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Protein Modeling and Docking of Curcurin Against Neuraminidase, Hemagglutinin Proteins of Pandemic Influenza H1N1/2009

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Abstract: A new approach to anti-viral therapy in recent years has been relying on the modeling and docking processes in the quest of novel inhibitors. Curcurin, an ribosome-inactivating protein, from *Jatropha curcas Linn*. (Euphorbiaceae), a herbal plant that has been used in traditional folk medicine in many tropical countries, was investigated against 2 proteins of Pandemic Influenza H1N1/2009. Although the structural properties of curcin are well documented in the literature, the modeling and docking studies by in-silico techniques with phytoproteins are limited. In this pursuit, the modeling of Curcurin was carried out by Phyre2 and docking with Influenza virus proteins by using Hex, Patchdock and FireDock server. Further, we applied the Molecular operating environment (MOE) to analyze the protein interactions between Curcin and Hemagglutinin and with Neuraminidase. The present work provides the structural insight into the binding mode of curcin protein and forms the basis for designing future inhibitors of Influenza proteins.

Keywoprds: Pandemic Influenza H1N1/2009**,** Curcurin, Protein Modeling, Docking.

INTRODUCTION:

Jatropha (Euphorbiaceae) is a genus of approximately 175 succulent plants, shrubs and trees (some are deciduous like *Jatropha curcas* L.). Irrespective of the species, extracts from different parts such as leaves, stem, bark and roots of the Jatropha plant have been used in ethno-medicines for a long time [1]. In the past two decades, study on the utilization of Jatropha oil (non-edible) as a feedstock for biofuel has gained a momentum, resulting in industrial scale cultivation. Apart from the seed oil, genus Jatropha is also a rich source of phytochemicals that can be utilized in agricultural, nutritional and pharmaceutical industries [2].
The toxicity of the whole seed from *Jatropha curcas* has been known for a long time. Its toxicity has been attributed to a protein component. A toxic protein was isolated from the seeds of *Jatrophacurcas* by Felke (1914), and was designated as “curcin” by him. He proposed that the curcin was a kind of toxalbumin [3], Barbieri (1993) reported the curcin was type I RIP, a single chain protein [4].
Many plants contain proteins that are capable of inactivating ribosome and accordingly are called ribosome-inactivating protein (RIP). Interests in RIP, particularly in Type I RIP have been growing since they are used as components of ‘immunotoxins’, one type of hybrid molecules consisting of a toxic peptide chain linked to an antibody [5]. Immunotoxins will be promisingly used to eliminate such targets as harmful cell, neoplastic, immunocompetent and parasitic cells.
Traditionally, RIPs are recognized to deadenylate the large ribosomal RNA with high site specificity and inactivate the ribosomes owing to their RNA N-glycosidase activity [6-8]. The potential of some of the RIPs to depurinate the rRNA at multiple sites [9] along with several other polynucleotide substrates [10] has been addressed. Broad and diverse pharmacological attributes have been associated with RIPs, as protein synthesis-inhibitory, immunosuppressive, anti-tumor, antiviral, anti-HIV and abortificient traits to mention a few [11]. Phytomolecules have long served a pivotal role as potent active components towards strengthening and widening the pharmaceutical options against various ailments.
Influenza is a respiratory infection caused by the influenza virus, which is transmitted mainly through airborne aerosols of respiratory secretions and direct or indirect

contact with infected people or their belongings. Influenza has caused several epidemics or pandemics, including the 1918 Spanish (H1N1), the 1957 Asian (H2N2), the 1968 Hong Kong (H3N2), and the 2009 Mexican pandemics (H1N1pdm) [12], due to its high mutation rate and its ability to cause cross-species infections. Influenza A virus belongs to the Orthomyxoviridae family, and contains eight negatively stranded RNA segments, which encode at least 12 proteins, including the RNA-dependent RNA polymerase complex (RdRp): PA, PB1, PB2, and NP, the outer-membrane proteins: M2, HA, and NA [13].

Currently, there are two classes of anti-influenza drugs, M2 and NA inhibitors. Amantadine and rimantadine are inhibitors of the M2 ion channel [14-15], which impedes the release of virus genome into the host. Oseltamivir and zanamivir (Relenza) are NA inhibitors that block NA from hydrolyzing the binding of host neuraminic acid and HA, thus preventing the virus from spreading. However, there have been an increasing number of cases of virus resistance being reported [16]. The rise of resistant viruses has become a serious problem, although several groups have demonstrated that a combination of oseltamivir and ribavirin treatment has reduced the death rate resulting from H5N1 infection in a mouse model of influenza [17].

In addition to combinations of available drugs, the development of new drugs is desperately needed. The in-silico method gives advantage in terms of economy and time by providing approximate result which can be easily replicated under lab conditions when done properly. Now a day's In-silico studies are carried out beforehand to provide an approximate idea whether those ligands or drugs can be used for further studies. Current research was focused on to search for unknown and potent phyto-ligands possessing an efficient docking ability with the viral inhibitors and furthermore, which can be used for further antiviral methodologies in in-vitro procedures.

MATERIALS AND METHODS:

Identification of potential therapeutic targets using Database:

The detection of Neuraminidase, Hemagglutinin and M2 protein are antigenically distinct entities of the influenza virus envelope [18]. From the Protein Data Bank (PDB) database 3TI4 (Neuraminidase), 3UBE (Hemagglutinin) and 2RLF (M2 protein) were selected and sequences were retrieved for further analysis. These particular structures, were selected as they exhibited high 3D resolution (>1.80 A˚) amongst other PDB structures of proteins and also on the basis of Ramachandran plot, complexed to potent inhibitors (LVO for 3TI4, NAG for 3UBE and 2RLF for M2 protein) reported to exhibit more than 75% promiscuity, i.e., a protein structure to which more than 75% of actives are docked correctly which was confirmed by using PDBSUM tool [19-21]. The M2 Protein was not selected for docking as it was not suitable enough as per the requirement of the study.

Phytoligand modeling using PHYRE2:

Jatropha curcas with the phytocomponent as Curcurin with NCBI Acession number ACO53803.1 was modeled using PHYRE and EM using ADT autodock tools. Curcurin sequence was retrieved from NCBI protein database and was further modeled using Phyre2 followed by structure refinement using ADT. The modeled and refined structure was further validated using Ramchandran's plot using RAMPAGE server.

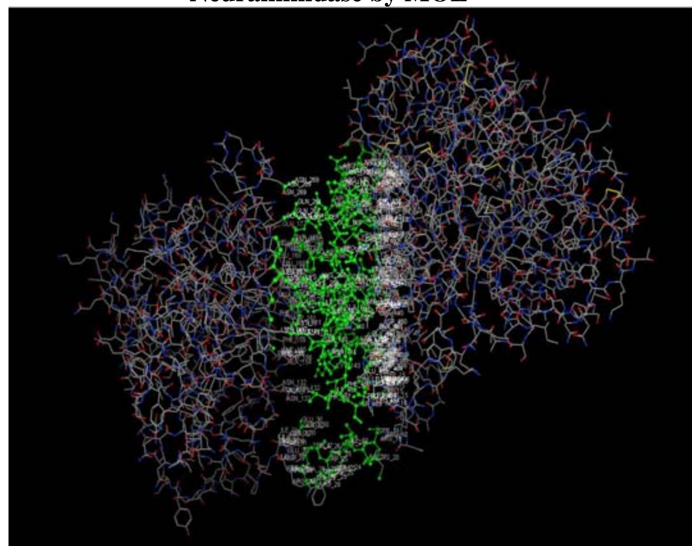
Dockability Studies and Visualization of protein-protein complex:

Different docking tools were used to predict binding affinities using docking algorithms. Initially, Hex [22] and Patchdock [23] server were used for docking and further refinement was done by using Firedock server. FireDock is an efficient method for refinement and re-scoring of rigid-body protein-protein docking solutions [24]. The interface study and analysis of the protein complexes was done using MOE (Molecular operating environment) [25]. MOE provides a collection of applications for visualizing and understanding details of receptor active sites and protein interactions. Visualization the protein-protein interaction and identification of amino acids was done using PISA and MOE.

RESULT AND DISCUSSION:

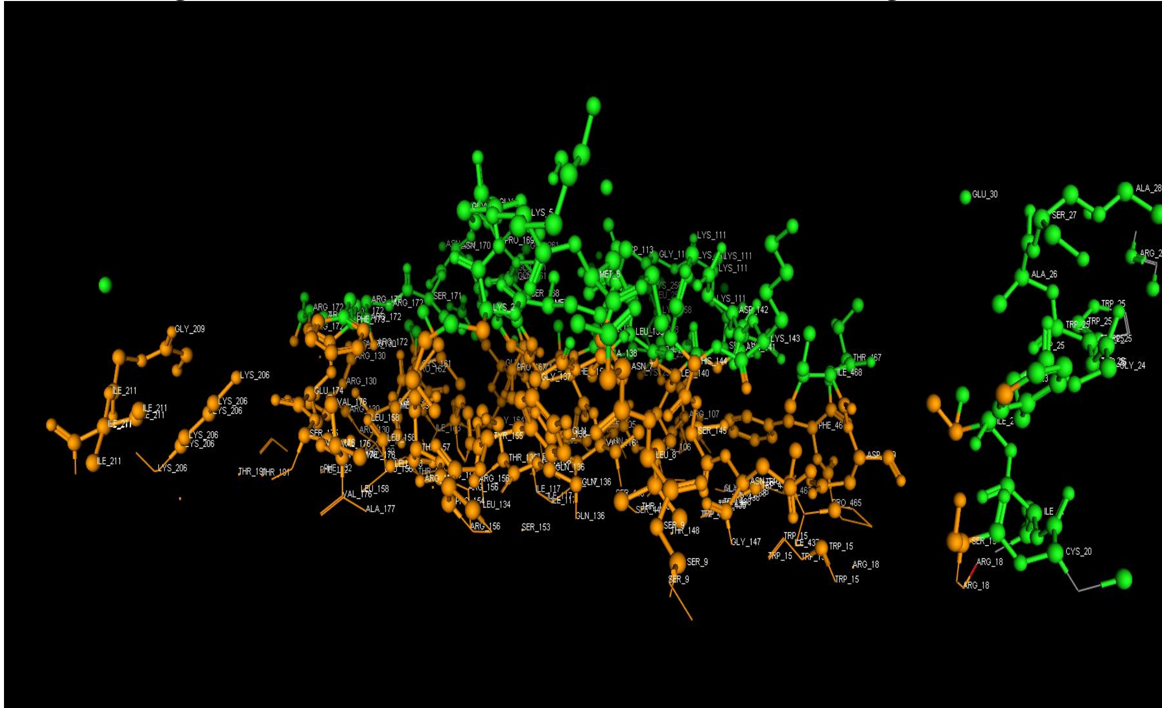
The interaction between Curcurin and Neuraminidase is shown in Figure 1, 2 and 3 respectively and interaction between Curcurin and Hemagglutinin shown in figure 4 and 5 respectively. **Accessible Surface Area** (*ASA*): ASA is the amount of protein surface area accessible to the solvent. Water is the solvent in this case (default probe radii 1.4 Å). **Buried Surface Area** (*BSA*): The amount of ASA buried for complex formation is denoted as the buried surface area. **Parentage Buried Surface Area**: Percentage buried surface area is the ratio of BSA to the total ASA of all the subunits multiplied by 100.

Figure 1: Interaction between Curcurin and Neuraminidase by MOE



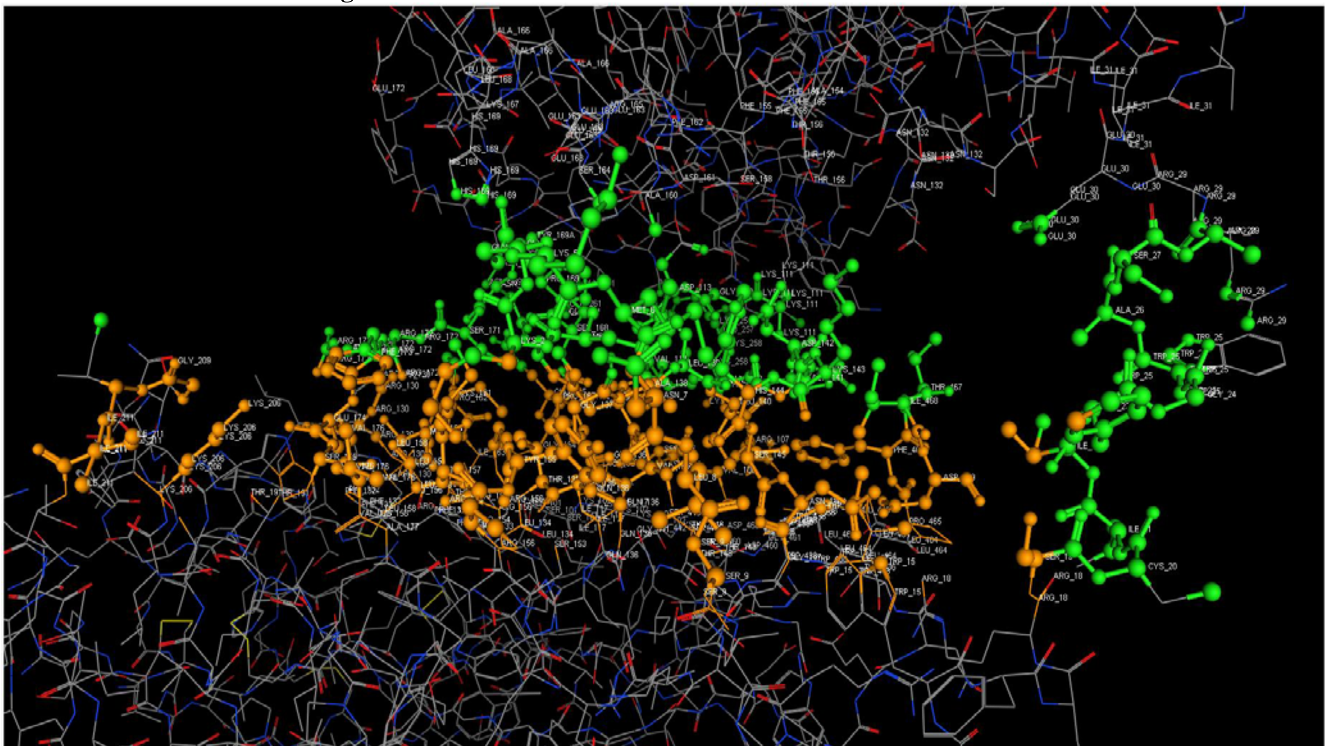
In the above Figure 1, the green color area denotes the amino acids which are interacting between the Curcurin and Neuraminidase.

Figure 2: Interaction between Curcurin and Neuraminidase using PISA tool.



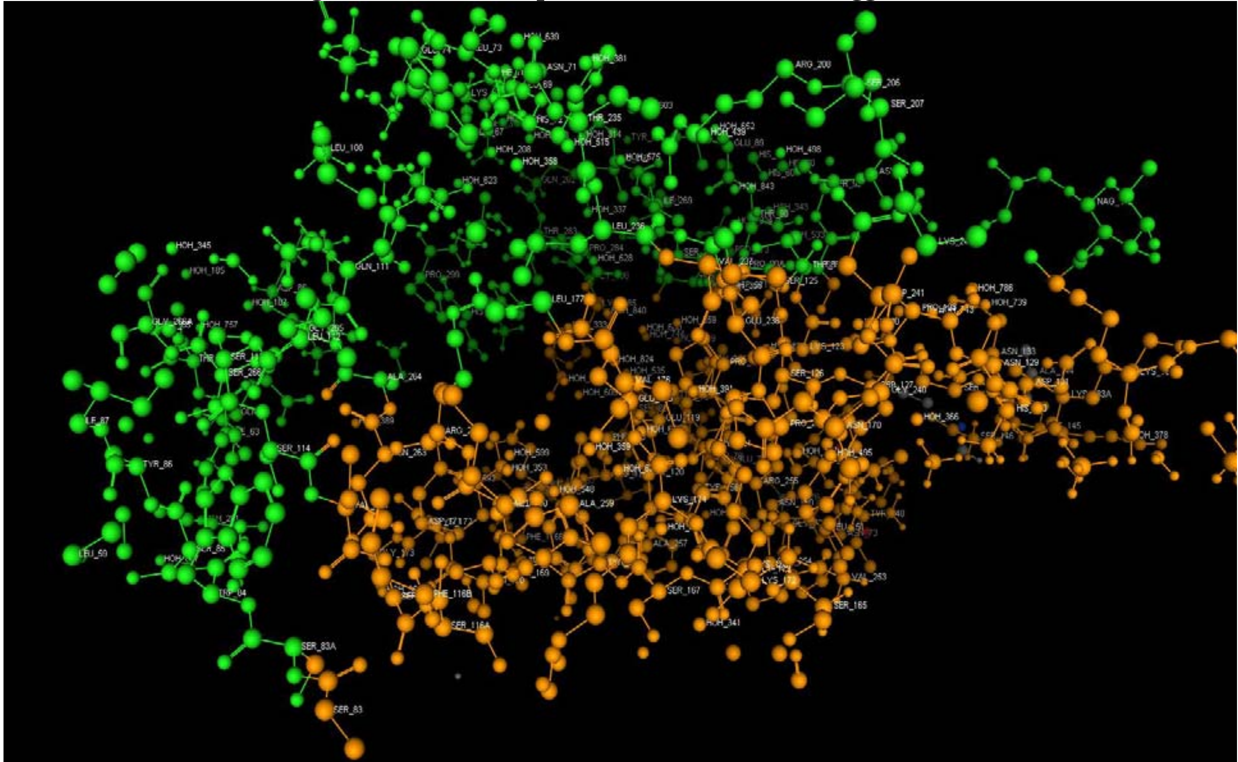
In the above Figure 2, the green and orange are indicate the interacting amino acids of the 2 proteins involved in the interaction.

Figure 3: Interaction between Curcurin and Neuraminidase



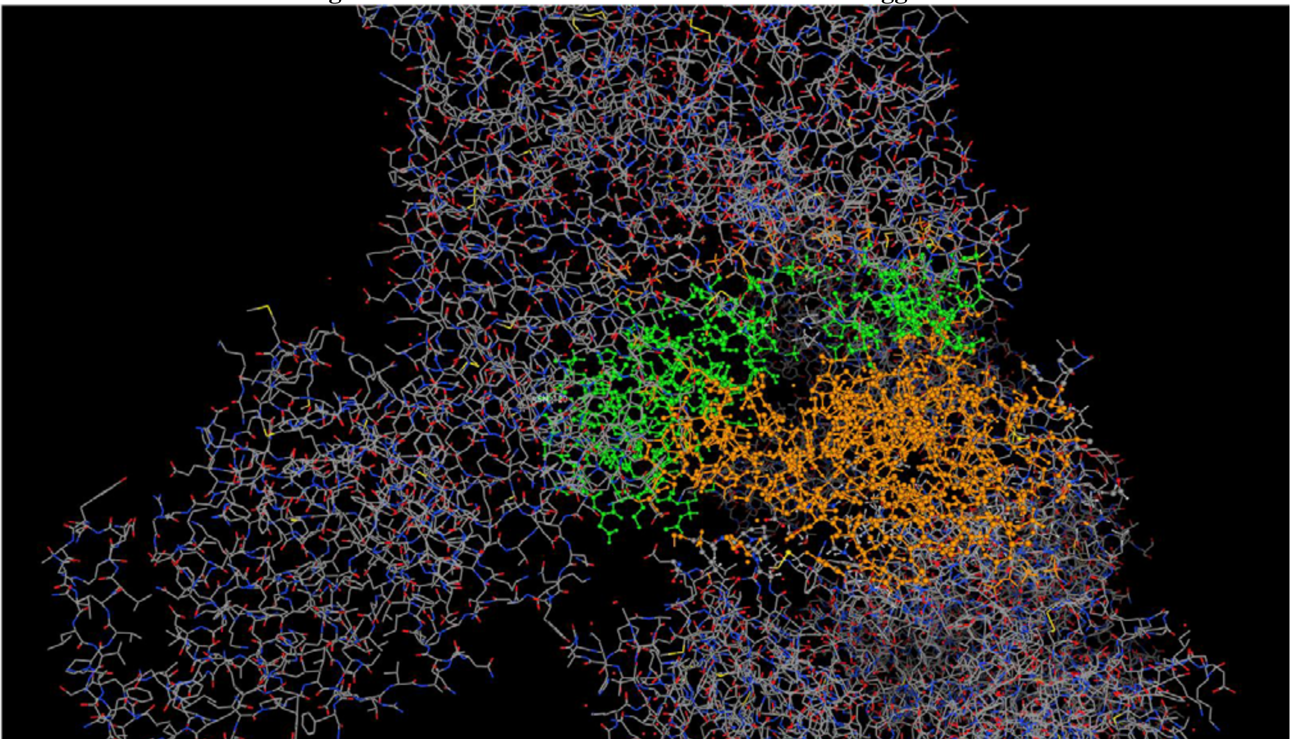
In the above Figure 3, the green and the orange area represents the interacting amino acids between the two proteins. Amino acids present in interface region are:Ala2, ser3, ile5, asp6, ile7, ser8, ile10, asn11, trp13, phe17, his23, ala26, leu30, lys31, ala35, asp36, leu108, ile126, arg129, leu135, tyr155, thr182, ala188, phe217, ile226, pro231, ley292, tyr350, glu364, tyr362. The amino acids: tyr350, glu364, tyr362, phe217, ile226, pro231, ley292, thr182 demonstrate high percentage buried surface area.

Figure 4: Protein complex of Curcurin and Hemagglutinin



In the above Figure 4, the orange and green are represents the interacting amino acids between Curcurin and Hemagglutinin

Figure 5: Interaction between Curcurin and Hemagglutinin



In the above Figure 5, the orange and green are represents the interacting amino acids between Curcurin and Hemagglutinin. Amino acids present in the interface region are Asp11, cys14, ala19, asn20, val29, thr37, val40, leu42, glu106, arg109, ser266, pro293, gly303, tyr308, leu314, leu316, val309, leu314, thr318, ile323.thr37, val40, leu42, glu106, arg109, ser266, pro293, gly303, tyr308, leu314, leu316, val309, leu314, thr318, ile323 show high percentage buried surface area.

Table 1: Binding Energy values 3TI4 (Neuraminidase) after docking with Curcurin:

Rank	Solution Number	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB	Structure show/hide
1	10	-2.62	-21.85	7.36	11.63	-4.50	☑
2	8	-1.36	-24.52	25.78	3.69	-3.75	☐
3	5	20.11	-5.72	3.63	3.76	0.00	☐
4	1	25.53	-41.68	52.12	9.27	-5.40	☐
5	7	43.71	-44.70	77.70	8.66	-5.80	☑
6	4	44.00	-25.69	45.16	9.00	-1.02	☐
7	3	167.09	-7.32	176.94	-0.26	0.00	☐
8	2	380.06	-31.20	434.07	20.83	-4.10	☐
9	6	523.90	-57.18	728.67	14.01	-5.64	☐
10	9	637.44	-26.48	756.62	21.33	-4.44	☐

Table 2: Binding Energy values 3UBE (Hemagglutinin) after docking with Curcurin:

Rank	Solution Number	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB
1	-11.20	-19.56	8.36	3.68	9.69	

In the Table 1: Ten different numbers of solutions were obtained by docking between 3TI4 and Curcurin, out of which Rank 1: Solution No 10 was the best solution, as shown in the figure for 3TI4 (Neuraminidase). The global binding energy was -2.62KJ/mol whereas Vanderwall's interaction for attraction and repulsion were -21.85 KJ/mol and 7.36 KJ/mol respectively.

In the Table 2: Ten different numbers of solutions were obtained by docking between 3UBE and Curcurin, out of which Rank 1 as shown in the Figure for 3UBE (Hemagglutinin).The global binding energy was -19.56 KJ/mol whereas Vanderwall's interaction for attraction and repulsion were 8.36 KJ/mol and 3.68 KJ/mol respectively.
With respect to the binding energy values of docking studies it can be implicit that binding affinity of Curcurin towards Hemagglutinin as a potent inhibitor is more as compared to Neuraminidase.

CONCLUSION:

Influenza virus continues to emerge and re-emerge and remains a major public health concern [26]. As an alternative to chemically synthesized antivirals such as amantadine [27] or oseltamivir [28], many plant extracts, and purified substances like phytochemicals have been tested and reported to have selective antiviral activities inhibiting influenza viruses [29-30]. In a similar manner within the reach for identifying novel antiviral substances of plant origin, the antiviral potential of seeds of *Jatropha curcas* was tested against influenza virus proteins by in-silico methodologies. By this investigation, we have successfully showed the potential of Curcurin in docking studies with the influenza proteins.
The current study explores the significance on docking and modeling of Curcurin as a valuable protein which can be used as potent inhibitors against the influenza virus. Furthermore the present findings persuade the need for invitro procedures to investigate the potential of Curcurin against the influenza virus.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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