EXPERIMENTAL WORKS

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COMPUTATIONAL INVESTIGATION OF HONEYBEE VENOM PROTEINS AS POTENTIAL OMICRON SARS-CoV-2 INHIBITORS

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Because of the catastrophic consequences of COVID-19 on the world population, there should be novel interventions to handle ongoing infections and daily death cases. The aim of the current study is to examine the effectiveness of HBV (Honeybee venom) proteins on spike protein RBD by in silico tools. The sequences of 5 HBV proteins were used for homology modeling by Phyre 2. The generated protein models were employed for protein-protein docking against Omicron Spike glycoprotein receptor binding domain (RBD) (PDB ID# 7T9L) through HDock and ClusPro platforms followed by prediction of binding affinity using PRODIGY web portal and PDBsum for revealing interaction details. It was found that all of the examined HBV proteins exhibited strong docking scores and binding affinity profiles toward RBD. The findings of the present study indicate the possible HBV as preventive as well as treatment options against Omicron SARS-CoV-2.

K e y w o r d s: COVID-19, SARS-CoV-2, RBD, Honeybee venom, docking.

As of July 13th, 2022, over 550 million cases of coronavirus disease 2019 (COVID-19) have been confirmed, of which around 6 million deaths globally [1]. The admitted patients with COVID-19 suffer from varying degrees of severity and duration of fever, fatigue with or without breathlessness [2]. Elderly people have been found to be at higher risk of infection as well as mortality rate due to their weak immune function [3]. The causative agent of the disease is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Once SARS-CoV-2 invades the host and reaches lung tissues, it must get its spike protein cleaved by host type-II transmembrane serine proteases (TTSPs) specially, TMPRSS2 prior to its binding to angiotensin-converting enzyme 2 (ACE-2) receptor. It is now recognized that TMPRSS2 is the major determinant of viral infectivity [4] whose blockade can prophylactically inhibit viral attachment to cells.

Because it is an RNA virus, the mutation rate is very high. This accounts for the development of 5 variants up to date. Alpha (B.1.1.7): the first variant of concern first reported in the United Kingdom (UK), Beta (B.1.351): first described in South Africa, Gamma (P.1) in Brazil, Delta (B.1.617.2) in India, and the last current variant Omicron (B.1.1.529) again first declared in South Africa in late November 2021 [5]. Interestingly, there are now sub-lineages of Omicron characterized by fast transmission and lower fatality most seen in the US and Europe [6].

The big pharma companies are racing for the development and subsequent huge production of the appropriate vaccine toward the corresponding emerging variants. Nonetheless, the genetic evolution observed in SARS-CoV-2 is faster than vaccine-manufacturing process. Moreover, the hesitancy of vaccine production puts individuals at risk of safety issues albeit there is a rise (71%) in vaccine approvals nowadays within society compared to past years [7]. This motivates scientists to explore other strategies to control the COVID-19 spread. Among which is the blockade of the binding of spike protein to its receptor ACE-2 [8-10].

During the genetic evolution SARS-CoV-2 was subjected, different mutations have been detected in the spike protein-coding gene of all the face variants. However, the Omicron variant got the largest percentage [11] of mutations on its spike protein [12]. Recent evidences suggested the possibility of reinfection by Omicron owing to (i) higher binding affinity toward ACE-2 and (ii) the sophisticated escape abilities of the virus and (iii) increased resistance to neutralizing antibodies [13-15].

Spike protein of SARS-CoV-2 serves dual functions, i.e. anchors the envelope of virion and on the other hand mediates its attachment to ACE-2 [16]. To ensures the dual function, spike protein is a large complex (1237 amino acids) comprised of 3 segments, intracellular C-terminal, transmembrane domain and extracellular N-terminal segments [17]. The receptor binding domain (RBD) resides in the N-terminal region of spike protein (Fig. 1) [18].

Honeybee venoms (HBV) are a complex mixture that now are used to treat different maladies such as rheumatoid arthritis, chronic pain, amyotrophic lateral sclerosis and Alzheimer's disease, among others [19, 20]. This is attributed to the anti-inflammatory of the venom enzymes and peptides. Recent reports suggest the exploitation of HBV as a complementary therapy option for COVID-19 [21] as demonstrated its possible benefit toward Ebola virus [22].

So, the purpose of this work is to examine the blocking efficacy of HBV peptides against RBD of Omicron spike protein via *in silico* docking platforms.

Materials and Methods

Design of study. The methodology of this work has multi-step approaches each employing different programs and webservers to increase the accuracy of results, get more information and in-depth analysis and discussion. The workflow of this study is represented in Fig. 2.

Homology modeling. 5 HBV enzymes and peptides amino acid sequences were accessed via UniProt database [23], namely, phospholipase A2 (PLA2, UniProt ID: P00630), apamin (UniProt ID: P01500), mast cell-degranulating peptide (MCDP, UniProt ID: P01499), melittin (UniProt ID: P01501) and secapin (UniProt ID: I1VC85) having 167, 46, 50, 70 and 77 amino acids. The obtained FASTA sequences were used for homology model HBV using Phyre2 web portal [24]. The best models then were refined exploiting the Galaxyrefine 2 server (refined models are shown in Fig. 3) [25]. Afterward, the refined models were used for docking studies after checking the refined conformation through PROCHECK web portal [26].

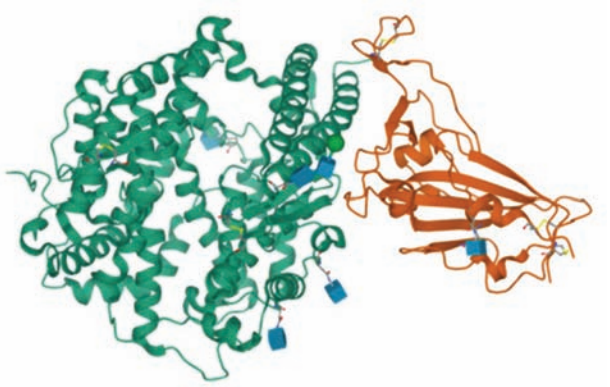

Fig. 1. Crystal structure of SARS-CoV-2 Omicron spike receptor-binding domain in complex with ACE2 (PDB ID: 7WSK) [18]

Protein-protein docking. The crystal structure of Omicron spike protein RBD was retrieved from protein data bank (PDB) using the PDB ID: 7T9L. The receptor was prepared prior to docking by removing the ACE-2 chain and other hetero-molecules using Discovery studio client 21 software [27]. Then, the docking study was performed by the online tools HDock [28] and ClusPro [29]. In each platform, the RBD was assigned as the receptor while the HBV proteins were uploaded as the ligand and the docking parameters were kept as default.

Post-docking analysis and visualization. The docking output of HDock as well as ClusPro servers were uploaded to PRODIGY web portal [30] to calculate PPI binding energy and other interaction parameters. Furthermore, by PDBsum webserver (http://www.ebi.ac.uk/pdbsum) the bonded and non-bonded interactions were detected. The 3D structures of the docked proteins were visualized through PyMOL [31] software.

Results

Homology modeling. Prior to protein-protein docking, homology modeling of the examined HBV proteins was performed in order to get the most favorable conformation of the selected proteins. We used Phyre 2 server for homology modeling, followed by refinement by Galaxyrefine 2 webserver and the best-refined models were checked for suitability via PROCHECK web portal. The best

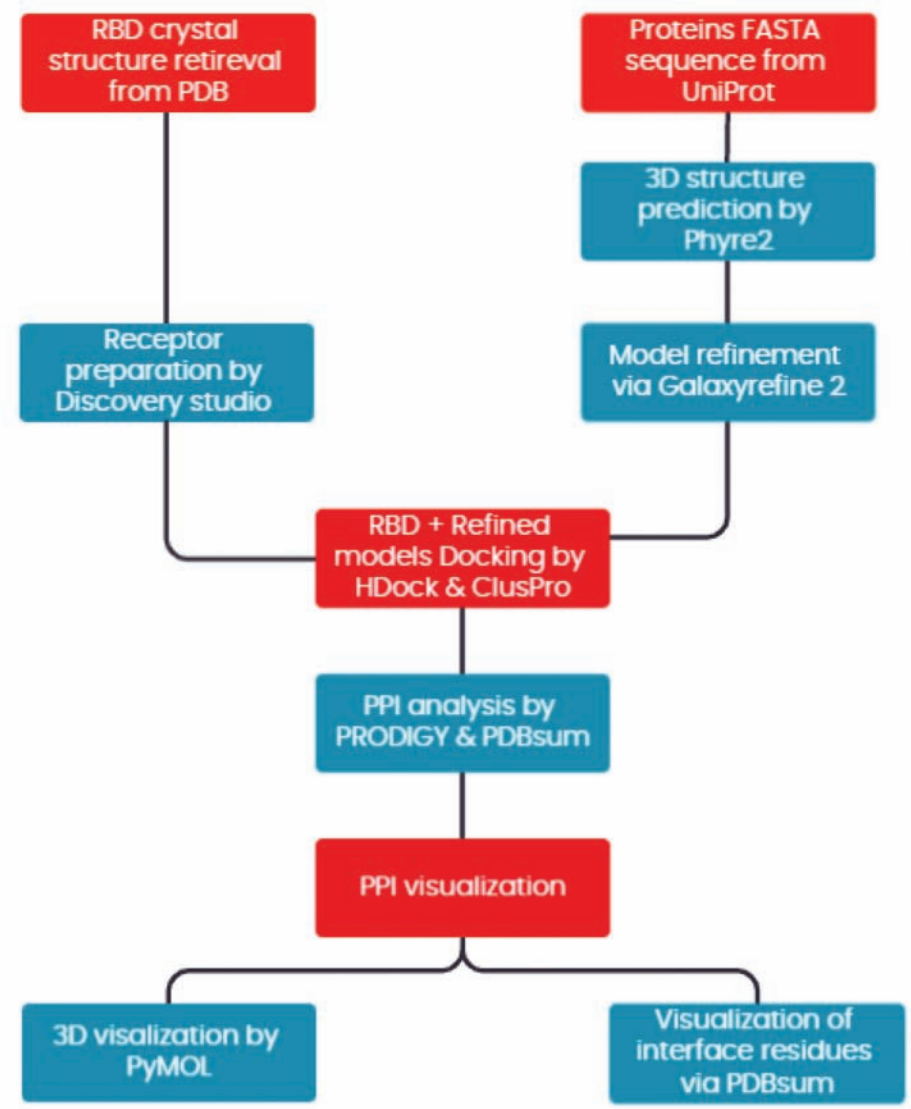

Fig. 2. Schematic flowchart representation of the methodology followed by this study. PPI: protein-protein interaction

refined models are elucidated in Fig. 3. However, the verified PROCHECK results are available in the Supplementary File.

After modeling HBV proteins and peptides, protein-protein docking was done via 2 servers, namely HDock and ClusPro.

The HBV proteins and peptides docked well to the Omicron RBD as reflected by the high docking score and free energy of binding (Table 1). In HDock server, the docking score ranged from -243 (for apamin) to -295 (for melittin). Nonetheless, the binding affinity of PPI calculated via PRODIGY web portal revealed different results. MCDP was the top-ranked in terms of binding affinity (-11.5 kcal/mole) while secapin was the lowest (-8.1 kcal/mole). The docking score of ClusPro matches closely that of HDock with the exception of PLA2 whose docking score in ClusPro was -838 kcal/mole but had the best ΔG value (-13.0 kcal/mole). Overall, HBV proteins showed strong binding affinity toward Omicron spike protein RBD which candidate themselves as significantly potent neutralizing inhibitors for SARS-CoV-2 Omicron spike protein.

Table 2 confirms the results of docking and free energy of binding represented in Table 1 with some details. Secapin had the least interface residues (18:10) which corresponded to the least docking results while PLA2 together with melittin showed the highest number of interface residues which account for the higher binding affinity of the two proteins.

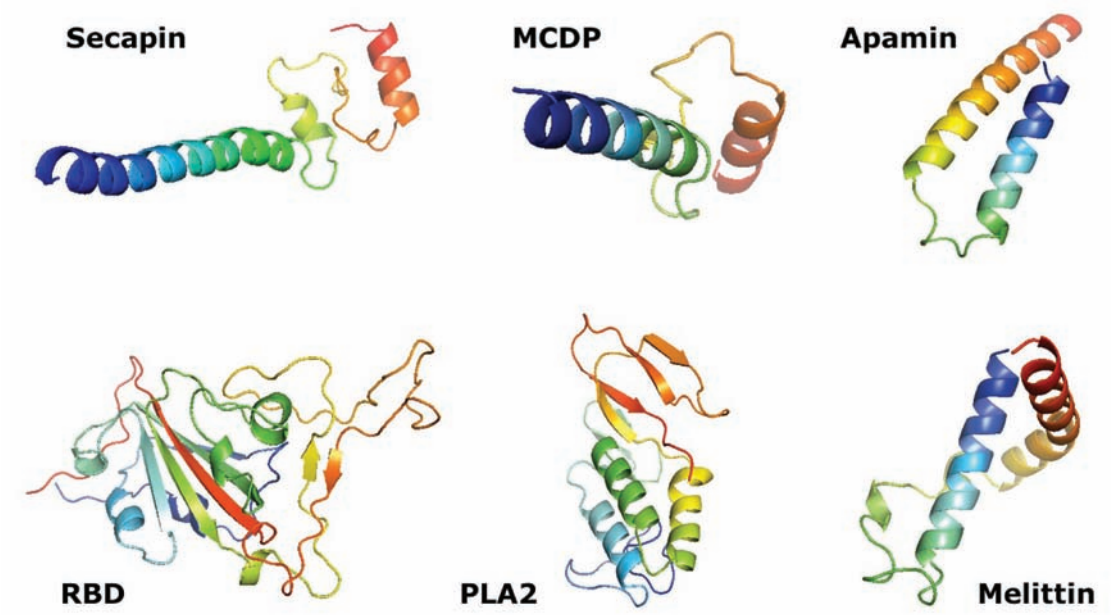

Fig. 3. Three-dimensional structure of tested HBV and the RBD of Omicron SP RBD

Table 1. Docking score and binding free energy of the docked HBV proteins to Omicron RBD

Ligand protein	HDock score	ΔG (kcal/mole)	ClusPro score	ΔG (kcal/mole)
Apamin	-243.76	-9.8	-814.6	-12.4
Melittin	-295.18	-10.9	-997.8	-9.1
MCDP	-287.66	-11.5	-955.5	-11.2
Secapin	-245.52	-8.1	-925.2	-9.0
PLA2	-295.12	-10.6	-838.5	-13.9

Table 2. Detailed interaction characters of PPI between HBV proteins and Omicron RBD calculated via PDBsum

Character	Apamin	MCDP	Melittin	Secapin	PLA2
No. of interface residues	18:15	18:14	22:14	18:10	22:15
Salt bridges	3	0	0	0	0
H-bonds	3	7	0	4	5
Non-bonded contacts	117	129	188	133	177

Apamin was the only HBV protein which formed salt bridges with the receptor. This can be attributed to the presence of charged residues on its surface. Although melittin exhibited strong binding affinity toward receptor, all of the bonding types were hydrophobic (no H-bonds nor salt bridges). MCDP formed 7 H-bonds with Omicron RBD followed by PLA2 (5 H-bonds) which account for the high binding energy of the two proteins. The docked models were represented in Fig. 4. As shown in Fig. 4, all the proteins of HBV bind RBD precisely at the binding site and form many H-bonds in common and salt bridges (only apamin) but most of the free energy of binding comes from non-bonded contact (hydrophobic interactions).

Discussion

Currently, traditional RNA vaccines and small organic anti-viral inhibitors are no longer enough to get viral pandemics under control due to the continual emergence of new variants resistant to the previously employed therapeutics [32]. Therefore,

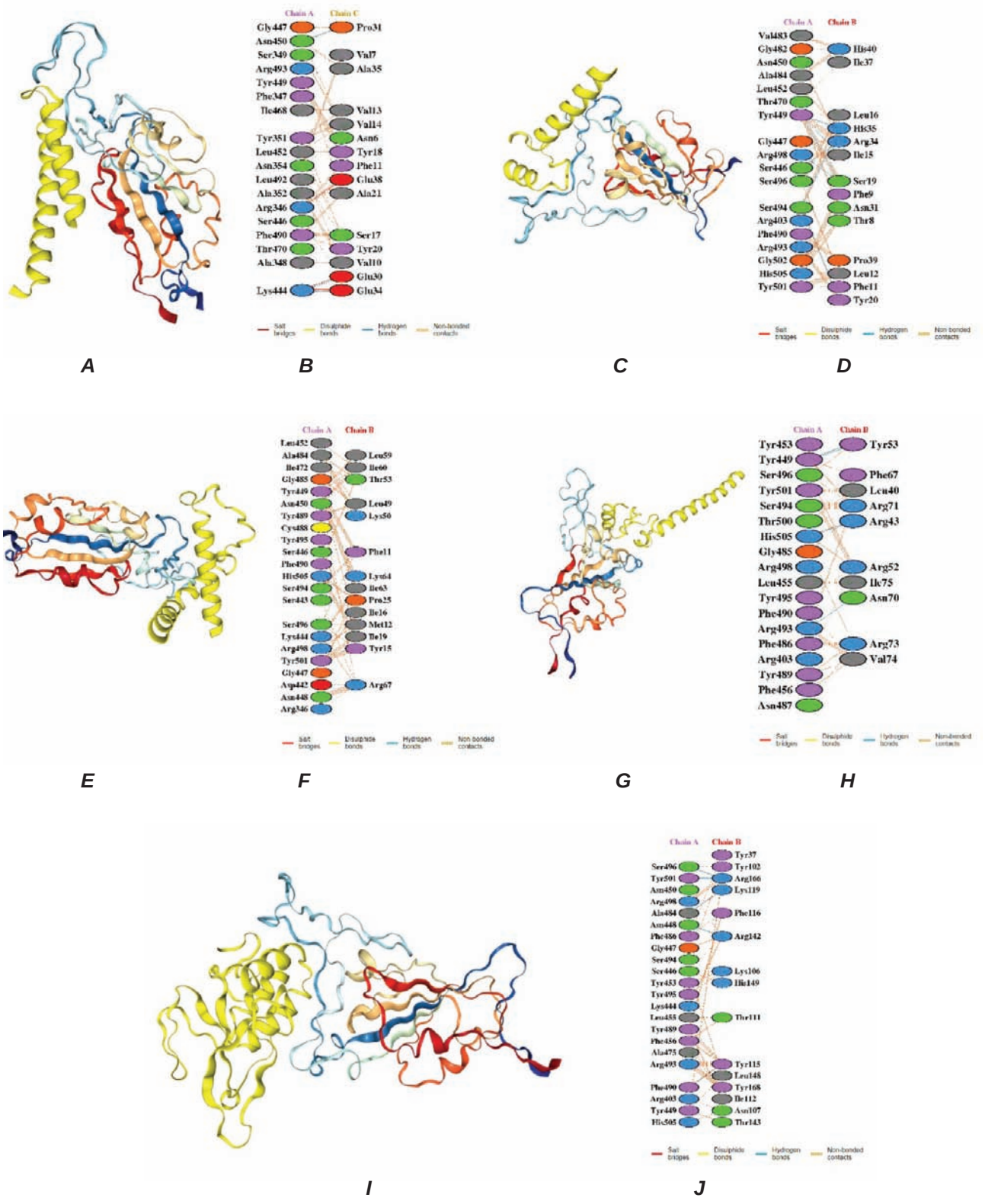

Fig. 4. 3D visualization of PPI between HBV proteins and Omicron RBD. HBV proteins are depicted in yellow whereas RBD is in rainbow. ***A***, ***C***, ***E***, ***G*** *&* ***I*** *represent the PPI of apamin, MCDP, melittin, secapin & PLA2 with spike protein RBD in 3D whilst* ***B***, ***D***, ***F***, ***H*** *&* ***J*** *illustrate the interacting residues at the interface of the corresponding complex*

natural peptides and proteins proved their efficacy in certain types of viral infections and deserve their investigation against COVID-19.

Bansal et al. [33] examined the capability of microbial non-ribosomal proteins to block the interaction between Spike glycoprotein with ACE2. They suggested Dactinomycin and Gramicidin S strongly bind to Spike protein with a binding affinity − 12.4 kcal/mol and − 11.4 kcal/mol, respectively. Similarly, Fakih [34] explored the natural antiviral peptide dermaseptin produced from the frog *Phyllomedusa* against Spike glycoprotein via protein docking approach. −792.93 kJ/mole was the docking energy via HPEPDOCK tool. In addition, He also tested the possibility of dermaseptin peptide, bounded to Spike glycoprotein, attachment to ACE2 receptor. Because the docking score was positive (517 kcal/mole), he concluded that dermaseptin peptide destabilized Spike protein-ACE2 interaction. The peptide LDAVNR derived from *S.maxima* exhibited significant binding and interaction energy (−113.456 kcal/mol and −71.0736 kcal/mol respectively) to target Spike protein of COVID-19 [35]. It is evidenced that HBV enhances both cellular and humoral immune systems. Moreover, HBV has been used for the management of respiratory and neurological disorders. Vaccination with HBV immunizes individuals against viral infections such as cytomegalovirus [21]. HBV proteins demonstrated *in silico* inhibition of Ebola virus Spike protein of the native and mutant type. Among all HBV proteins screened, PLA2 gave the strongest inhibition suggesting its antiviral effectiveness [22].

The binding affinity of Omicron RBD to its receptor (angiotensin-converting enzyme 2 (ACE2)) was found to be -11.8 kcal/mole [36]. This figure is close to the data obtained in the current study, indicating a strong possibility for HBV to destabilize the interaction that would take place between Omicron Spike glycoprotein RBD to ACE2 receptor. This in turn would be useful as preventive as well as treatment options against COVID-19.

Conclusion. HBV proteins and peptides examined in the present study demonstrated their efficacy toward blockade of the interaction between Omicron Spike protein and its host receptor ACE2 via strong docking score and the corresponding binding affinity toward Spike protein RBD. On the basis of binding affinity, PLA2 was the best (binding affinity -13.9 kcal/mole) followed by apamin>MCDP>melittin>secapin. All of the in showed strong enough docking scores and binding affinities as well. Nevertheless, experimental validation should substantiate the obtained findings.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

КОМП'ЮТЕРНЕ ДОСЛІДЖЕННЯ ПРОТЕЇНІВ ОТРУТИ МЕДОНОСНОЇ БДЖОЛИ ЯК ПОТЕНЦІЙНИХ ІНГІБІТОРІВ OMICRON SARS-CoV-2

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Через катастрофічні наслідки COVID-19 для населення планети необхідно постійно шукати нові способи боротьби з наявними інфекціями та щоденними смертельними випадками. Метою даного дослідження було вивчення ефективності впливу протеїнів отрути медоносної бджоли (HBV) на рецептор-зв'язувальний домен (RBD) шипоподібного протеїну за допомогою інструментів *in silico.* Послідовність 5 протеїнів HBV використовували для моделювання гомології за допомогою сервісу Phyre 2. Створені моделі протеїнів застосовували у протеїн-протеїновому докінгу проти глікопротеїнового рецепторного домену (RBD) Omicron Spike (PDB ID# 7T9L) із використанням платформ HDock та ClusPro, з подальшим прогнозуванням афінності зв'язування і встановленням особливостей взаємодії за допомогою веб-порталу PRODIGY та PDBsum. Виявлено, що всі досліджені протеїни HBV демонструють високий показник докінгу та профіль афінності зв'язування до RBD. За результатами досліджень протеїни HBV можуть бути застосовані як профілактичний та лікувальний засіб проти Omicron SARS-CoV-2.

Ключові слова: COVID-19, SARS-CoV-2, рецептор-зв'язувальний домен (RBD), отрута медоносної бджоли, докінг.

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