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In silico binding studies with b-sitosterol and some of its fatty acid esters to 3C-like protease of SARS-CoV-2

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Abstract
COVID-19, a coronavirus (SARS-CoV-2) caused disease has turned into a pandemic with no therapeutics in the form of drugs or vaccines yet in sight. The objective of this study was to evaluate in molecular docking studies the binding energies of b-sitosterol (a phytosterol) and some of its fatty acid esters to the main protease of COVID-19, otherwise known as the 3C-like protease or $3CL^{pro}$, (PDB ID: 6LU7) in an attempt to discover possible lead compounds or drugs against the virus as a means to contain the pandemic. Molecular docking (blind) was done with the help of Autodock Vina. Seven fatty acid esters of b-sitosterol (a major phytosterol) were evaluated. While b-sitosterol gave a binding energy of -7.0 kcal/mol, b-sitosterol-acetate and b-sitosteryl-ferulate gave binding energies of -6.9 and -7.8 kcal/mol, respectively. The other esters gave lower binding energies. As a result, the ferulic acid ester of b-sitosterol has a greater probability of being a COVID-19 therapeutic.

Keywords: Molecular docking, b-sitosterol, COVID-19, pandemic, fatty acid esters

Introduction
The ongoing pandemic [1] caused by a coronavirus SARS-CoV-2 has already as of September 14, 2020 resulted in 29,180,055 cases of infection and 928,212 deaths throughout the world. The disease first was recognized at a seafood market in Wuhan, China in the latter part of December 2019 [2]. The Director General of the World Health Organization (WHO) declared the outbreak on January 20, 2020 as a "public health emergency of international concern" (PHEIC). On February 11, 2020 WHO announced the designation of the current coronavirus disease as COVID-19. Altogether seven coronaviruses have been found affecting humans including the present virus or SARS-CoV-2 and all of them are zoonotic, that is they originally were transmitted from animals to humans prior to human to human transmissions. Prior to SARS-CoV-2, six CoVs were known to infect humans including HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV. Though SARS-CoV (Severe Acute Respiratory Syndrome coronavirus, emerged in 2002) and MERS-CoV (Middle East Respiratory Syndrome corona virus, emerged in 2012) have resulted in outbreaks with high mortality, others remain associated with mild upper respiratory tract illnesses only [3]. The name severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was named by the International Committee on Taxonomy of Viruses (ICTV).

Despite the increasing number of fatalities and infections caused by SARS-CoV-2 and the severe economic disruptions caused by this virus throughout the world, and despite frantic efforts by scientists to have a safe vaccine or drug against this virus, no such discoveries have been made till now. A number of drug targets in the virus have been identified, of whom the most promising target has been mentioned as the chymotrypsin like protease (or main protease M^{pro}) or 3C-like protease for which the crystal structure is known [4]. This is a conserved protease with 96.1% similarity with the main protease of SARS-CoV. Inhibition of this homodimeric cysteine protease can inhibit replication of the virus through stoppage of cleavage of viral polyproteins into individual polypeptides necessary for replication and transcription [5-9].

The catalytically active 3C-like protease is a dimer with the His41-Cys145 playing the major role in the proteolytic process [10]. Dimerization has been postulated to provide a 'substrate-binding cleft' between the two monomers [11]. However, in the dimer the His-Cys dyads are located symmetrically at opposite ends of the cleft, suggesting that they act independently [12]. Three domains are present in the main protease monomer. Domains 1 and 2 comprise of residues 8-101 and 102-184, respectively; they form a chymotrypsin-like fold responsible for catalysis [13]. Domain 3 comprises of residues 200-303. The first seven amino acids at the N-terminus reportedly form the N-finger and play a significant role in the formation of the active site of the 3C-like protease [14].

In silico approaches like molecular docking can play an effective role in the identification of compounds, which can be therapeutics for COVID-19. A number of such approaches have been tried with natural plant-derived compounds like phytochemicals binding to 3C-like protease with some promising results, but as of yet no clinical trials or availability of drugs. What is of importance is that the phytochemicals reported to inhibit $3CL^{pro}$ (betulinic acid, kaempferol, quercetin) mainly entered the regions between domains 2 and 3 [15]; this region is important for $3CL^{pro}$ to form a dimer [10]. We had also been screening phytochemicals from various plant sources through *in silico* studies to evaluate their binding energies to the 3C-like protease of SARS-CoV-2 with the assumption that a strong binding can lead to inhibition of the protease with consequential inhibition of viral replication [16-20].

b-Sitosterol is one of the most abundant phytosterols in the plant kingdom. The compound is known for having diverse pharmacological activities including anti-pyretic, antioxidant and anti-inflammatory activities [21]. The objective of the present study was to determine the binding affinity of b-sitosterol and some of its fatty acid esters to 3C-like protease of SARS-CoV-2 using molecular docking as the *in silico* tool in our search for a COVID-19 therapeutic.

Methods

Three-dimensional structure of COVID-19 and SARS major protease (3C-like protease)

We have used the pdb file (6LU7) of the main protease or SARS-CoV-2 3C-like protease (or main protease M^{pro}) as published by Professor Rao and his colleagues [22]. Inhibitor (called N3) was removed from the pdb file before using the protein's structure in our molecular docking studies. Monomeric form of protein was used for molecular docking.

Compounds used in docking studies

We have studied b-sitosterol and seven of its fatty acid esters (b-Sitosterol-acetate, b-Sitosterol-behenate, b-Sitosterol-hydrogen succinate, b-Sitosterol-isostearate, b-Sitosteryl-ferulate, b-Sitosteryl-oleate, and b-Sitosteryl-palmitate). Ligand molecules were downloaded from Pubchem [23] in sdf format. They were optimized with the force field type MMFF94 using Openbable softwares and saved as pdbqt format.

Ligand molecular docking studies

We have conducted molecular docking (blind) using AutoDock Vina [24]. We report ΔG values as an average of values from five independent runs of the docking program. In our figures, we show the pose of phytochemicals bound to SARS-CoV-2 main protease as obtained from PyMOL and displayed in Discovery Studio [25].

Results and Discussion

The structures of b-sitosterol and its seven fatty acid esters, namely, b-Sitosterol-acetate, b-Sitosterol-behenate, b-Sitosterol-hydrogen succinate, b-Sitosterol-isostearate, b-Sitosteryl-ferulate, b-Sitosteryl-oleate, and b-Sitosteryl-palmitate were obtained from PubChem. The structures of these eight compounds (b-sitosterol and its seven fatty acid esters) are shown in Figure 1 and their binding energies (ΔG = kcal/mol) is shown in Table 1.

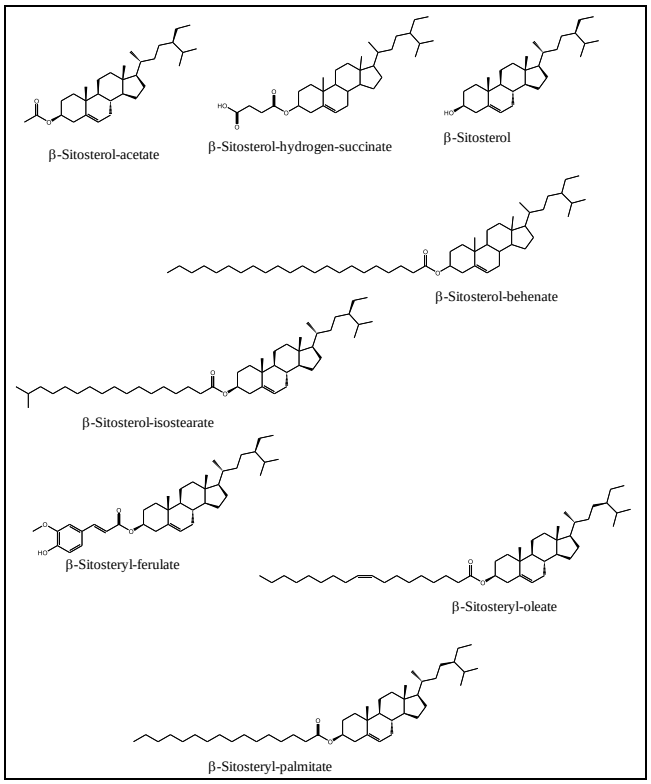

Fig 1: Structures of b-sitosterol and its fatty acid esters

Interestingly, b-sitosterol gave a binding energy of -7.0 kcal/mol; on the other hand, b-sitosterol-acetate and b-sitosteryl-ferulate gave binding energies of -6.9 and -7.8 kcal/mol, respectively. The other esters gave lower binding energies (Table 1). The lowest binding energy of -5.2 kcal/mol was observed with b-Sitosterol-behenate. It appears that the length of the carbon chain of the fatty acid in the ester has an inverse relationship with binding energy; however, the relationship is directly not proportional indicating other factors are also present. In this regard, the other three esters with long carbon chains, namely b-Sitosterol-isostearate, b-Sitosteryl-palmitate, and b-Sitosteryl-oleate gave binding energies of -5.3, -6.0 and -6.1 kcal/mol, respectively.

Table 1: Binding energies of b-sitosterol and its fatty acid esters to 3C-like protease

Compounds	Binding energy (ΔG = kcal/mol)	Interacting residues
b-Sitosterol-acetate	-6.9	Val202, His246, Ile249, Pro252, Pro293, Phe294, Val 297
b-Sitosterol-behenate	-5.2	Pro108, His246, Ile249, Pro293
b-Sitosterol-hydrogen succinate	-6.3	Val202, Glu240, Ile249, Pro293, Phe294
b-Sitosterol-isostearate	-5.3	Lys102, Ile249, Pro293, Phe294, Val297
b-Sitosterol	-7.0	Leu141, Gly143, Ser144, Pro168
b-Sitosteryl-ferulate	-7.8	Pro108, Pro132, Val202, Glu240, His246, Ile249, Pro252, Pro293, Phe294, Val297
b-Sitosteryl-oleate	-6.1	Val104, Ile106, Phe294
b-Sitosteryl-palmitate	-6.0	Val104, Asp153, Ser158, Val202, His246, Ile249

The 2-D interaction of b-Sitosterol-behenate, b-Sitosterol, and b-Sitosteryl-ferulate are shown in Figures 2-4, respectively. The interacting amino acids of the 3C-like protease and all the tested compounds are given in Table 1. It is to be noted that while interacting residues with b-Sitosterol are in domain 2, most interacting residues of b-Sitosterol-behenate and b-Sitosteryl-ferulate are in domain 3 of the protease. However, it is possible that binding to domain 3 of the protease can bring conformational changes leading to inhibition of the enzyme. It is further to be noted that both b-Sitosterol-behenate and b-Sitosteryl-ferulate also interact with Pro108 in domain 2.

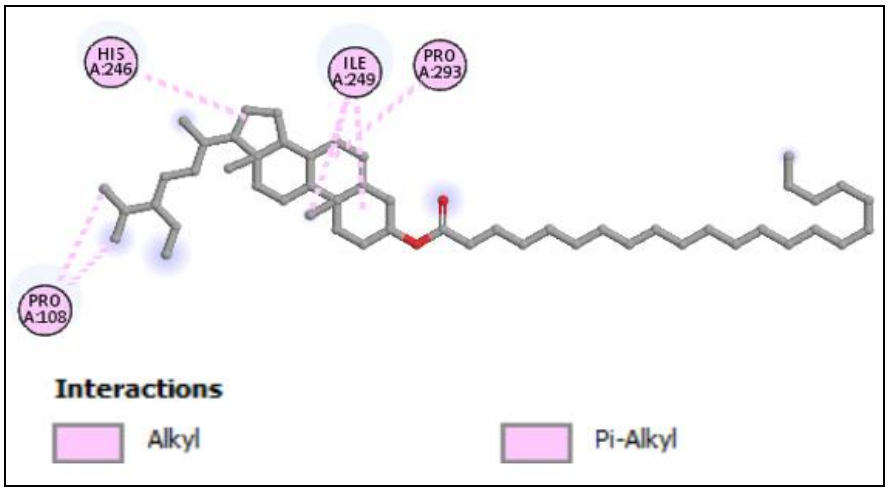

Fig 2: Interaction of b-Sitosterol-behenate with 3C-like protease of SARS-CoV-2.

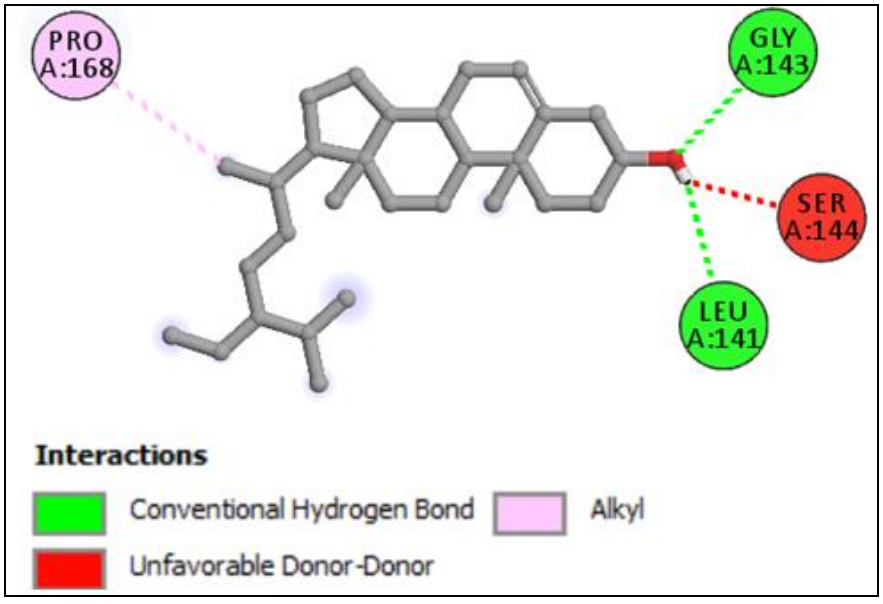

Fig 3: Interaction of b-Sitosterol with 3C-like protease of SARS-CoV-2.

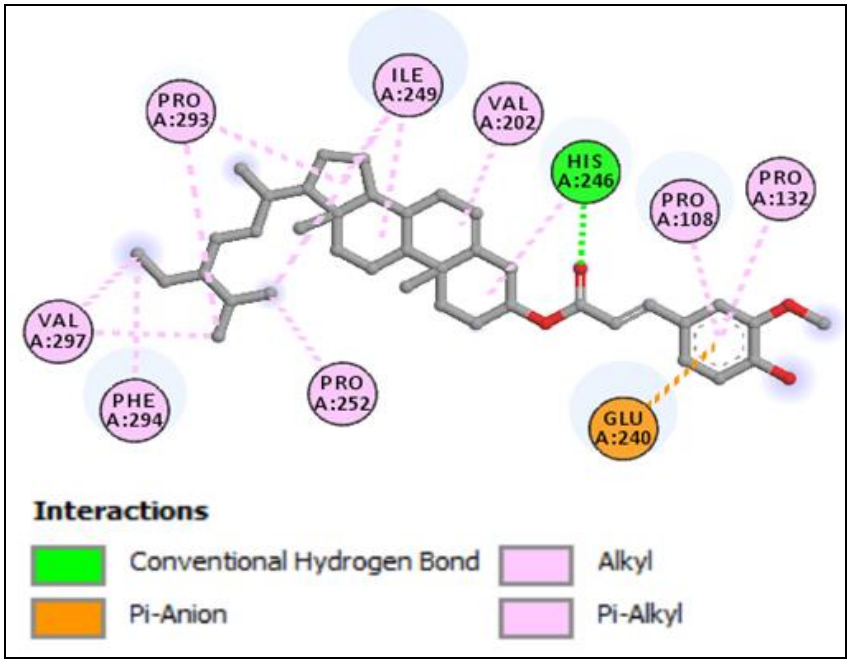

Fig 4: Interaction of b-Sitosterol-ferulate with 3C-like protease of SARS-CoV-2.

Ferulic acid and derivatives have antiviral properties. Substituted ferulic acid amide derivatives and corresponding hydrogenated ferulic acid amide derivatives reportedly showed activity against tobacco mosaic virus [26, 27]. *trans*-Ferulic acid derivatives containing acylhydrazone moiety also showed activity against tobacco mosaic virus [28]. Novel myricetin derivatives containing ferulic acid amide scaffolds were also found to be active against tobacco mosaic virus [29]. It appears that other compounds conjugated with ferulic acid may have antiviral activities. The present study suggests that antiviral activity of b-Sitosteryl-ferulate deserves studying as a possible therapeutic for COVID-19.

Conclusion

Molecular docking studies indicate that b-Sitosteryl-ferulate may be a useful therapeutic for COVID-19.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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