

## BIOTECHNOLOGY APPROACH TO PERFORM A COMPUTATIONAL MODEL OF BEE VENOM AGAINST COVID-19

By

EMAN ALAAELDIN ABDELFATTAH<sup>1</sup>, MAMDOUH I. NASSAR<sup>1\*</sup>,  
SOHA M. MOHAMED<sup>2</sup>, DINA H. ABD EL-MONEM<sup>1</sup>, SAMAR M. IBRAHIM<sup>3</sup>,  
MAI YOUSSEF<sup>4</sup> AND HADEER OSAMA FATHY<sup>4</sup>

Department of Entomology<sup>1</sup>, Faculty of Science, Hospital Misr University for Science and Technology<sup>2</sup>, Micro-analytical Center<sup>3</sup> and Biotechnology Program<sup>4</sup>, Faculty of Science, Cairo University<sup>1,3,4</sup>, Giza, P.O. Box 12613, Egypt

(\*Correspondence: mamdouh@sci.cu.edu.eg or mmnassar2002@yahoo.com  
ORCID: 0000-0002-4624-740X)

### Abstract

Arthropod venoms have multiple effect on antiviral and antibacterial infection especially, bee venom (BV), which has a specific character among other due to its ant-proliferative potential, antiviral, antimicrobial and antitumor activity. Venom-based drugs as Captopril<sup>®</sup> & Ribavirin<sup>®</sup> are used as antiviral and anticancer drug. Empathetic the action mechanism of venom-peptides increase the drug specificity against virus cells. A new coronavirus (SARS-CoV-2) leads to an incipient coronavirus disease (COVID-19). COVID-19 virus belongs to genus *Betacoronavirus* virus that includes MERS-CoV & SARS-CoV. The study evaluated venom derivatives and antiviral drugs against COVID-19 envelope protein (EP) and spike protein (SP) sequences. In this study, sequence analysis, protein modeling, & molecular docking were used for COVID-19 EP, and SP, to build a model of testing venom derivatives and antiviral drugs against COVID-19 EP, and SP. Approved drugs includes Remdesivir<sup>®</sup>, Ledipasvir<sup>®</sup> (antiviral drugs), Captopril<sup>®</sup>, & Ribavirin<sup>®</sup> (as venom antiviral drug derivatives).

**Key words:** COVID-19, Venom antiviral, Coronavirus, Multiple sequence alignment, Molecular docking, Protein modeling.

### Introduction

Bee Venom (BV) which extracted from honeybee (*Apis mellifera L.*) contains biochemically active compounds such as enzymes, and polysaccharides mixtures. The commonest antimicrobial peptide is melittin (C<sub>131</sub>H<sub>229</sub>N<sub>39</sub>O<sub>31</sub>). Inhibition of free radicals especially superoxide anion radicals leads to decrease oxidative stress action on host cells (Abdelfattah *et al*, 2017). Mansour *et al.* (2021) reported that the therapeutic properties of bee venom (BV) and its active component, melittin (Mel), made them suitable candidates as potential anti-cancer agents or as adjuvants for cancer chemotherapy. They added that both BV & Mel have a synergistic anticancer effect with Sorf on HepG<sub>2</sub> that represent a new enhancing strategy for hepatocellular carcinoma treatment. It induced degranulation of neutrophil, and was used as a potent anti-inflammatory agent (Mousavizadeh and Ghasemi, 2021).

The new human  $\beta$ -corona-virus (COVID-19) is a coronavirus disease of the worldwide distribution. WHO (2020) added that it is a spherical virus compacted with an envelope coated positive the single strand RNA (ssRNA). RNA linked with a nucleoprotein covered with a matrix protein capsid. From taxonomic point of view it is similar to the severe acute respiratory human virus or SARS HCoV (Chan *et al*, 2015). HCoVs are single-stranded positive RNA viruses with two characteristic protein groups; structural and non-structural proteins. Structural protein includes spike (S), nucleocapsid (N), matrix (M) and envelope (E), but the non-structural one includes RNA dependent RNA polymerase or RdRp (Elfiky *et al*, 2017). Spike protein is the most virus effective part, and the principle goal for deactivating antibodies produced by the infected host's immune system. Spike protein is divided into two functional subunits:

Globular S1 subunit, & S2 subunit. S1 is responsible in receptor recognition, but S2 subunit-participates in membrane fusion facilitation. Corona-virus needs the spike protein to infect the host cell that binds to a host cell receptor called the angiotensin-converting enzyme 2 (ACE2) on surface, and fuses with host cell membrane and release its genetic material into cell (Hofmann *et al*, 2004).

In severe acute respiratory syndrome corona-virus, and Middle Eastern respiratory syndrome coronavirus (MERS-CoV), envelope protein has a short, hydrophilic amino-terminus consists of 7-12 amino acids (van Regenmort *et al*, 2000). Thus, with a large hydrophobic transmembrane domain (TMD) of 25 amino acids, and ends with a long, hydrophilic carboxyl terminal group that contains the majority of protein (Schoeman and Fielding, 2019). RdRp is the essential enzyme in the life cycle of RNA coronaviruses. It has an active site with two highly conserved sequential aspartate residues protruding from a beta-turn structure making surface very nearby via nucleotide channel (Ganesan and Barakat, 2017). Lasioglossins extracted from the eusocial bee venom, *Lasioglossum*

#### Materials and Methods

**Multiple sequence alignment:** The first available full genome COVID-19 sequence was recovered from the National Center for Biotechnology Information (NCBI) nucleotide database (NCBI, 2020). The multiple sequence alignments of envelope protein (EP) sequences and spike protein (SP) sequences in three  $\beta$ - Human Corona virus (HCoV) strains (Middle East respiratory syndrome (MERS), Severe acute respiratory syndrome 1 (SARS), and Severe acute respiratory syndrome 2 (Covid-19) were done using Clustal Omega (McWilliam *et al*, 2013).

**Protein modeling:** Swiss Model web server is used to build the EP & SP model (Bienert *et al*, 2014). NMR structure of SARS coronavirus E- protein (PDB Chain Id: A, B, C, D & E) used as a model sequence with COVID-19 EP. While, electron microscopy

*lossum laticeps* has anti-microbial activity (Nassar and Elzayat, 2019). Also, Captopril<sup>®</sup> used as anti-hypertensive drug is an analogue of dipeptide Ala-Pro and effectively bind to the angiotensin-converting enzyme (ACE) active site (Russell *et al*, 2004). The FDA has approved antiviral drugs as Sofosbuvir<sup>®</sup>, and Ribavirin<sup>®</sup> for HCV treatment (Doublet and Ellenberger, 1998). They are nucleotides derivative competing with physiological SP active site and nucleotide for RdRp active site (Yang *et al*, 2011). Ribavirin<sup>®</sup> showed that the ( $EC_{50}$ ) against Covid-19 was 109.5 $\mu$ M, and its ( $IC_{50}$ ) against Dengue fever virus was 8 $\mu$ M (Markland *et al*, 2000). Remdesivir<sup>®</sup>  $EC_{90}$  *in vitro* was 1.76  $\mu$ M for COVID-19 (Nassar *et al*, 2020; Wang *et al*, 2020).

This study aimed to perform and build the COVID-19 spike, and envelope model by comparing available sequence structures in the protein data Bank, as well as molecular docking modeling of antiviral drugs; Ledipasvir<sup>®</sup> & Remdisvir<sup>®</sup> as well as bee venom derivatives drugs; Captopril<sup>®</sup> & Ribavirin<sup>®</sup> against Covid-19 Spike and envelope proteins.

structure of SARS-protein (PDB Chain Id: A, B, & C) was used as a template and shares sequence of SP COVID-19. The molprobit web server of Duke University, and the structure analysis and verification server (SAVES) of the California University, Los Angeles (UCLA) tested these models (Williams *et al*, 2018, SAVES, 2020). To test models validity, verify 3D (Eisenberg *et al*, 1997), ERRAT (Hooft *et al*, 1996), Prove (Pontius *et al*, 1996), and Pro-check (Laskowski *et al*, 1996) web apps confirmed their validities. After the validation step the computational chemistry workspace scigress was done to complete experiments of molecular docking (Elfiky and Elshemey, 2018).

**Molecular docking:** The molecular docking was used for initial prediction of receptor (protein)-ligand (drug) molecular recognition binding (Dar and Mir, 2017). Docking test performed the efficacy of six different co-

mpounds (Uracil triphosphate (UTP), Cinnamaldehyde, Remdisivir, Iedipasvir, Captopril, and Ribavirin) for Covid-19, SARS E P & SP by using the Auto-Dock software (VINA, 2010), implemented in Scigress (Trot and Olson, 2010). Ribavirin was used as antiviral (Gane *et al*, 2013) and captopril was used as competitive inhibitor of angiotensin-converting enzyme (ACE). Thus, it was used as an anti-hypertension (Smith and Vane, 2003). Remdisivir was used to treat coronavirus infection (Warren *et al*, 2016). But, Cinnamaldehyde was used as negative control drug. After docking, the structures were tested by using the protein-ligand interaction profiler (PLIP) web server (Technical University of Dresden), and tabulated for comparison (Salentin *et al*, 2015).

### Results

Results were given in figures (1, 2, 3, & 4).

### Discussion

In this study, sequence identity between the Covid-19 and SARS in both spike and Ribavirin bind to Covid-19 spike and envelope protein, with binding affinity of -3.9, -5.8, -8.7, & -6.4 kcal/mol for envelope protein, and -5.3, -6.4, -7.2, & -6.3kcal/mol respectively for spike protein. These antivirals venom-based drugs could decrease binding affinity of new strain coronavirus to human host cells. Envelope proteins sequences were high-quality model. Multiple sequence alignment (MSA) of spike and envelope proteins from different strains of  $\beta$ - human coronavirus (MERS) caused severe acute respiratory syndrome of Covid-19 & SARS. Red highlights indicated identical residues, secondary structures at top of PDB for SARS envelope protein (BMRB: 19845). While the surface accessibility at bottom (blue: highly accessible, but white was buried). Multiple alignment sequence showed that identity of Covid-19 spike protein sequence was 77.38% & 31.93% for SARS and MERS respectively. Identical sequence of Covid-19 envelope protein was 96% & 35% for SARS and MERS respectively. Sequence identity of complete nucleotide ge-

nome of Covid-19 was 79.81% with SARS & 54.19% with MERS. The Covid-19 of spike and envelope protein model by Swiss Model web server, showed a very high model (99.50%, & 91.38%) sequence identity for spike protein & envelope protein, respectively. Ramachandran plot showed that model validity was accepted, and Mol-Probity score was 1.26, & 1.98 for spike and envelope proteins respectively.

After check of transition state, optimization step was formed using the density functional theory (DFT) quantum mechanics (Stewart, 2007). The molecular docking experiments was performed using Auto-Dock software with A grid box (25Å×25 Å×25Å) centered at (26: 42: 25) Å & (34: 22: 19) Å for Covid-19 spike protein & envelope protein respectively, and centered at (28: 51: 27) Å and (32: 20: 16) Å for SARS spike and envelope proteins respectively. The docking score values for Covid-19 & SARS showed that physiological compounds (ATP) binding affinity for Covid-19 was -8.6 & -9 kcal/mol in envelope and spike proteins respectively. Negative control one gave low binding affinity (-6.2, & -6.1kcal/mol) in envelope and spike proteins respectively. The post molecular docking analysis ensured the binding mode to Covid-19 spike protein and envelope protein. Differences in binding affinity, and docking complexes were checked using PLIP web server. Interactions between ligands UTP, Ribavirin, Captopril, Remdisivir, and Iedipasvir against Covid-19 envelope protein were shown in spike protein after docking.

Based on the binding affinity after minimize the energy of each test drug, the best ligands in descending order were Iedipasvir (-8.7kcal/mol), Ribavirin (-6.4kcal/mol), Remdisivir (-5.8 kcal/mol), and Captopril (-3.9kcal/mol). Moreover, Iedipasvir caused highest binding affinity to the Covid-19 spike protein (-7.2kcal/mol, followed by Ribavirin (-6.3 kcal/mol), then Remdisivir (-6.4kcal/mol), and lastly Captopril (-5.3kcal/mol).

## Conclusion

The four bee venom derivatives Covid-19 drugs (Captopril, Remdesivir, Iedisivir, & Ribavirin) bind to Covid-19 spike and envelope protein. The ongoing molecular docking to newly emerged antimicrobial peptides from bee venom by high-quality model of Covid-19 might give a feasible vaccine

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### Explanation of figures

Fig. 1: Multiple sequence alignment of  $\beta$ - Human corona virus (HCoV) strains (MERS, Covid-19 & SARS) arranged respectively from first to third line, Envelope protein sequences(a) and spike protein sequences(b) (from 1 to 170). Red highlights indicate identical residues, secondary structures at top of PDB for SARS envelope protein (BMRB: 19845), surface accessibility at bottom (blue: highly accessible while white is buried). Alignment by using Clustal omega web server and represented by ESript 3.

Fig. 2: Newly emerged Covid-19 Envelope protein model (a) Spike protein model built by Swiss Model. II. Ramachandran plot for Covid-19 envelope, and Spike protein model (b) Spike protein model built by Swiss model I. (color in figure legend, referred to article).

Fig. 3: Binding Affinity calculated by AutoDock Vina for Cinnamaldehyde (-ve control), UTP (as a physiological nucleotides), Remdesivir, Ledipasvir (Antivirals), Captopril, and Ribavirin (venom antiviral drug derivatives) against Covid-19 and SARS HCoV envelope protein (a) and spike protein (b)

Fig. 4: Overall 3D structure complexes: Protein in green cartoon for envelope protein (a), cyan color cartoon for spike protein (b) and ligands in red. Interactions established after docking four drugs against Covid-19 envelope protein, and spike protein.



