

# Beta-Sitosterol: As Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein

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**Received date:** July 31, 2020, **Accepted date:** August 24, 2020

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## Abstract

As an extension to our recently published research work in *Asian Journal of Pharmaceutical and Clinical Research*, entitled “B-Sitosterol: Isolation from *Muntingia Calabura* Linn. Bark Extract, Structural Elucidation, and Molecular Docking Studies as Potential Inhibitor of SARS-CoV-2 M<sup>pro</sup> (COVID-19)”, we have investigated the role of  $\beta$ -sitosterol as immunostimulant, antioxidant and inhibitory potential against Receptor Binding Domain (RBD) of SARS-CoV-2 Spike Glycoprotein with the aid of molecular docking. There are many studies which reveals the antioxidant and immune boosting role of  $\beta$ -sitosterol especially in viral infection including pneumoniae. This commentary emphasis on further potential of  $\beta$ -sitosterol in treatment of COVID-19 through molecular docking studies. We have targeted RBD of spike glycoprotein and performed molecular docking studies of  $\beta$ -sitosterol to find out its inhibitory potential of SARS-CoV-2.  $\beta$ -sitosterol have showed binding affinity - 7.8 kcal/mol with 0 RMSD lower and upper bound. It formed one hydrogen bond with Ala-B:419 with bond length of 2.16Å.  $\beta$ -sitosterol has formed five alkyl bonds with Pro-C:384 (5.0Å, 4.66Å, 5.23Å, 4.27Å) and with Lys-C:378 (4.66Å). From present commentary, we have concluded that  $\beta$ -sitosterol can be used to enhance immunity against the SARS-CoV-2 infection as well as to restrict the viral invasion into the host cell through angiotensin converting enzyme-2 (ACE-2) by inhibiting spike glycoprotein. If we can increase the dietary intake of  $\beta$ -sitosterol and other phyosterols it can modulate the immunity which is today's need to face COVID-19.

**Keywords:**  $\beta$ -sitosterol, SARS-CoV-2 spike glycoprotein, Molecular docking, 6VSB

## Introduction

This article is an extension to our recently published article in *Asian Journal of Pharmaceutical and Clinical Research*, entitled “B-Sitosterol: Isolation from *Muntingia Calabura* Linn. Bark Extract, Structural Elucidation, and Molecular Docking Studies as Potential Inhibitor of SARS-CoV-2 M<sup>pro</sup> (COVID-19)” [1]. The article describes detailed procedure for the isolation (by Column Chromatography) and structural characterization (by FTIR, UV-Visible Spectroscopy and HPTLC) of  $\beta$ -sitosterol from *Muntingia Calabura* bark. The  $\beta$ -sitosterol was docked on SARS-CoV-2 M<sup>pro</sup> to study the binding affinity (kcal/mol) in comparison with favipiravir. It has been found that favipiravir has a less binding affinity, i.e. 5.7 kcal/mol than  $\beta$ -sitosterol which has 6.9 kcal/mol. The number of hydrogen bonds formed by the favipiravir is much more, i.e., 4 than  $\beta$ -sitosterol which formed only 01 hydrogen bond with SARS-CoV-2 M<sup>pro</sup>.

As an extension to this published research work, we have investigated the role of  $\beta$ -sitosterol as immunostimulant, antioxidant and inhibitory potential against receptor binding domain (RBD) of SARS-CoV-2 Spike Glycoprotein with the aid of molecular docking. There are many studies which reveals the antioxidant and immune boosting role of  $\beta$ -sitosterol especially in viral infection including pneumoniae. This commentary emphasized on further potential of  $\beta$ -sitosterol in treatment of COVID-19 through molecular docking studies.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel, zoonotic, positive-sense [2], single-stranded RNA beta-coronavirus [3] (sub-genus *Sarbecovirus*, sub-family *Orthocoronaviridae*) [4]. The disease caused due to SARS-CoV-2 is termed as COVID-19 [5]. Almost every country of the world is now affected by SARS-CoV-2 infection. The World Health Organization (WHO) declared it a Public Health

Emergency of International Concern on January 30, 2020, and on February 28, it upraised the worldwide threat of COVID-19 to the utmost level [6]. A global pandemic was declared on March 11, 2020 [7]. The N, E, M, and S proteins are the four structural proteins encoded by SARS-CoV-2 [8]. This S protein of SARS-CoV-2 i.e. SARS-CoV-2 spike glycoprotein causes invasion to the host cell after binding with angiotensin converting enzyme-2 (ACE-2) [9]. The SARS-CoV-2 spike glycoprotein is cleaved into two subunits during entry [10]. The S1 subunit contains a receptor binding domain (RBD) and attaches to ACE-2 [11]. The S2 subunit then facilitates membrane fusion [12-14]. Therefore, we have targeted RBD of spike glycoprotein and performed molecular docking studies of  $\beta$ -sitosterol to find out its inhibitory potential of SARS-CoV-2.

### **$\beta$ -sitosterol as Immunostimulant, as Antiviral and as Antioxidant**

Cheng et al. investigated the effects of dietary  $\beta$ -sitosterol at different levels on serum lipid levels, immune function, oxidative status, and intestinal morphology in broilers. They have concluded that dietary  $\beta$ -sitosterol supplementation could regulate serum cholesterol level, promote immune function, and improve intestinal oxidative status and morphology in broilers [15]. Fraile et al. reported that  $\beta$ -sitosterol can be considered an immunomodulator in pigs [16]. Bouic and Lamprecht reported that this phytosterol complex seems to target specific T-helper lymphocytes, the Th1 and Th2 cells, helping normalize their functioning and resulting in improved T-lymphocyte and natural killer cell activity. The re-establishment of these immune parameters may be of help in numerous disease processes relating to chronic immune-mediated abnormalities, including chronic viral infections, tuberculosis, rheumatoid arthritis, allergies, cancer, and autoimmune diseases [17]. Bouic et al. concluded that phytosterols could be used to prevent the subtle immunosuppression associated with excessive physical stress [18]. There are many studies which have reported the immunostimulant activity of the phytosterols [19-24].

Li et al. reported that  $\beta$ -sitosterol is a candidate for the development of anti-virulence agents against pathogens that rely on cholesterol-dependent toxins for successful infections [25]. Zhou et al. reported that  $\beta$ -sitosterol blocks the immune response mediated by RIG-I signaling and deleterious IFN production, providing a potential benefit for the treatment of influenza [26]. Parvez et. al. reported the antioxidative and hepatoprotective efficacy of *G. senegalensis* leaves extract. HPTLC analysis of  $\beta$ -amyrin,  $\beta$ -sitosterol, lupeol and ursolic acid strongly supported the anti-HBV efficacy of GSLE via abating the cellular oxidative stress molecules [27]. There are many studies that have reported the antioxidant activity of  $\beta$ -sitosterol [27-34].

### **Molecular Docking Studies of $\beta$ -sitosterol with RBD of SARS-CoV-2 Spike Glycoprotein**

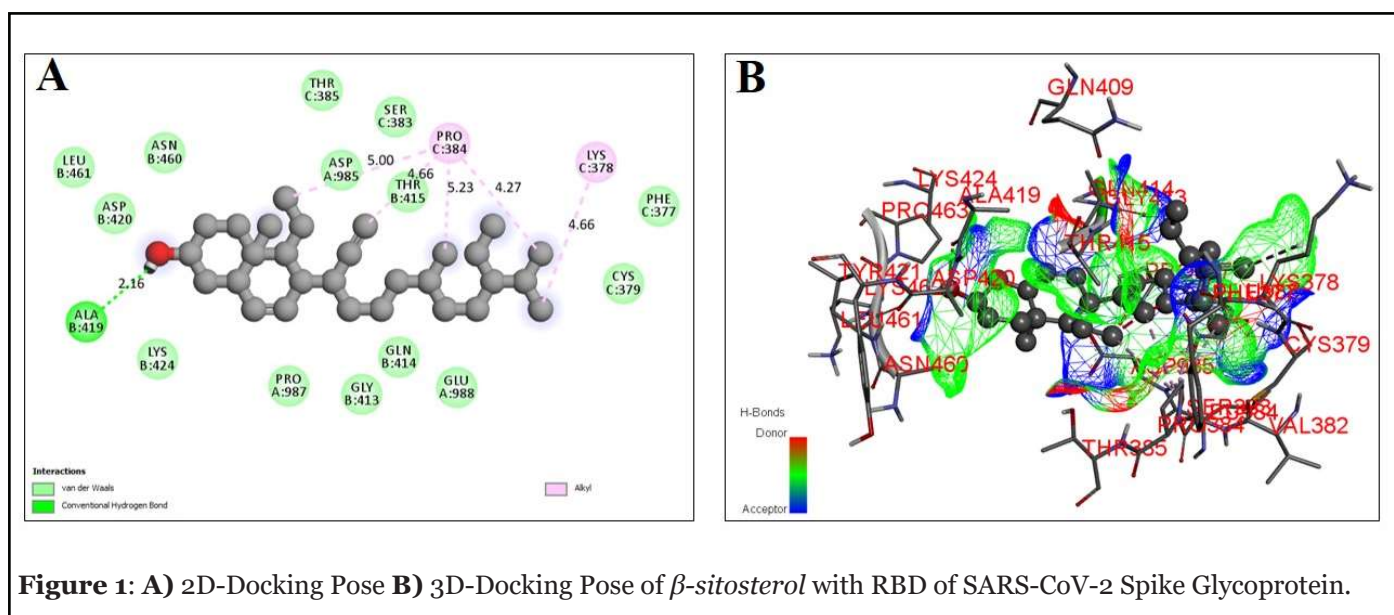
Autodock vina 1.1.2 in PyRx-Virtual Screening Tool 0.8 were used to perform the docking studies [35]. The active amino acid residues in the protein were identified and noted using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287) [36]. The complete docking procedure along with ligand preparation and target preparation have been performed as described in the reference article [1]. The recently elucidated structure pre-fusion 2019-nCoV (SARS-CoV-2) spike glycoprotein with a single receptor-binding domain up was obtained from the RCSB Protein Data Bank (PDB ID: 6VSB) which was released on 26 February 2020 (<https://www.rcsb.org/structure/6VSB>) [37]. RBD from SARS-CoV-2 spike glycoprotein was identified from the official website of Protein Data Bank in Europe (EMBL-EBI) (<https://www.ebi.ac.uk/pdbe/entry/pdb/6vsb>). There were three sequence domains in the 6VSB crystal structure; Spike receptor binding domain; Spike glycoprotein N-terminal domain; Coronavirus spike glycoprotein S1, C-terminal, along with three chains in the structure (Chain A, B, C). For molecular docking simulation, the three-dimensional grid box (size\_x = 76.1623A<sup>o</sup>; size\_y = 84.3011A<sup>o</sup>; size\_z = 62.4413A<sup>o</sup>) was designed (to define area for interactions) around the RBD (to occupy) using Autodock tool 1.5.6 with exhaustiveness value of 8 [35,36].

### **Results and Discussion**

RBD comprises of amino acid residues from chain A, chain B, and chain C as well, therefore, the most potent inhibitor will be the one which interacts with amino acid residues from all the chains.  $\beta$ -sitosterol has showed a binding affinity of 7.8 kcal/mol with 0 RMSD lower and upper bound. The 2D- and 3D-Docking poses of the  $\beta$ -sitosterol represented in figure 1A & 1B respectively. It formed one hydrogen bond with Ala-B:419 with bond length of 2.16A<sup>o</sup>.  $\beta$ -sitosterol has formed five alkyl bonds with Pro-C:384 (5.0A<sup>o</sup>, 4.66A<sup>o</sup>, 5.23A<sup>o</sup>, 4.27A<sup>o</sup>) and with Lys-C:378 (4.66A<sup>o</sup>). It also shows van der Waals attraction with Thr-C:385, Ser-C:383, Asp-A:985, Thr-B:415, Phe-C:377, Cys-C:379, Glu-A:988, Gln-B:414, Gly-B:413, Pro-A:987, Lys-B:424, Asp-B:420, Leu-B:461, Asn-B:460. As  $\beta$ -sitosterol is interacting with amino acids from every chain (A, B, C), it indicates that it is a potent inhibitor of RBD of SARS-CoV-2 spike glycoprotein. The interacting residues, bond length, and binding affinity are represented in table 1.

### **Conclusion**

Currently, there is no specific treatment available for SARS-CoV-2 infection. Use of immunostimulants, antivirals, and antioxidants can help to reduce the risk of COVID-19. Literature supports the immunostimulant, antiviral, and antioxidant activity of the  $\beta$ -sitosterol.



**Figure 1:** A) 2D-Docking Pose B) 3D-Docking Pose of  $\beta$ -sitosterol with RBD of SARS-CoV-2 Spike Glycoprotein.

Name of Molecule	Binding Affinity (kcal/mol)	Types of Bond	Active Amino Residues with Bond Length (Å°)
$\beta$ -sitosterol	-7.8	Hydrogen Bond	Ala-B:419 (2.16Å°)
		Alkyl Bond	Pro-C:384 (5.0Å°, 4.66Å°, 5.23Å°, 4.27Å°), Lys-C:378 (4.66Å°)
		Van Der Waals	Thr-C:385, Ser-C:383, Asp-A:985, Thr-B:415, Phe-C:377, Cys-C:379, Glu-A:988, Gln-B:414, Gly-B:413, Pro-A:987, Lys-B:424, Asp-B:420, Leu-B:461, Asn-B:460

**Table 1:** Binding Affinity (kcal/mol), types of bond and active amino acid residues with bond length (Å°).

Also, molecular docking studies have found very good binding affinity of  $\beta$ -sitosterol with RBD of SARS-CoV-2 spike glycoprotein which can restrict the viral invasion into the host cell. It has formed one hydrogen bond with Ala-B:419, which is good for better inhibition. From present commentary, we have concluded that  $\beta$ -sitosterol can be used to enhance immunity against the SARS-CoV-2 infection as well as to restrict the viral invasion into the host cell through angiotensin converting enzyme-2 (ACE-2) by inhibiting spike glycoprotein. If we can increase the dietary intake of  $\beta$ -sitosterol and other phytosterols it can modulate the immunity which is today's need to face COVID-19.

### Conflict of Interest

The authors have no conflicts of interest.

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