The potential use of chitosan deduced from the results of *in silico* analysis

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Abstract. One type of coronavirus, SARS-CoV-2, is currently the most feared virus globally because it causes pulmonary infectious diseases that can cause a risk of death. Researchers have tried together by conducting massive research and finding an antidote, especially those sourced from natural ingredients. Chitosan is a natural ingredient isolated from crustaceans. This compound has long been studied and proven to have the ability to inactivate various types of viruses. Therefore, in this study, chitosan’s ability to inhibit several proteins and enzymes from SARS-CoV-2 was evaluated *in silico*. The evaluation results suggested that chitosan has *in silico* excellent activity in preventing the entry of SARS-CoV-2 into the cells and inhibiting its replication.

1. Introduction

Since the beginning of the year, the new coronavirus SARS-CoV-2 has been spreading worldwide from China. The pathogen triggers lung disease COVID-19 and is classified as a pandemic since March 11, 2020. Researchers around the world are working on developing vaccines and drugs against this virus. SARS-CoV-2 is an enveloped, positive-stranded RNA virus with 14 ORFs encoding 27 proteins [1]. One of the proteins that play an essential role in entering this virus into the human body is the spike surface glycoprotein (S-protein) [2]. The receptor-binding domain (RBD) located at position 319-510 of the S-protein mediates viral binding with angiotensin-converting enzyme 2 (ACE2), which acts as a receptor located on the surface of cells susceptible to viral infection [3]. This binding process triggers endocytosis
so that with the entry of nucleocapsids into the cell, then the virus will hijack and customize the host cell machinery in order to translate all the components needed to produce mature virions [5].

The COVID-19 is categorized as the most feared disease today because it has caused a high global death rate. That is why the search for agents that can inhibit the entry of the virus and viral replication in human cells is a priority. The bioactive compounds that have been studied so far are targeted at the proteins and enzymes encoded by these viral genes, including Spike Glycoprotein, Spike Ectodomain, Receptor Binding Domain (RDB), Endoribonuclease, Envelope (E) protein pentameric ion channel, RNA-dependent RNA polymerase (RdRp), Papain-like protease (PLpro), and Main Protease (Mpro) [6].

Indonesia is a country with a very high level of flora and fauna diversity, even in the third rank in the world. Of the many studies on the development of drugs for infectious diseases, more has been directed at the potential of plants. Many plants are reported to have ethnomedicinal properties due to their antioxidant [7], antimicrobial and antiviral activity [8], [9], [18], [19], [10]–[17]. However, there are also compounds of animal origin, which have medicinal benefits according to previous studies. One of them is chitosan, which is very abundant in crustaceans [20]. Chitosan is a linear polysaccharide produced by the deacetylation of chitin, a naturally occurring polymer [21]. Chitosan has been studied to have antioxidant, antibacterial, antifungal, and antiviral activities [22–26]. Chitosan has also been reported to have other medical uses in biomedical and biotechnological applications as drug delivery systems [27]–[29]. Based on information regarding chitosan’s medical properties, in this article we report the results of the in silico analysis of the interaction of chitosan with several essential proteins and enzymes required by SARS-CoV-2 to enter and replicate in cells.

2. Material and methods
2.1. Determination of receptors
Eight essential proteins from SARS-CoV-2 selected as receptors in this study were Spike Glycoprotein (close state) (PDB code: 6VXX), Spike Ectodomain Structure (open state) (PDB code: 6VYB), Receptor Binding Domain (RDB) (PDB code: 6YLA), Endoribonuclease (PDB code: 2H85), Envelope (E) protein pentameric ion channel (PDB code: 5X29), RNA-dependent RNA polymerase (RdRp) (PDB code: 6M71), Papain-like protease (PLpro) (PDB code: 6WX4), dan Main Protease (Mpro) ((PDB code: 6LU7).

2.2. Ligand and receptor preparation
Chitosan polymer acts as a ligand in this in silico analysis. Its structure was retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov) (Figure 1A). The .sdf format of the ligand was converted into .pdb format using Open Babel. The torque was adjusted, and the file was saved in .pdbqt format. The anhydrous form of chitosan (β-chitosan) (Figure 1B) was also used as a ligand, which was obtained from the Database of Polysaccharide 3D structures (http://polysac3db.cermav.cnrs.fr/db-connect.php?number=75). The 3D structure of anhydrous β-chitosan was converted into SMILES using an online translator (https://cactus.nci.nih.gov/translate/), then the SMILES was converted into 2D dimension using Swiss ADME (http://www.swissadme.ch). The ADME properties of each ligand were also evaluated.

The three-dimensional structures of the receptors were retrieved from Protein Data Bank (http://www.rcsb.org). The files were opened with BIOVIA Discovery Studio Visualizer
2020. Each water molecules and native ligands were removed, and receptors were stored in .pdb format.

![Chemical structure of chitosan](image)

**Figure 1.** Chemical structure of chitosan (PubChem CID: 71853) (A); anhydrous β-chitosan generated using Swiss ADME (B).

2.3. Receptor-ligand docking and visualization
The docking process was executed using Autodock Vina. The .pdbqt formats of the ligands and receptors were copied into the Vina folder. The configuration of vina was typed in notepad and saved as conf.txt. A command prompt was used to run the Vina program. The selection of the best docking pose is an *in silico* evaluation process. The pose with the most negative Gibbs' free energy binding is the best pose from the molecular docking process. Biovia Discovery Studio 2020 was used to visualize the interaction between the ligand and the receptors.

3. Results and discussions
COVID-19 pandemic presents significant challenges modern medicine has ever faced. Scientists are trying very hard to find treatments and drugs that can save people's lives and prevent people's exposure to this disease. Researchers are also continuing to conduct research to study SARS-CoV-2, the virus that causes this disease, in detail. For example, the findings are in the form of potential active compounds from nature and their potential target receptors on viruses.

In order to summarize the course of new drug discoveries, computer simulations are essential. *In silico* drug discovery is in great demand these days because of the convenience it offers to simulate interactions between drug candidates and receptors. The choice of chitosan as a ligand in this study is because this compound is a natural biopolymer and is the second most abundant natural polysaccharide. Besides, chitosan has many medicinal benefits due to its immunogenic property, as well as its antitumor, anti-inflammatory, antibacterial, antifungal, and antimicrobial activities [30].

Tables 1 and 2 show *in silico* analysis results between the large polymer of chitosan and anhydrous β-chitosan with several proteins and enzyme of SARS-CoV-2, which serve as receptors. The interacting amino acids and the type of the bonds formed between the ligands and the receptor are presented in Figure 2 for chitosan polymer and Figure 3 for anhydrous β-chitosan. The *in silico* analysis results in this study indicate a very strong interaction between the chitosan polymer and the SARS-CoV-2 proteins and enzymes. The very negative binding affinity value indicates this. Based on the binding affinity value shown by the chitosan
polymer with the receptor, there are indications that chitosan polymer interacts strongly with S-protein, with values ranging from the highest to the lowest as follows: -14.9 kcal/mol with 6VXX, -14.2 kcal/mol with 6VYB, and -14.1 kcal/mol with 6YLA. The lowest value is indicated by the affinity binding between the polymer chitosan and Mpro (-9.9 kcal/mol). However, in the anhydrous form, the ability of β-chitosan to bind to SARS-CoV-2 proteins and enzymes is lower, as shown in Table 2. Nevertheless, the ability to bind to the receptor shows the same pattern, that the highest interaction is observed between 6VXX, 6VYB, and 6YLA with ligands. These results indicate that chitosan, both in the form of polymer and anhydrous, has strong interactions with S-protein, especially on RDB.

Table 1. Binding analysis of chitosan polymer with SARS-CoV-2 receptors.

| PDB ID | Name of Receptors | Binding Free Energy (kcal/mol) | No. of H-bonds | Interacting residues and H-bond formation |
|--------|-------------------|-------------------------------|----------------|------------------------------------------|
| 6VXX   | Spike Glycoprotein (closed state) | -14.9                        | 7              | Conventional H-bond: Glu(C1017), Ser(C1021), Asn(A1028), Thr(B1027), Glu(A725), Asp(A1041); Carbon H-bond: Ser(C1021); van der Waals: Ala(C1020), Leu(C1024), Pro(C728), Lys(C947), Asn(B1023), Lys(A1028), Leu(A727), Leu(A1024), Thr(A1027), Ala(A1026), Ala (B1026), Ser(B1030), Gln(B784). |
| 6VYB   | Spike Ectodomain Structure (open state) | -14.2                        | 8              | Conventional H-bond: Arg(C1039), Thr(A1027), Ser(A1030), Ala(A1026), Gln(A784), Gly(A889), Phe(A888); Carbon: H-bond Glu(C1017); van der Waals: Arg(A1039), Leu(B1024), Arg(B1039), Thr(C1027), Asn(C1023), Ala(C1020), Leu(C1024), Ser(C1021), Asn(A1023), Gly(C1044), Ala(A783), Leu(A1034), Lys(A786), Gly(A891). |
| 6YLA   | Receptor Binding Domain (RDB) | -14.1                        | 9              | Conventional H-bond: Asn(H203), Tyr(B80), Lys(H205), Gly(B8), Glu(B10), Gly(H42), Met(H40), Gly(L47); Carbon H-bond: Ser(B7); van der Waals: Ser(H157), Thr(H155), Ser(B21), Lys(H210), Ser(B17), Leu(B18), Lys(B12), Ile(B20), Pro(H153), Lys(H43). |
| PDB ID | Name of Receptors | Binding Free Energy (kcal/mol) | No. of H-bonds | Interacting residues and H-bond formation |
|--------|-------------------|-----------------------------|----------------|-----------------------------------------|
| 2H85   | Endoribonuclease  | -12.8                       | 9              | Pro(H41), Gln(H39), Tyr(95). Conventional H-bond: Asn(A163), Tyr(A88), Thr(A195), Thr(A274), Ser(A273), Asp(A296), Lys(A70), Asp(A272), Asp(A199); van der Waals: Val(A294), Tyr(A278), Arg(A198), Lys(A276), Thr(A166), Lys(A89), Ile(A295), Val(A165), Gly(A164), Lys(A89), Trp (A58). |
| 5X29   | Protein pentameric ion channel | -11.8                       | 3              | Conventional H-bond: Arg(D61), Thr(E30); Carbon H-bond: Ala(A32); van der Waals: Ala(A32). Conventional H-bond: Asp(A618), Lys(A621), Tyr(A619), Ser(A759), Ser(A682), Asp(A684), Asn(A496), Arg(A569), Lys(A500); van der Waals: Lys (A798), Pro(A620), Cys(A622), Asn(A691), Thr(A680), Thr(A687), Ala(A688), Ala(A685), Gly(A683), Gly(A590), Ile(A589), Leu(A758). |
| 6M71   | RNA-dependent RNA polymerase (RdRp) | -11.7                       | 10             | Conventional H-bond: Asp(A618), Lys(A621), Arg(A553), Tyr(A619), Ser(A759), Ser(A682), Asp(A684), Asn(A496), Arg(A569), Lys(A500); van der Waals: Lys (A798), Pro(A620), Cys(A622), Asn(A691), Thr(A680), Thr(A687), Ala(A688), Ala(A685), Gly(A683), Gly(A590), Ile(A589), Leu(A758). |
| 6WX4   | Papainlike protease (PLpro) | -10.9                       | 7              | Conventional H-bond: His(D73), Gln(D174), Ser(D170), Met(D206), Glu(D203), Lys(D232); Carbon H-bond Met(D206); van der Waals: Thr(D75), Thr(D74), Tyr(D171), Val(D202), Arg(D166), Leu(D185), Phe(D216), Tyr(D207), Leu(D199), Arg(D183), (Asn(D128). |
| 6LU7   | Main Protease (Mpro) | -9.9                        | 4              | Conventional H-bond: Thr(A190), Gly(A143), Cys(A145); Carbon H-bond Gly(A143); van der Waals: Asn(A119), Thr(A45), Thr(A24), Ser(A46), Ser(A44), Met(A49), Asn(142), Tyr(A118), Thr(A26), Pro(A168), Glu(A166), Gln (A189), Met(A165), Ala(A191), Pro(A168), Gln(A192), Glu(A166), Gln(189). |
Table 2. Binding analysis of anhydrous β-chitosan with SARS-CoV-2 receptors.

| PDB Code | Name of Receptors                  | Biding Free Energy (kcal/mol) | No. of H-bonds | Interacting residues and H-bond formation |
|----------|------------------------------------|------------------------------|----------------|------------------------------------------|
| 6VXX     | Spike Glycoprotein (close state)   | -8.4                         | 7              | Conventional H-bond: Thr(A549), Leu(A572), Arg(B1000), Met(B740), Tyr(B741), Asn(B856); Carbon H-bond: Gly(B44); van der Waals: Leu(B966), Ser(B875), Thr(A572), Val(B976), Thr(A573), Leu(A546), Thr(A547), Asn(B978), Phe(A541), Gly(A548), Gly(B744), Phe(B855). |
| 6VYB     | Spike Ectodomain Structure (open state) | -8.2                         | 10             | Conventional H-bond: Ser(A968), 4Ser(A967), 2His(A49), Arg(A44), Asp(C571); Carbon H-bond: Asp(C571); van der Waals: Gly(A757), Ser(B758), Lys(A964), Arg(C567), Ile(C569), Ser(A50). |
| 6YLA     | Reseptor Binding Domain            | -8.3                         | 7              | Conventional H-bond: Lys(B19), Ser(H157), Ser(H160), Ser(B17); Carbon H-bond: Lys(B19), 2Thr(B155); van der Waals: Gln(B82), Gly(H161), Leu(B18), Lys(B12), Lys(H205), Ser(B7), Thr(H155), Asn(H203), Lys(H210), Tyr(B80). |
| 2H85     | Endoribonuclease                   | -7.7                         | 10             | Conventional H-bond: Glu (A:201), Asp (A:199), 4Tyr (A:278), Ser (A:273), Lys (A:70), Asp (A:272); van der Waals: Thr(A195), Asp(A296), Ser(A197), Val(A294), Lys(A276), Arg(A198), Met(A251), Lys(A89), Leu(A200), Leu(A265). Conventional H-bond: 3Thr(B30); van der Waals: Leu(B39), Leu(B34), Ala(C36), Val(29), Leu(B27), Ala(C32), Ile(B46), Ile(C33). |
| 5X29     | Envelope protein pentameric ion channel | -6.5                         | 3              | Conventional H-bond: Ser(A709), Lys(A47), 2Tyr(A129), Asp(A711), Thr(A710); Carbon H-bond: Lys(A47); van der Waals: Tyr(A32), Lys(A714), Ala(A46), Gln(A773), Gly(A774), His(A133), Lys(A780), Asn(A781), Ser(A784). |
| 6M71     | RNA-dependent RNA polymerase (RdRp) | -7.8                         | 7              | Conventional H-bond: Ser(A709), Lys(A47), 2Tyr(A129), Asp(A711), Thr(A710); Carbon H-bond: Lys(A47); van der Waals: Tyr(A32), Lys(A714), Ala(A46), Gln(A773), Gly(A774), His(A133), Lys(A780), Asn(A781), Ser(A784). |
| PDB Code | Name of Receptors | Biding Free Energy (kcal/mol) | No. of H-bonds | Interacting residues and H-bond formation |
|----------|-------------------|-----------------------------|----------------|------------------------------------------|
| 6WX4     | Papain-like protease | -6.6                        | 11             | Conventional H-bond: 2Ser(D212), Tyr(D213), Gly(D214), 2Tyr(D305) Thr(D257), Lys(D254), Gly(D252), Lys(D218); Carbon H-bond: Tyr(D251); van der Waals: Thr(D251), Val(D303), Phe(D258), Leu(D253), Gln(D215). | 
| 6LU7     | Main Protease     | -6.8                        | 6              | Conventional H-bond: Met(A165), 2Glu(A166); Gly(A143); van der Waals: Cys(A145); Carbon H-bond: His(A41); van der Waals: Arg (A188), Asp(A187), Gln(A189), His(A163), His(A164), Leu(A141), Ser(A144), Cys(A145), Asn(A142), Thr(A26), Leu(A27), Thr(A25), His(A41), Met(A49). | 

Figure 2. The diagrams that show the type of interactions form between the chitosan polymer and receptors of SARS-CoV-2.

Chitosan polymer has very strong interactions with the proteins and enzymes of SARS-CoV-2, which are indicated by their energy binding values. The score far exceeds the value of the interaction between anhydrous β-chitosan and SARS-CoV-2. This score indicates that the polymer chitosan does have the ability to inhibit viral replication, including SARS-CoV-2, as
reported by previous researchers [23], [30], [31]. The linear form of chitosan shows the best binding energy with the RDB of the virus [32].

Most of the interactions that occur between anhydrous β-chitosan and the receptors are conventional hydrogen bonds. This type of bond greatly determines the bonds’ stability because the more hydrogen bonds formed with the amino acid residues in the receptors, the interaction will be more stable, so that the energy binding score will be lower [33]. Table 2 shows that the ligands 6WX4, 2H85, and 6VYB have the most hydrogen bonds with the receptors. From the value of the binding energy score and the presence of hydrogen bonds, the most stable interaction is between the ligand and the spike ectodomain structure (open state) (6VYB). According to Wrapp et al., [34], the SARS-CoV-2 infection rate is very high due to the strong interaction between the ectodomain of SARS-CoV-2 and ACE2, so this domain is one of the main targets for inactivation with antiviral drugs.

![Figure 3](image)

**Figure 3.** The diagrams that show the type of interactions form between the anhydrous β-chitosan and receptors of SARS-CoV-2.
Chitosan polymer  

Anhydrous Chitosan

**Formula**  

C$_{56}$H$_{103}$N$_{9}$O$_{39}$  

C$_{12}$H$_{26}$N$_{2}$O$_{10}$

**Molecular weight**  

1526.45 g/mol  

358.34 g/mol

**Num. H-bond acceptors**  

47  

12

**Num. H-bond donors**  

29  

8

**Water Solubility**  

Highly soluble  

Highly soluble

**Pharmacokinetics**  

GI absorption  

Low  

Low

Cytochrome P450 (CYP) inhibitors  

No  

No

**Druglikeness**  

Lipinski violations  

3  

2

Bioavailability Score  

0.17  

0.17

Although, according to calculations, the bioavailability scores of these two compounds are low (0.17), chitosan is often used as a drug carrier [35]. As a mucoadhesive polymer, it is known to increase permeation or absorption of many compounds, thus improving oral bioavailability [36]. The nanostructures of chitosan were observed to enhance molecular intestinal adsorption throughout the small intestines [37]. Moreover, chitosan can be used in oral, vaginal, nasal, pulmonary, and ocular routes [38]. Many successful studies have shown that chitosan nanoparticles can be used as a drug carrier because they can increase the bioavailability of active compounds, including cyanocobalamin (VB12) [39], tea polyphenols [40], carvedilol (an anti-hypertensive drug) [41], and curcumin [42].

Nanochitosan-based pharmaceutical products are currently designed against COVID-19, as reported by Cavalcanti and Nogueira [43]. They reported that chitosan nanoparticle, called Novochizol, has been developed for aerosol application to treat COVID-19 infection by providing a therapeutic dose to a patient for a period of 25 min to 3 h. A current study has also examined the antiviral effectiveness of HTCC (N- (2-hydroxypropyl) -3-trimethylammonium chitosan chloride) against the new coronavirus SARS-CoV-2 and MERS-CoV in vitro (on Vero and Vero 6 cells), and ex vivo on the human respiratory
epithelium (HAE) [31]. Alitongbieke et al., [44] have currently reported that β-chitosan neutralized RBD of SARS-CoV-2 S-protein because of its function that resembles an antibody, so that S-protein was no longer able to bind to hACE2 mice. Chitosan nanoparticle has also been studied at the University of Brasilia for application in facial respirators because it can reduce particle permeability and has virucidal activity against various viruses, including SARS-CoV-2.

The potential use of chitosan, in fact, is not limited only as an antiviral, but can also be used as a drug carrier [27], [28], [45] an immunomodulating adjuvant [46], [47] and pharmaceutical excipient [48]. Thus chitosan has a package of advantages for handling infectious diseases, including COVID-19, if it is developed optimally.

4. Conclusion

The finding of this research suggests that chitosan polymer and anhydrous β-chitosan have the potential to prevent SARS-CoV-2 infection and can also be used as a therapeutic agent for COVID-19. The best interaction has been observed between chitosan and S-protein.

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