoV [67] and Kaempferol prevent the early stage of HCoV-22E9 infection, including viral attachment and penetration [68]. In other studies, lutein and zeaxanthin are a group of carotenoids, reported to have anti-viral properties. in the Hepatitis B virus, Lutein can effectively suppress HBsAg secretion from HepG2 2.2.15 cells and can suppress the amount of extracellular HBV DNA. In addition, Lutein also shows inhibitory properties on the activity of HBV promoters, indicating that lutein has anti-viral properties through the process of inhibiting HBV transcription in viruses [69]. In addition, chili peppers have a lot of bioactive compounds that act potentially against COVID-19.

We presented novel findings of a secondary metabolite which were not well described in previous studies in term of direct inhibition SARS-CoV-2 infection. There were 17 new molecules that matched to direct action towards the viral inhibition, comprising 11 new compounds involving in direct action and 6 compounds which play either indirect (antibody enhancements) action or direct action. Carotenoids and organic acids are the biggest new member of unidentified active compounds. Moreover, all carotenoids which were inferred to have direct inhibition were relying on hydrophobicity to interact with the active sites whereas hydrogen bonds were commonly found on organic acids and flavonoids. Hydrophobicity of carotenoids has been assumed rather a disadvantage due to less utilization in medicine and food chemistry and water dispersibility requirements for an effective administration [70].

Meanwhile, binding energy formed by protein-ligand interaction is influenced by several parameters such as solvation/desolvation energy ($\Delta G_{solvent}$), changes in energy receptor-ligand due to complex formation (ΔG_{conf}), specific energy interaction protein-ligand (ΔG_{int}), and movement energy (rotational, translational, vibrational) (ΔG_{motion}) [71]. The specific interaction of energy-protein-ligand (ΔG_{int}) is influenced by the number and type of chemical bonds. Bonds formed from docking are

hydrogen, hydrophobic, electrostatic, van der Waals and unfavorable interactions [72]. Hydrogen bonds are the strongest bonds followed by hydrophobic, electrostatic, van der Waals, and unfavorable interactions. Unfavorable interactions are included in unstable interactions, so the more unfavorable interactions, the more unstable the protein-ligand interactions [73].

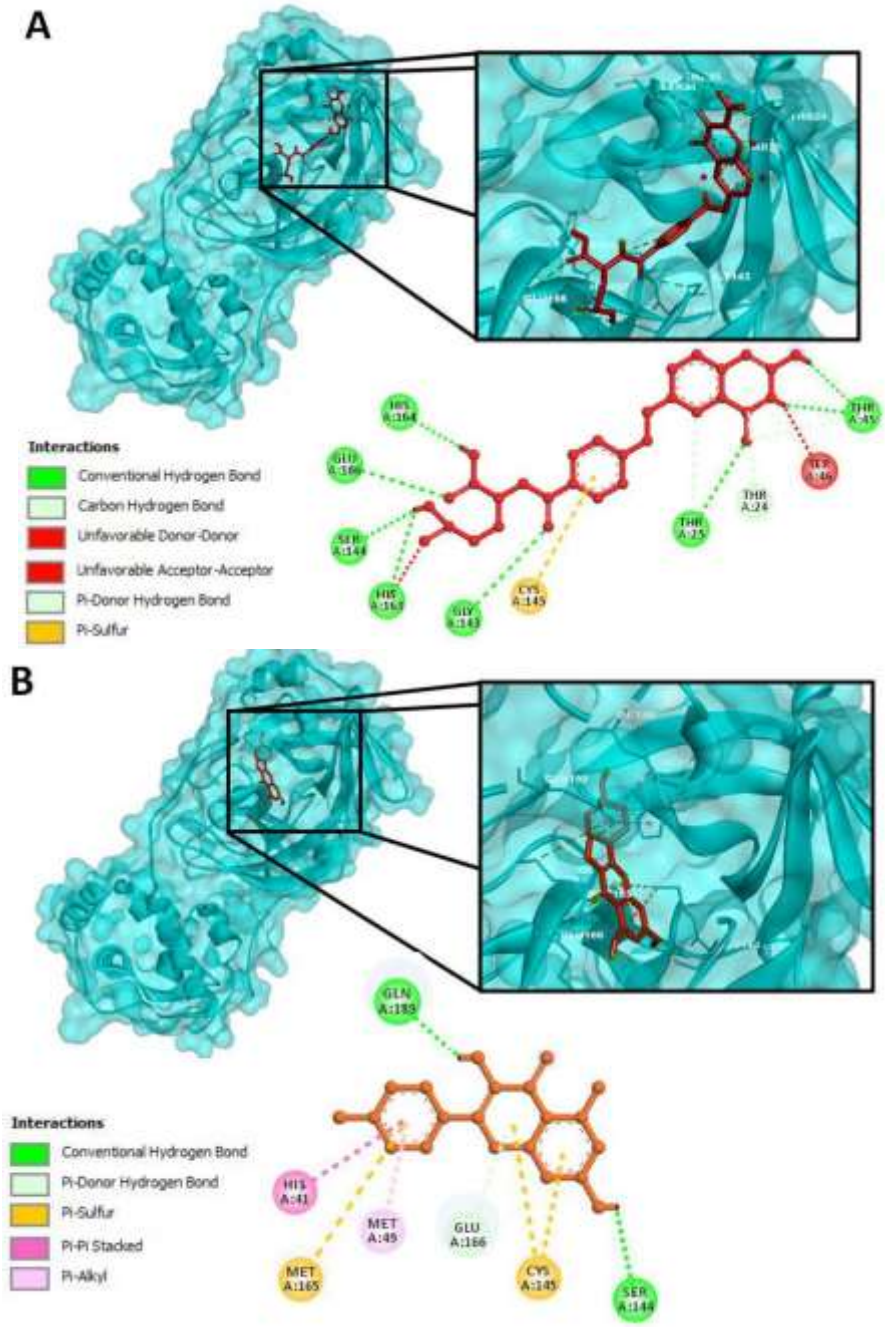

Figure 9. 3CLPro was docked with folate (A) and kaempferol (B). Each figure is shown in 3D (above) and 2D view (below) with the type of interactions explained.

5. Conclusion

This study revealed organic acids and flavonoids were the most abundance in *C. chinense* while hydrophobic compounds (carotenoids and fatty acids) were most highly found in *C. annuum.* Seventeen novel compounds in 4 chili pepper species actively restrained SARS-CoV-2-

related proteins, such as ACE-2, TMPRSS2, and 3CLPro which were dominated by the carotenoids group. Based on the binding energy and the binding pose of the compounds contained in chilies, this study implied that the most potent compound to inhibit coronavirus infection, namely folate, kaempferol, lutein, zeaxanthin, and quercetin according to the highest concentration abundance and binding energy of protein-ligand interaction. Another approach for the investigation of a fixed period of protein-ligand interaction should be performed through molecular dynamics analysis. Bridging molecular docking and molecular dynamics (MD) may transform the rigid view of the ligand-binding process from pepper bioactive compounds into a detailed explanation of ligand flexibility into docking.

Conflict of interest

The authors declare that there is no conflict of interest

Consent for publication

The authors submit and transfer this article to International Journal of Advanced and Biomedical Science. The authors accept responsibility for releasing this material including reprints, translations, photographic reproductions, and electronic forms.

Availability of data and material

Author have confirmed that all relevant data are included in the article which is supported by supplementary information files.

Author contribution

Estri Laras Arumingtyas (ELA): Reviewing and feedback, taking responsibility in all manuscript, funding, research design and concept. Nur Rahmattullah (NR): Research design and concept, manuscript draft, data collection and analysis, literature search. Muhammad Hermawan Widyananda (MHW): Bioinformatics analysis, discussion draft, data analysis, figure editing, literature search. Alhuda Niftakhul Ahyar (ANA): Discussion draft, data collection, figure and graphics editing. Imam Tabroni (IT): Reference formatting and citation management, data collection and analysis, results conception.

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Ethics approval and consent to participate

The authors declare that we did not use human or animals in this research.

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