Innov Biosyst Bioeng, 2023, vol. 7, no. 3, 21–31
doi: 10.20535/ibb.2023.7.3.285245

Original article

POTENTIAL ALPHAVIRUS INHIBITORS FROM PHYTOCOMPOUNDS – MOLECULAR DOCKING AND DYNAMICS BASED APPROACH

M. Sharma, A. Bansal, S. Suman, N.R. Sharma*

Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

*Corresponding author: neeta.raj@lpu.co.in

Received 21 July 2023; Accepted 4 September 2023

Background. Alphaviral diseases are an economic burden all over the world due to their chronicity and distribution worldwide. The glycoproteins E1 and E2 are important for binding to the surface of the host cell by interacting with the receptors and non-structural proteins named nsP2 and nsP4 are important for the replication of virus, so can be an important drug discovery target.

Objective. We are aimed to explore the *in silico* interaction between plant-based compounds (phytocompounds) and specific protein targets, such as nonstructural protein nsP4 and glycoprotein E2 of Sindbis virus (SINV), nsP2 and E2 of Chikungunya virus (CHIKV), and glycoproteins E1 and E2 of Ross River virus (RRV).

Methods. A library of phytochemicals from Indian medicinal plants was prepared using databases and converted to 3D structures. Protein structures (nsP2, nsp4, E1, E2) were obtained and refined, followed by molecular docking with AutoDock Vina. Promising ligands were evaluated for properties, cytotoxicity, and mutagenicity, considering drug-likeness and potential issues. Molecular Dynamics simulations assessed complex stability.

Results. We analyzed 375 phytocompounds against these targets using molecular docking, modeling, and molecular dynamics for SINV, CHIKV, and Ross River (RRV) virus proteins. Granatin A has been found to successfully bind to the target sites of SINV nsP4, CHIKV E2, and CHIKV nsP2 with binding affinity values of –16.2, –20.6, and –18.6 Kcal/mol respectively. Further, stability of CHIKV E2 – Granatin A complex was done by performing molecular dynamic simulation and the complex was stable at 60ps.

Conclusions. This research provides valuable insights into the development of effective antiviral drugs against alphaviruses, emphasizing the importance of natural compounds and their interactions with viral proteins. This study might pave the way for further exploration of these small molecules as effective anti-alphaviral therapeutic agents.

Keywords: alphaviral diseases; glycoproteins; nsP2; nsP4; molecular docking; granatin A.

Introduction

The emergence and spread of viral diseases are serious concerns globally. Many diseases produced by viruses like Chikungunya virus (CHIKV), Ross River virus (RRV), Mayaro virus (MAYV), O'nyong-nyong virus (ONNV), Barmah Forest virus (BFV), Semliki Forest virus (SFV), Venezuelan equine encephalitis virus (VEEV) emerging as a great risk. Another important issue regarding viruses is the ability to mutate their genomes and develop resistance against antiviral drugs [1]. To address the side effects of antiviral drugs, the development of plant-based antivirals with potentially fewer side effects is being considered, though it's worth exploring other approaches as well. The goal is to find effective solutions with minimal side effects [2]. So, plant-based bioactive compounds could be investigated for their antiviral properties and may be used with the already existing therapies with a different mode of delivery to supplement the effectiveness of antiviral [3].

There are no effective antivirals available for arthritogenic alphaviruses, whose infection in humans leads to fever, arthralgia, myalgia, and skin rash. Involvement of joints varies from tenderness to restricted joint movement with a lot of redness and swollen joints of hand, wrist, and ankle, sometimes involving larger joints like Knee and shoulder, leading to pain just like rheumatoid arthritis reducing the quality of life [4]. One of the alphaviruses, Sindbis virus (SINV) outbreaks were noted in South Africa in 1963 and 1974. In Finland, from 1974–2002, epidemics took place at an interval of 7 years [5]. In 2013, an upsurge was observed in north area of Sweden [6] showing the northward spread of SINV.

Sporadic episodes of Chikungunya fever by Chikungunya virus (CHIKV) were reported across Africa and Asia since the 1960s [7]. It's spread was reported in the entire Indian Ocean region in the year 2000. In 2013, it was introduced into the Caribbean. Number of cases exceeded one million in the year of introduction of CHIKV to the Ameri-

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cas, and it is currently endemic in South and Central America and the Caribbean [7, 8].

Ross River virus (RRV) symptoms of arthritis generally last from six weeks to six months, sometimes a year, creating a burden on the public health system [9]. Number of cases of epidemic polyarthritis due to RRV reported in Australia exceeded 4500 in 1992, with RRV ranked as fifth most prevalent arthropod borne viral disease. RRV epidemics occurred in the Cook Islands, Fiji, New Caledonia, and Samoa in 1979–80 [10].

Debilitating and widely disseminated diseases caused by specific viruses, particularly Alphaviruses, impose significant financial burdens. Therefore, there is an urgent need to develop effective measures against such viral infections. Currently, only symptomatic treatment is being provided for the patients, underscoring the importance of exploring antiviral strategies. One crucial aspect in this context is the viral attachment process, which relies on the interaction of envelope glycoproteins E1 and E2 with host cell receptors [11].

Since E1 and E2 glycoproteins are important proteins used by the virus to enter the host cell these can be an important target for drug development against the alphaviruses. Non-structural protein nsP2 has RNA helicase activity and acts as a viral protease for nonstructural polyproteins. nsP2 has a methyl transferase-like domain [12] in addition to its helicase and protease activity. In addition, it also obstructs the synthesis of host cellular macromolecules, thereby hindering various antiviral responses [13]. So nsP2 can be a possible target for developing antiviral drugs effective in Alphaviral infections. RNA polymerase function of nsP4 due to GDD motif has been found to be consistent with the findings of genetic studies conducted [14], making nsP4 also an important drug target.

The purpose of this study was to explore the *in silico* interaction between plant-based compounds (phytocompounds) and specific protein targets, such as glycoproteins nsP4 and E2 of SINV, glycoproteins nsP2 and E2 of CHIKV, and glycoproteins E1 and E2 of RRV. This exploration was motivated by the lack of effective antivirals against arthritogenic alphaviruses and the availability of protein assemblies in the Protein Data Bank. The researchers utilized molecular docking and molecular dynamics to identify lead molecules, followed by *in silico* absorption, distribution, metabolism, and excretion (ADME) and toxicity profiling of these lead molecules. These *in silico* findings aimed to facilitate subsequent experimental investigations and potentially support future clinical trials.

Materials and Methods

The sequence of the methodology used and the various software employed in the present study are presented in Fig. 1.

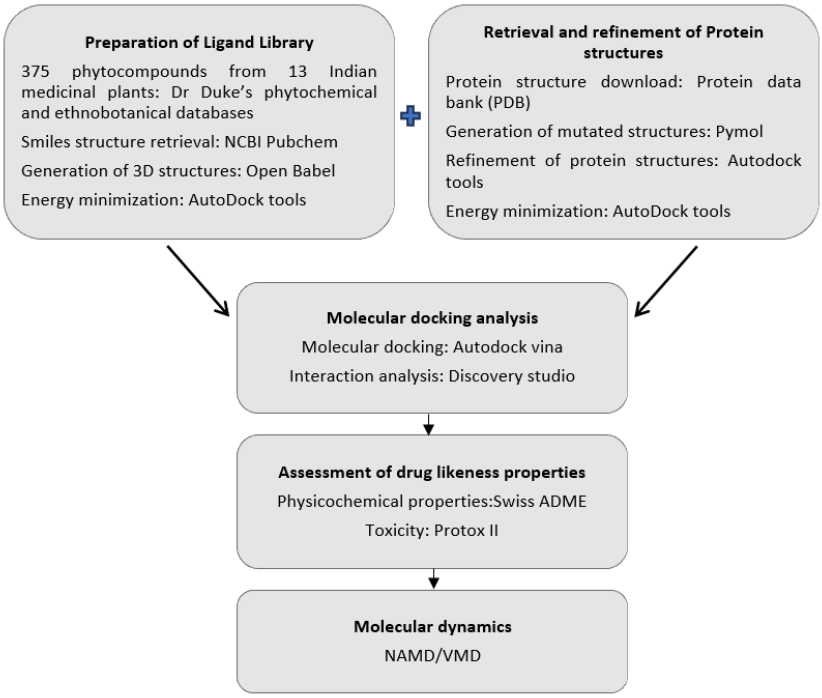


Figure 1: Flow chart of the methodology, software, and web servers in the current study

Preparing the ligands

From Dr. Duke's Phytochemical and Ethnobotanical Databases, a library of 375 compounds, from 13 Indian plants with medicinal properties, was prepared (https://phytochem.nal.usda.gov/phytochem/search/list). From the NCBI PubChem database, the SDF files of the phytocompounds were downloaded (https://pubchem.ncbi.nlm.nih.gov/). In order to generate the 3D structures of these compounds, the Open Babel software [15] was used. The AutoDock Tool [16] was employed to add Gasteiger charges and generate pdbqt format of ligands.

The protein structure retrieval and refinement

The macromolecule used in the study were the nsP2 protein of CHIKV, the nsp4 protein of SINV, the E1 protein of RRV, and the E2 protein of CHIKV, SINV, and RRV. The three-dimensional structures of the proteins were salvaged from https://www.rcsb.org/ in PDB format with PDB IDs: 4ztb, 7vw5, 6vyv (E1), 6w09, 1z8y, and 6vyv (E2) respectively. The PyMOL molecular graphics system version 2.0.6 was used for opening and preparing the protein structure. AutoDock Tool [16] was used for addition of Kollman charges and Polar hydrogen atoms.

Molecular docking

Molecular docking was carried out using the AutoDock Vina software [17]. A grid-based approach with flexible protein receptors and ligand docking procedure was followed. At this point the grid box boundary for the macromolecule was set under which the ligand was seen for its potential binding. The following values were entered at exhaustiveness = 8 to set the grid box as shown in Table 1. The Discovery Studio server was used for visualizing molecular interactions.

Assessing the drug-likeness properties of the promising molecules

The molecules with a binding energy value <–10 Kcal/mol were further studied for their physicochemical and ADMET properties using the SWISS ADME server [18], and their cytotoxicity and mutagenicity were predicted with the help of Protox-II web server [19]. The promising inhibitory candidates were further evaluated for their physicochemical properties to assess their potential as a lead molecule taking into consideration the Lipinski's rule of fives, which is helpful in predicting the bioavailability following oral route of administration and pharmacokinetic properties of the compound like membrane permeability. Pan-assay interference structures (PAINS) are an important source of false positive signals in the process of drug-discovery, which include redox reactivity, fluorescence of small molecules and covalent modifications of target protein. In biological screening assays, PAINS compounds are responsible for activity artefacts due to their tendency to form colloidal aggregation and chemically reactive nature in the conditions used for assay.

Molecular Dynamics simulations

In this work, we have used protein-ligand complex PDB and PSF files which were merged for more processing. Then, by creating a water box covering the whole structure, the process of solvation was performed. A simulation of 100 ps with an output frequency of 50000 steps was used, to attain equilibrium in the Ligand receptor system, and an NVT ensemble was used; the temperature and pressure initially fixed was 330K and 0.001 atm respectively. The result was provided in terms of RMSD that is Root mean square deviation. In order to evaluate the trajectory, NAMD full setup and coarse-grained Brownian dynamics were employed.

Table 1: Grid box size of protein prepared for docking

Virus protein	Protein PDB id	Center			Size (Å)		
		X	Y	Z	X	Y	Z
SINV E2	1z8y	72.690	68.898	239.381	199.661	181.463	25.000
SINV nsP4	7vw5	5.829	40.210	–7.174	102.692	93.984	25.000
CHIKV E2	6w09	90.812	88.125	285.892	230.999	275.438	25.000
CHIKV nsP2	4ztb	24.339	28.522	–24.972	148.600	189.827	25.000
RRV E1	6vyv (E1)	86.550	82.945	281.929	226.508	274.189	25.000
RRV E2	6vyv (E2)	90.6994	89.583	281.929	236.300	312.391	25.000

Results

Worldwide distribution of alphaviruses, increase in global travel and wide variety of hosts and vectors of alphaviruses necessitate the need for development of effective antiviral drugs against alphaviruses. In the current work, the binding predictions of 375 compounds were investigated with E2 glycoproteins of SINV, CHIKV and RRV and nsP4 of SINV, nsP2 of CHIKV and E1 glycoprotein of RRV alphaviruses.

The phytocompounds with minimum binding energy scores have been tabulated in Table 2A, 2B, and 2C for SINV, CHIKV and RRV respectively displaying their binding affinities for all the target proteins. The molecules with binding affinity score of less than –10Kcal/mol were further evaluated for their physicochemical properties.

The results of the molecular docking with nsP2 of CHIKV, nsP4 of SINV and E2 glycoprotein of SINV, CHIKV and RRV indicated that Granatin A phytocompound showed effective binding with all of them.

Interaction of the phytocompounds with E2 glycoprotein of Sindbis virus associated with attachment to the host cell

Granatin A (PubChem ID: 131752596) has the highest affinity with low free energy value of –16.6 Kcal/mol with the E2 glycoprotein of SINV (PDB ID: 1z8y). Tercatain had a moderate binding energy of –13.7 Kcal/mol, whereas Granatin A breaches three of the four Lipinski's rules (Table 3), which are standards for determining a compound's drug-likeness. It may, however, be explored for future *in vitro* research, particularly when evaluating

Table 2A: Phytocompounds interacting with the respective viral proteins of SINV with the binding energy scores less than –10 Kcal/mol

Name of the molecule	Chemical formula	1z8y (E2 SINV) Binding Energy (Kcal/mol)	7vw5 (nsP4 SINV) Binding Energy (Kcal/mol)
Granatin A	**$C_{34}H_{24}O_{22}$**	**–16.6**	**–16.2**
Tercatain	$C_{34}H_{26}O_{22}$	–13.7	–13.7
2-O-Galloylpunicalin	$C_{41}H_{26}O_{26}$	–	–10.5
Casuariin	$C_{34}H_{24}O_{22}$	–	–10.6
Punicalagin	$C_{48}H_{28}O_{30}$	–	–11.3
Punicalin	$C_{34}H_{22}O_{22}$	–	–10.3

Table 2B: Phytocompounds interacting with the respective viral proteins of CHIKV with the binding energy scores less than –10 Kcal/mol

Name of the molecule	Chemical formula	6w09 (E2 CHIKV) Binding Energy (Kcal/mol)	4ztb (nsP2 CHIKV Binding Energy (Kcal/mol)
Granatin A	**$C_{34}H_{24}O_{22}$**	**–20.6**	**–18.6**
2-O-Galloylpunicalin	$C_{41}H_{26}O_{26}$	–11.1	–10.2
beta-Amyrin	$C_{30}H_{50}O$	–10.5	–
Corilagin	$C_{27}H_{22}O_{18}$	–10.7	–
Ellagitannin	$C_{44}H_{32}O_{27}$	–12.0	–
F-Gitonin	$C_{50}H_{82}O_{23}$	–10.4	–
Friedelin	$C_{30}H_{50}O$	–10.3	–
Maslinic acid	$C_{30}H_{48}O_4$	–10.8	–
Oleanolic Acid	$C_{30}H_{48}O_3$	–10.4	–
Punicalagin	$C_{48}H_{28}O_{30}$	–10.8	–
Punicalin	$C_{34}H_{22}O_{22}$	–12.9	–
Punigluconin	$C_{34}H_{26}O_{23}$	–10.3	–10.1
Strictinin	$C_{27}H_{12}O_{18}$	–11.7	–
Taraxerol	$C_{30}H_{50}O$	–10.1	–
Tercatain	$C_{34}H_{26}O_{22}$	–17.8	–
Tigogenin	$C_{27}H_{44}O_3$	–10.1	–
1,3,6-tri-O-galloyl-beta-D-glucose	$C_{27}H_{24}O_{18}$	–	–10.1
Apiumoside	$C_{29}H_{30}O_{12}$	–	–10.3
Casuariin	$C_{34}H_{24}O_{22}$	–	–10.6

Table 2C: Phytocompounds interacting with the respective viral proteins of RRV with the binding energy scores less than –10 kcal/mol

Name of the molecule	Chemical formula	6vyv (E1 RRV) Binding Energy (Kcal/mol)	6vyv (RRV E2) Binding Energy (Kcal/mol)
Granatin A	**$C_{34}H_{24}O_{22}$**	**–22.1**	**–21.7**
(-)-Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	–10.9	–10.8
1,2,4,6-tetragalloylglucose	$C_{34}H_{28}O_{22}$	–10.3	–10.8
2-O-Galloylpunicalin	$C_{41}H_{26}O_{26}$	–12.7	–12.4
beta-Amyrin	$C_{30}H_{50}O$	–10.2	–10.2
Casuariin	$C_{34}H_{24}O_{22}$	–11.0	–11.1
Corilagin	$C_{27}H_{22}O_{18}$	–11.2	–11.2
Ellagitannin	$C_{44}H_{32}O_{27}$	–10.4	–10.9
F-Gitonin	$C_{50}H_{82}O_{23}$	–10.7	–10.7
Friedelin	$C_{30}H_{50}O$	–10.5	–10.5
Homoorientin	$C_{21}H_{20}O_{11}$	–10.6	–10.2
Maslinic acid	$C_{30}H_{48}O_4$	–10.1	–10.2
Oleanolic Acid	$C_{30}H_{48}O_3$	–10.1	–10.1
Procyanidin	$C_{30}H_{26}O_{13}$	–11.0	–10.3
Punicafolin	$C_{41}H_{30}O_{26}$	–10.3	–11.2
Punicalagin	$C_{48}H_{28}O_{30}$	–11.2	–11.5
Punicalin	$C_{34}H_{22}O_{22}$	–12.3	–10.4
Punigluconin	$C_{34}H_{26}O_{23}$	–11.5	–11.3
Strictinin	$C_{27}H_{12}O_{18}$	–11.3	–
Tercatain	$C_{34}H_{26}O_{22}$	–18.7	–
Tigogenin	$C_{27}H_{44}O_3$	–10.1	–10.1
Ursolic acid	$C_{30}H_{48}O_3$	–10.2	–
Taraxerol	$C_{30}H_{50}O$	–	–20.1

Table 3: Physiochemical properties and drug-likeness features of selected best-hit compounds

Name of the compound	MW (<500) g/mol	LogP (<5)	#H bond acceptors (<10)	#H bond donors (<5)	TPSA (Å²)	Lipinski's rule# violation	PAINS #alert	Mutage-nicity	Cyto-toxicity
Granatin A	784.54	–2.17	22	12	374.26	3	1	Inactive	Inactive

Notes. MW – molecular weight, TPSA – topological polar surface area, NA – results not obtained.

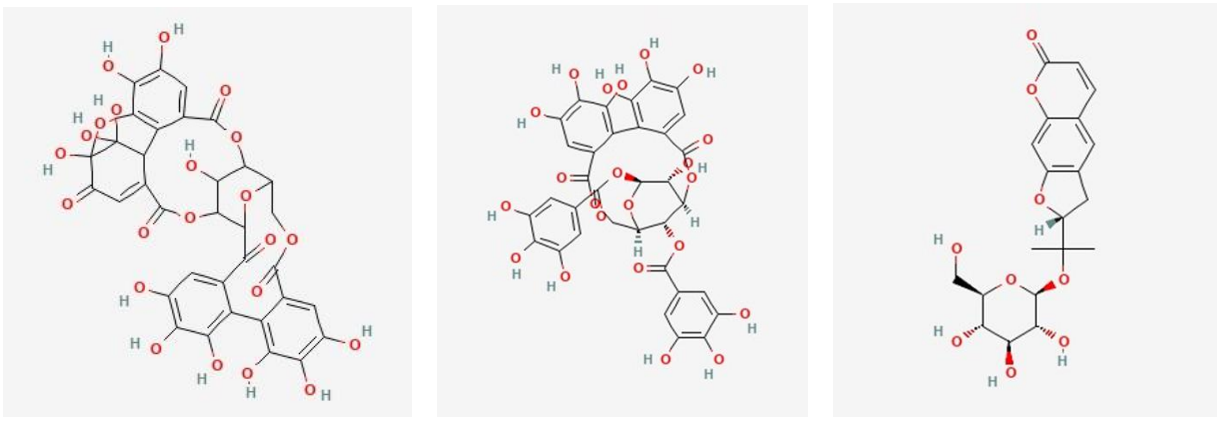
alternative routes of administration other than the oral route.

Interaction of phytocompounds with nsP4 protein of Sindbis virus associated with replication complex

Granatin A (Fig. 2A) had the strongest interaction with the RNA-dependent RNA polymerase nsP4 protein of SINV, with a minimum binding energy score of –16.2 Kcal/mol. Tercatain (Fig. 2B) came in second with a binding energy of –13.7 Kcal/mol, and Punicalagin came in third with a binding energy of –11.3 Kcal/mol. Fig. 3 C,D demonstrates the interactions of Granatin A with the SINV nsP4 protein, and Table 2A has further information. THR 141B, GLN 191B, ASN 192B, ASN 196B, SER 377B, TYR 573A, and salt bridges produced between THR 134B and GLN 195B are among the amino acids implicated in hydrogen bond interaction (Table 4).

Interaction of the phytocompounds with E2 glycoprotein of Chikungunya virus associated with receptor binding

Granatin A binds to the E2 glycoprotein of the CHIKV with a binding affinity of –20.6 Kcal/mol. GLU (10J), LYS (12J), ILE (20J), MET (48J), ILE (83J), PHE (95J), CYS (96J), and THR (114J) were the amino acids implicated in the interaction. Tercatain and Punicalin both demonstrated efficient binding with the E2 glycoprotein, with binding energy values of –17.8 and –12.9 Kcal/mol, respectively.



A B C

Figure 2: Structure of promising ligand molecules: (A) Granatin A, (B) Tercatain, (C) Nodakenin [20]

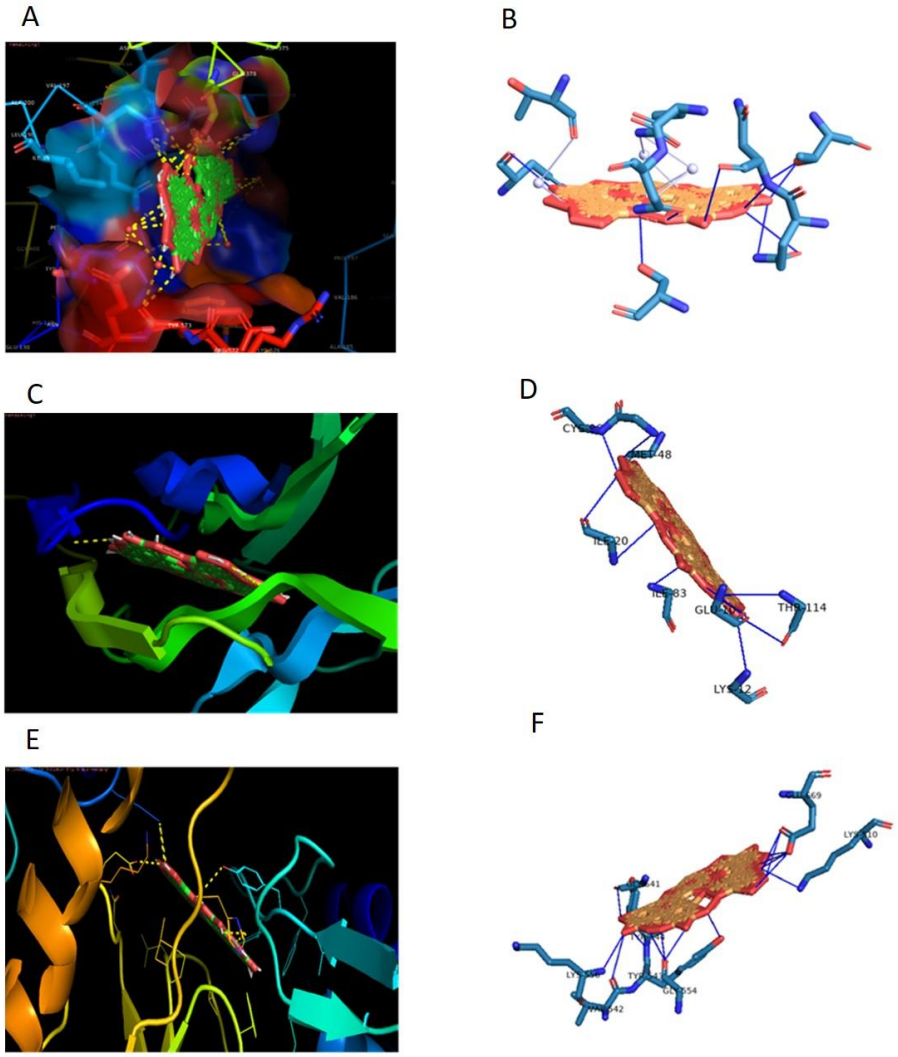


Figure 3: Interaction of ligand molecules with different proteins: (A) Granatin A with nsP4 protein of Sindbis (PDB ID-7vw5), (B) various amino acid residues of nsP4 of Sindbis showing hydrophobic interactions with Granatin A, (C) Granatin A with E2 glycoprotein of Chikungunya (PDB ID-6w09), (D) various amino acid residues of E2 of Chikungunya showing hydrophobic interactions with Granatin A molecule, (E) Granatin A with nsP2 protein of Chikungunya (PDB ID-4ztb, (F) various amino acid residues of nsP2 of Chikungunya showing hydrophobic interactions with Granatin A

Table 4: Binding energy values of Granatin A with all the proteins

Protein	Binding Energy Scores (Kcal/mol)
SINV nsP4 (7vw5)	–16.2
SINV E2 (1z8y)	–16.6
CHIKV E2 (6w09)	–20.6
CHIKV nsP2 (4ztb)	–18.6
RRV E1 (6vyv)	–22.1
RRV E2 (6vyv)	–21.7

Interaction of the phytocompounds with nsP2 protein of Chikungunya virus

Granatin A demonstrated efficient interaction with the CHIKV nsP2 protein, with a binding energy of –18.6 Kcal/mol. It established H-bonds with nsP2 protease residues 510D LYS, 542A VAL, 543A TYR, 544A TYR, 554A GLY, 556A LYS, 641D GLY, and 669D GLU. Tercatain and Casuariin also bound to the nsP2 protein effectively, with binding energy scores of –16.5 and –10.6 Kcal/mol, respectively.

Interaction of the phytocompounds with E2 and E1 glycoprotein of Ross River virus associated with receptor binding and fusion with the membrane

Granatin A binds to both the E2 and E1 glycoproteins of the RRV. E2 had a binding energy of –21.7 Kcal/mol and E1 had a binding energy of –22.1 Kcal/mol. Tercatain and 2-O-Galloylpunicalin also bound to E2 glycoprotein effectively, with binding energy scores of –20.1 and –12.7 Kcal/mol, respectively. With a binding energy score of –12.4 Kcal/mol, 2-O-Galloylpunicalin likewise demonstrated efficient binding with E1 glycoprotein.

Mutagenicity and cytotoxicity

Majority of the compounds with binding scores less than –10 Kcal/mol were not mutagenic, except Nodakenin, Apiumoside, Homoorientin, and Isovitexin. No compound was predicted to be cytotoxic. Nodakenin followed all the rules of Lipinski.

Molecular Dynamics simulation

Throughout the simulations, the root means square deviation (RMSD) values were monitored for the protein as seen in Fig. 4. A lower RMSD value indicates a better agreement between the simulated structures. A high RMSD value may be indicating that the simulation has not correctly reproduced the experimental structure, or that the system has undergone important conformational changes during the simulation. The results shown in Fig. 4 depict that the RMSD of a protein increased quickly up to 1.3 Å. At that point RMSD flattened off and remained at approx. 1.3 Å till 70 ps and thereafter RMSD decreased and protein came in a relaxed state. However, the deviation in the RMSD was observed in the protein-ligand complex. Initially, the RMSD value increased to 9.5 Å which could be due to the thermal motion or force fields applied in the simulation but after 60 ps the structure showed a relaxed state via decreasing the RMSD.

Therefore, it could be concluded that the ligand and protein complex showed some significant interaction under the suitable range and with fewer fluctuations at 60 ps indicating the good integrity of docked structures.

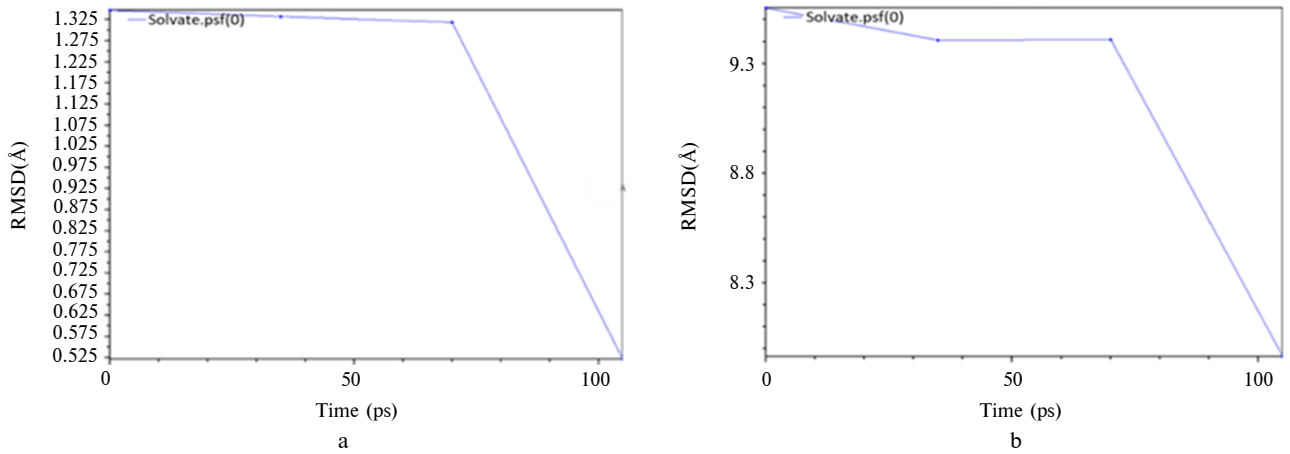


Figure 4: Root Mean Square deviation (RMSD) versus time: RMSD value of (a) protein and (b) protein-ligand complex

Discussion

This study's findings offer important insights into the potential creation of effective antiviral medicines against alphaviruses. Several potential compounds were discovered by studying the binding predictions of 375 compounds with critical proteins from three alphaviruses (SINV, CHIKV, and RRV). Granatin A, a naturally occurring chemical discovered in Punica granatum (pomegranate), has the highest affinity with low free binding energy with major viral proteins from all three alphaviruses, indicating that it is a good option for further antiviral research. It is a very weak basic elligatannin found in the pericarp of Pomegranate. It is known to be antihepatotoxic and antiperoxidant. Satomi *et al.*, 1993 [21] reported the inhibitory effects of elligatannins on carbonic anhydrase. Zahin *et al.*, 2010 [22] reported the antioxidant and antimutagenic activity of the active fraction obtained from the peel extracts of *Punica granatum* L. Sindbis E2 glycoprotein is a 423 amino acid Type I transmembrane protein that is responsible for cell receptor interaction during the life cycle of SINV [23], and the structure of E2 glycoprotein 1z8y of SINV was published by Mukhopadyay *et al.*, 2006 [24] by 9 Å resolution cryo-EM reconstruction of SINV. Granatin A, PubChem ID 131752596, bonds most efficiently with the E2 Glycoprotein of SINV virus (PDB ID:1z8y) with a binding affinity of –16.6 Kcal/mol, followed by Tercatain with a binding affinity of –13.7 Kcal/mol and Nodakenin with a binding affinity of –9.1 Kcal/mol. Granatin A breaks three of Lipinski's four rules and can be considered for additional *in vitro* research while keeping other modes of administration in mind rather than the oral route.

Nodakenin (see Fig. 2C), isolated from Angelica biserrate roots, interacted with the CHIKV nsP2 protein efficiently, demonstrating its potential as a multitargeted antiviral therapy. It possesses anti-inflammatory, antimicrobial, and antineoplastic activities. Lim *et al.*, 2020 [25] has reported Nodakenin's effects as an adjuvant treatment for preventing liver injury. Rim *et al.*, 2012 [26] discussed Nodakenin's neuroprotective and memory-enhancing properties. During virus replication, the Sindbis RNA-dependent RNA polymerase nsP4 enzyme generates minus strand RNA from plus strand templates and adds adenosine residues to the 3' end of an RNA substrate [27]. Tan *et al.*, 2022 [28] published the crystal structure of the SINV nsP4 protein. Furthermore, Punicalin and Punicalagin, both produced from *Combretum Glutinosum* and *Punica granatum*, displayed high binding with RRV E2 glycoproteins, which are recognized for their antioxidant and chemoprotective properties [29]. These chemicals have incredible antiviral uses, but further *in vitro* testing and inquiry are needed to determine their efficacy and safety in lowering viral growth and infection.

The surface envelope glycoprotein E2 of CHIKV present as E2-E1 heterodimer was reported to have a heparan sulfate binding pocket which is structurally conserved in alphaviruses and is positively charged [30]. E2 glycoprotein of CHIKV was expressed in Mesocricetus auratus and the structure was obtained using electron microscopy at a 5.3 Å resolution with PDB ID 6w09 [31]. Granatin A binds effectively with E2 glycoprotein of the CHIKV with a binding affinity of –20.6 Kcal/mol. The amino acids involved in interaction are shown in Fig. 3, C,D. It was followed by effective binding of Tercatain and Punicalin with energy score –17.8 and –12.9 Kcal/mol respectively.

Punicalin and Punicalagin showed effective binding with the protein targets. These are found in *Combretum Glutinosum*. A decoction from the roots, stems, and leaves of this plant is known to be used in hypertension, as a laxative, in hepatitis, etc. The leaves are also used for cough. "Dokhan" obtained from the burnt roots and stems of this plant is used as a medicine traditionally [32].

nsP2 glycoprotein of CHIKV is concerned with protease, RNA triphosphatase, NTPase, and RNA helicase activities and is also significant for suppressing the host cell transcription and counters the cellular antiviral responses [33]. nsP2 protein of CHIKV crystal structure with PDB ID: 4ztb was resolved at 2.59 Å with two subdomains which are N-terminal protease and C-terminal methyl transferase [34]. Granatin A also binds effectively with a binding energy of –18.6 Kcal/mol forming H-bond with AA residues of CHIKV nsP2 (Fig. 3, D,F). It was followed by Tercatain and Casuariin with binding energy scores of –16.5 and –10.6 Kcal/mol respectively. Casuariin is a natural product found in *Quercus salicinai* and *Osbeckia chinensis* [35].

Interaction of the phytocompounds with E2 and E1 glycoprotein of Ross River virus associated with receptor binding and fusion with membrane:

E1 and E2 glycoproteins of RRV present in the form of heterodimers in the envelope are responsible for receptor binding and membrane fusion as revealed by recent studies. The structure of envelope glycoprotein of RRV with PDB ID 6vyv was found using electron microscopy with 6.33 Å

resolution. Molecular docking in the present study indicated that the phytocompounds Granatin with binding energy –21.7 Kcal/mol, followed by Tercatain with binding energy –20.1 Kcal/mol and 2-O-Galloylpunicalin with a binding affinity score of –12.4 Kcal/mol bound effectively with E2 glycoprotein of RRV. Granatin A also showed effective binding with the E1 Glycoprotein of RRV with a binding energy score of –22.1 Kcal/mol. Tercatain and 2-O-Galloylpunicalin followed Granatin A with binding energy scores of –18.7 and –12.7 Kcal/mol respectively. 2-O-Galloylpunicalin is a natural product found in *Punica granatum* and *Terminalia macroptera* [35].

Majority of the compounds with binding score less than –10 Kcal/mol are not mutagenic except Nodakenin, Apiumoside, Homoorientin and Isovitexin. No compound was predicted to be cytotoxic. Protox-II server could not predict the results regarding cytotoxicity or mutagenicity of F-Gitonin and Maslinic acid. Out of all the compounds, only Nodakenin followed all the rules of Lipinski. The range of molecular weight for all these compounds was from 408.4−1084.72 g/mol with Nodakenin being the lightest and Punicalagin the heaviest. Friedelin showed the minimum TPSA of 17.07 Å and Punicalagin the maximum of 518.76 Å.

While our work focused on the binding affinity of these chemicals with viral proteins, it is critical to correlate our findings with molecular docking findings from other similar studies to further validate our findings. Such comparisons could add to the expanding body of knowledge in this sector by providing more insights into the potency and selectivity of the identified compounds.

To predict the interaction between the drugs and viral proteins, we used *in silico* approaches such as molecular docking and molecular dynamics simulations. While these computational approaches provide useful preliminary data, it is critical to recognize their limits. Real-world experimental validations are required to confirm the observed interactions and biological activities because *in silico* simulations do not fully reproduce the complexity of biological systems. Furthermore, a significant drawback of our work is the lack of *in vivo* and *in vitro* experiments employing cell culture or animal models. *In vitro* studies could provide vital information on the compounds' antiviral activity and cytotoxicity, while *in vivo* studies could help researchers grasp their pharmacokinetics and safety profiles.

Additionally, the potential effects of other physiological factors, such as metabolism and distribution in the body, should be considered in the context of drug development.

Finally, our research paves the way for the creation of novel antiviral medications that target alphaviruses by utilizing natural chemicals with established binding affinities to essential viral proteins. To develop these molecules towards therapeutic applications, however, intensive experimental studies and a thorough understanding of their pharmacological characteristics are required. Furthermore, future research should include broader partnerships, such as comparisons with other docking studies, to improve the robustness and translational potential of our findings. By overcoming these restrictions, we can pave the path for potential solutions to alphavirus-related global health concerns.

Conclusions

Finally, the purpose of this study was to investigate the binding predictions of 375 phytocompounds with essential proteins of alphaviruses, specifically the E2 glycoproteins of SINV, CHIKV, and RRV, as well as nsP4 of SINV and nsP2 of CHIKV. To evaluate the interactions between the compounds and target proteins, molecular docking, modelling, and molecular dynamics simulations were used. The results revealed that Granatin A had effectively bound with all target proteins, including SINV nsP4, CHIKV E2, and CHIKV nsP2. A molecular dynamics simulation of the combination of CHIKV E2 and Granatin A revealed that it remained stable for 60 ps. Despite breaching several of the rules, Granatin A, a natural chemical discovered in Punica granatum, has promising binding capabilities. Tercatain and Nodakenin also demonstrated effective binding with the target proteins. The findings suggest that these phytocompounds could be potential candidates for further investigation as anti-alphaviral therapeutic agents. Granatin A, in particular, displayed strong binding affinity with critical proteins involved in virus-host cell interactions and viral replication. The study also highlighted the potential biological activities of the identified phytocompounds, such as anti-inflammatory, antioxidant, antimutagenic, and hepatoprotective properties, which further support their potential as therapeutic agents.

Interests disclosure

Authors don't have any conflict of interests to declare.

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М. Шарма, А. Бансал, Ш. Суман, Н.Р. Шарма

Кафедра біотехнології, Школа біоінженерії та біонаук, Благосний професійний університет, Фагвара, Пенджаб, Індія

ПОТЕНЦІЙНІ ІНГІБІТОРИ АЛЬФАВІРУСІВ ІЗ ФІТОСПОЛУК: ПІДХІД НА ОСНОВІ МОЛЕКУЛЯРНОГО ДОКІНГУ ТА МОЛЕКУЛЯРНОЇ ДИНАМІКИ

Проблематика. Альфавірусні захворювання є економічним тягарем у всьому світі через їх хронізацію та глобальне поширення. Глікопротеїни E1 і E2 важливі для зв'язування з поверхнею клітини-господаря шляхом взаємодії з рецепторами, а неструктуровані білки nsP2 і nsP4 важливі для реплікації вірусу, тому можуть бути важливим цільовим об'єктом для розробки ліків.

Мета. Дослідити взаємодію *in silico* між рослинними сполуками (фітосполуками) та специфічними білками-мішенями, такими як протеїн nsP4 і глікопротеїн E2 віруса Сіндбіс (SINV), nsP2 і E2 віруса Чікунгунья (CHIKV) та глікопротеїни E1 і E2 віруса Росс Рівер (RRV).

Методика реалізації. З використанням баз даних підготовлено бібліотеку фітохімічних речовин з індійських лікарських рослин, яку перетворено на 3D-структури. Визначено й уточнено структури білків (nsP2, nsp4, E1, E2), після чого проведено молекулярне докінгування за допомогою AutoDock Vina. Перспективні ліганди оцінювали за властивостями, цитотоксичністю та мутагенністю, враховуючи подібність до ліків і потенційні проблеми. Моделювання методом молекулярної динаміки дало змогу оцінити стабільність комплексу.

Результати. З використанням молекулярного докінгу, моделювання та молекулярної динаміки проаналізовано 375 фітосполук проти цих мішеней для білків вірусів SINV, CHIKV і RRV. Встановлено, що гранатин А успішно зв'язується з цільовими сайтами SINV nsP4, CHIKV E2 і CHIKV nsP2 зі значеннями афінності зв'язування –16,2, –20,6 і –18,6 Ккал/моль відповідно. Крім того, стабільність комплексу CHIKV E2 – гранатин А була досягнута завдяки виконанню молекулярно-динамічного моделювання, і комплекс залишався стабільним при 60 пс.

Висновки. Наше дослідження надає важливі дані для розробки ефективних противірусних препаратів проти альфавірусів, акцентуючи увагу на важливості природних сполук та їх взаємодії з вірусними білками. Отримані результати відкривають шлях для подальшого вивчення цих малих молекул як ефективних антивірусних терапевтичних препаратів проти альфавірусів.

Ключові слова: альфавірусні захворювання; глікопротеїни; nsP2; nsP4; молекулярне докінгування; гранатин А.