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Binding affinities of disinfectants and the disinfectant effects of chlorhexidine against SARS-CoV-2

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Abstract

Objective: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a single stranded positive sense RNA enveloped virus. The spike glycoprotein on this virus surface binds to the human angiotensin- converting enzyme2 receptor hence leads to cell entry into the host. Its primary route of entry is the upper respiratory tract or the facial muscosal surfaces. Disinfectants are chemical agents that destroys the structural proteins of SARS-CoV-2 thereby decreasing the decree of infection and reducing the emission of variants. The study investigated the binding affinities of disinfectants against SARS-CoV-2 by *in silico* docking simulations.

Methods: We searched for COVID-19 protein target, 6VSB was selected in Protein Data Bank. The protein was prepared and saved as pdbqt. The approved disinfectants were selected and downloaded from pubchem. The mol2 file of the disinfectants were prepared and saved as pdbqt. Validation of docking protocol was done. The molecular docking was done using Autodock Vina-4.2.6 in the Linux operating system (ubuntu). The docking process was repeated four times for the protein and each of the ligand for the calculation of the average and standard deviation. The docking results were analysed and visualized using PyMol-v1.3r1-ed win32.

Results: The results obtained shows that chlorhexidine had binding affinities of -7.23 and cetrimide had binding affinities of -4.28.

Conclusion: The study concludes that chlorhexidine has a strong disinfectant effects against SARS-CoV-2

Keywords: SARS-CoV-2; Disinfectants; Binding Affinities; *in silico* docking simulations; COVID-19

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1. Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the causative pathogen for the COVID-19. It emerged first in Wuhan, China in December, 2019 and in January 30 2020, WHO declared it a pandemic. SARS-CoV-2 is an enveloped, positive-sense, single RNA virus of the genus Betacoronavirus [15]. SARS-CoV-2 enters the cell by the binding of its spike protein and its receptor, angiotensin- converting enzyme-2 (ACE2) and subsequently membrane fusion. SARS-CoV-2 is transmitted typically by respiratory droplets. It can be transmitted via contact by touching contaminated surfaces, followed by touching the mouth, nose, or eyes. The pandemic caused so many global challenges, economically and otherwise. Scientists and researchers worked tirelessly to put an end to the pandemic, then came the discovery of the vaccines against SARS-CoV-2 and the need to be vaccinated. Gradually, everything seemed to have returned back to normal and seemed that the SARS-CoV-2 has been eradicated from the surface of the earth. But, that is not so as the virus still exist between us, some many people are been infected and some are dying. During the pandemic, variants such as Alpha, Beta, Gamma, Delta emerged from the number of substitutions in the SARS-CoV-2 spike N-terminal domain (NTD) and receptor- binding domain (RBD) [4]. This virus mutates a lot and has developed many variants. The most recent variant is the omicron variant which first appeared in late November 2021 in South Africa [10].

Even after been vaccinated, WHO encourages us the need to wear face masks, maintain social distancing, frequent hand washing, use of hand sanitizers, and the use of disinfectants on surfaces and floors. Preventive measures help to reduce the emergence of new variants of SARS-CoV-2 [14].

Disinfection of floors and surfaces such as office door handles, stair rails, toilet handles, and so many on, though undermined plays important role in reducing the virus infection. Disinfectants are chemical agents that tend to destroy or reduce microbes on inanimate objects. Disinfectants destroy the structural proteins of SARS-CoV-2 thereby preventing its binding to the angiotensin converting enzyme 2 (ACE2) receptors of the host cells leading to the decrease of infection.

The improper selection and inadequate use of disinfectants plays a significant role in the cross- transfer and spread of pathogens resulting in additional public health concerns [1]. Thereby conducting molecular docking studies, will examine which disinfectants work best against SARS-CoV-2. Molecular docking is an arm of computer aided drug design (CADD). Deployment of computer aided drug design, a specialized discipline that uses computational methods to stimulate drug- receptor interactions [12]. The binding affinity (free energy) of a drug for a receptor describes how avidly the drug binds to the receptor [12]. In this study, focus will be on discovering the best disinfectants against SARS-CoV-2 *in silico* using their binding affinities.

2. Material and Methods

The research work requires certain materials, tools, software and essentially useful web sites:

A personal computer with internet access and Linux operating system (Ubuntu- 12.04), windows operating system.

2.1. Tools and Softwares

- An autodock tool vs-1.5.6 downloaded from http://mgltools.edu. It is used for proteins and ligands preparation and conversion of file format.
- Chimera – 1.9. it is used in the preparation of proteins (removal of residues, water, and other unwanted components of the protein).
- PyMol- v1.3rl-edu downloaded from http://delsci.com. It is used for viewing 3D structures of proteins and ligands.
- Molinspiration downloaded from www.molinspiration.com. This tool is used to sketch compounds from their SMILES format, predict biological activities and determine the physio-chemical properties of the compounds.
- Protein databank (PDB) gotten from www.rcsb.org. it is used for molecular characterization of proteins, download of 3D structures and fasta file format of biological molecules.
- ZINC database gotten from zinc.docking.org. it is used for physio-chemical properties prediction, download the mol2 file format and SMILE of ligands.

2.2. Literature and Bioinformatics mining

Literature mining on disinfectants, antiseptics, formulations was done for better understanding. A search for the SARS-CoV-2 drug targets was done to identify the potential drug targets. Literature mining was done for better understanding of the individual drug targets on the SARS-CoV-2. Sequence analysis of the drug target was also done.

2.3. Selection and Preparation of protein structure

After the identification of drug target, literature mining and analysis, suitable receptors (proteins) were selected. The target was checked in the protein data bank (PDB).

The protein was prepared by downloading the experimental crystal structures of SARS-CoV-2 bound to the ligand with PDB codes from protein data bank (www.rcsb.org) in PDB format. The PDB code used was 6VSB. The downloaded file was opened in Chimera-1.10.1; the unnecessary chains and unwanted components were deleted and saved as PDB file. The saved file was opened in Autodock tools, where kollman charges, polar hydrogen and macromolecules were added and saved as pdbqt file. The grid search spaces was also determined using the grid box.

2.4. Ligand Selection, Downloading and Preparation

The 3D structure of chlorhexidine and other approved disinfectants were downloaded from pubchem. Openbabel on Ubuntu was used to convert the downloaded files from sdf to mol2 which is the accepted format. The mol2 file for each ligand to be prepared was selected and opened on Autodock tools by clicking on the ligand option on the toolbar followed by input then open. The ligand was edited by adding compute gasteiger charges. The ligand option was selected again and torsion tree was selected and the choose torsion and finally all active bonds were made non-rotatable by the selecting the option. The ligands were saved as pdbqt by clicking on the tool bar then clicking on output to save. The above procedure was repeated for all the selected ligands respectively.

2.5. Validation of Docking protocol

Here, the experimental crystal structure of the target bound to their reference drugs were superimposed on the PDB crystal structure. This process is done to look for the binding pocket on the protein. Autodock tool-1.5.6 is used to fuse the protein and the reference ligand together to locate the exact binding pocket.

2.6. Molecular Docking

The molecular docking was done using Autodock Vina-4.2.6 in the Linux operating system (ubuntu). The file format used for the prepared protein and ligand was pdbqt. Binbash.sh was used for docking and all the necessary information including the center, size, protein name etc. were added into the configuration text. The docking process was repeated 4 times for the protein and each of the ligand for the calculation of the average and standard deviation.

2.7. Post Docking Analysis

The docking results were analysed and visualized using pymol . The binding pocket of the protein was called up and the different drugs where colored respectively to be able to view the affinity within the binding pocket. Snapshot were made for easy visualization of the affinity within the binding pocket in solid surface and saved as png.

3. Results

Table 1: shows the grid box position for the molecular docking. This set boundary where docking occurred.

Table 2 shows the disinfectants docked, their binding energies in Kcal/mol, the average of their binding energies and their standard deviation. The affinity of the ligand to the binding site of the receptor was visualized in PyMol.

Table 1 The grid box parameters

Size x	22
Size y	18
Size z	20
Center x	-12.778
Center y	-0.611
Center z	19.944

Table 2 The binding energies of the disinfectants docked

Chemical	**Dock_1**	**Dock_2**	**Dock_3**	**Dock_4**	**Average Dock**	**STD (n=3)**
Chlorhexidine	-7.20	-7.20	-7.30	-7.20	-7.23	0.05
Hexachlorophene	-5.40	-5.40	-5.40	-5.40	-5.40	0.00
2_Methoxy_6_Phenol	-5.20	-5.30	-5.30	-5.30	-5.28	0.05
Phthalaldehyde	-4.40	-4.40	-4.50	-4.40	-4.43	0.05
Cetrimide	-4.60	-4.10	-4.00	-4.40	-4.28	0.28
2_Iodophenol	-4.00	-4.00	-4.00	-4.00	-4.00	0.00
Povidone_Iodine	-3.60	-3.60	-3.60	-3.60	-3.60	0.00
Glutaraldehyde	-3.30	-3.30	-3.30	-3.30	-3.30	0.00
Peracetic_Acid	-3.10	-3.10	-3.10	-3.10	-3.10	0.00
Isopropyl_Alcohol	-2.40	-2.40	-2.40	-2.40	-2.40	0.00
Ethanol	-2.00	-2.00	-2.00	-2.00	-2.00	0.00
Hdrogen_Peroxide	-2.00	-2.00	-2.00	-2.00	-2.00	0.00
Chloramine	-1.70	-1.70	-1.70	-1.70	-1.70	0.00
Chlorine	-1.60	-1.60	-1.60	-1.60	-1.60	0.00
Iodine	-1.50	-1.50	-1.50	-1.50	-1.50	0.00
Formaldehyde	-1.40	-1.40	-1.40	-1.40	-1.40	0.00

STD= Standard deviation

4. Discussion

From the literature review done, the SARS-CoV-2 structure is divided into different parts but we are more interested in the structural proteins especially the spike protein for this research. The spike (S) proteins are present on the surface

of the SARS-CoV-2 and are responsible for the binding of the virus to the receptors on the host cells [13]. Another reason why this research is solely interested on the spike protein is the ability for spike protein to mutate a lot. According to Duchene S. [2], mutations at the S protein affect the virulence and differentiation mechanisms of SARS-CoV-2 and how it spreads and evolve.

The selection of the preferred PDB structure of COVID-19 which was 6VSB was done based on it is a spike glycoprotein with a good resolution which was 3.46Å and its prominent chains A, B, C. the study was conducted with the total of seventeen (17) chemical compounds obtained from pubchem website. Table 1 shows the parameters of the 3-D structural position of the 6VSB. This helps in accurate docking results.

Table 2 shows the list of the docked chemical compounds with their average binding energy levels. From the results obtained, the docking process were ranked according to their docking scores and their corresponding energy level [7]. The higher the negative value of the drugs, the better may be the disinfecting activity of the chemical compounds against SARS-CoV-2.

It has been proven scientifically that the lower the free binding energy, the more stable the ligand-target interactions. The binding affinity of a chemical compound explains its degree of interaction with the receptor site. The lower the free binding energies, the lower the binding affinities.

Chemical compounds with very low free binding energies have very low binding affinities and as such have complete interaction with the receptor site of COVID-19 and can prevent entry of COVID-19 into the host cells. From table 2, the docked chemical compounds, chlorhexidine had the highest binding affinity against SARS-CoV-2 than the rest of the other chemical compounds. Followed by hexachlorophene, followed by 2-methoxy-6-phenol, followed by phthalaldehyde, followed by cetrimide, followed by 2-iodophenol, followed by povidone-iodine, and so on.

The improper selection and inadequate use of disinfectants plays a significant role in the cross- transfer and spread of pathogens resulting in additional public health concerns [1]. This explains the importance of computational chemistry, molecular docking. With this method you can get the proper selection of disinfectants without any kind of chemical wastage.

This research is interested in the selection of disinfectants with high binding affinities and also very safe and available. Chemical compounds such as hexachlorophene, 2-methoxy-6-phenol, phthalaldehyde, 2- iodophenol have high binding affinities but they are neither safe for use nor available.

The three chemical compounds with high binding affinities and are safe for use and are readily available are chlorhexidine, cetrimide and povidone-iodine.

Chlorhexidine belongs to the class of biguanides. It is used as a mild disinfectant and preservative. Chlorhexidine is not considered a particularly effective antiviral agents [5]. This research contradicts that because it is clearly seen that chlorhexidine is an effective antiviral agent especially against SARS-CoV-2 and other lipid enveloped viruses. It acts by causing damage to the outer cell layers [3].

Cetrimide belongs to the class of quaternary ammonium compounds. They are membrane active agents [6]. This research agrees with what Springthorpe V. [11], that cetrimide has an eefect on lipid, enveloped viruses. It acts by inducing disintegration and morphological changes of the enveloped viruses resulting in the loss of infectivity [8].

Povidone – iodine belongs to the class of halogen – releasing agents. The research agrees with Prince H. [9] that povidone –iodine is more sensitive to lipid enveloped viruses than non-enveloped viruses. It acts by attacking the surface proteins of the enveloped viruses [11].

5. Conclusion

The present work showed the binding affinities of disinfectants against SARS-CoV-2. It showed that chlorhexidine has a very strong binding affinity against SARS-CoV-2 and as such can be used as the best disinfectant against SARS-CoV-2. Combination of chlorhexidine and cetrimide will give a synergistic effect against SARS-CoV-2.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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