Journal of the Korean Chemical Society
2023, *Vol. 67, No. 4*
Printed in the Republic of Korea
https://doi.org/10.5012/jkcs.2023.67.4.226

Flavonoids as Novel Therapeutic Agents Against Chikungunya Virus Capsid Protein: A Molecular Docking Approach

E. Vadivel*, Gundeep Ekka, and J. Fermin Angelo Selvin†

*Post - Graduate Chemistry Department, Dnyanprassarak Mandal's College and Research Center, Assagao - Bardez, Goa-403 507, India. *E-mail: velpharmamsu@gmail.com*
†Department of Physics, NMSSVN College, Madurai, Tamilnadu- 625019, India.
(Received May 23, 2023; Accepted July 8, 2023)

ABSTRACT. Chikungunya fever has a high morbidity rate in humans and is caused by chikungunya virus. There are no treatments available until now for this particular viral disease. The present study was carried out by selecting 19 flavonoids, which are available naturally in fruits, vegetables, tea, red wine and medicinal plants. The molecular docking of selected 19 flavonoids was carried out against the Chikungunya virus capsid protein using the Autodock4.2 software. Binding affinity analysis based on the Intermolecular interactions such as Hydrogen bonding and hydrophobic interactions and drug-likeness properties for all the 19 flavonoids have been carried out and it is found that the top four molecules are Chrysin, Fisetin, Naringenin and Biochanin A as they fit to the chikungunya protein and have binding energy of -8.09, -8.01, -7.6, and 7.3 kcal/mol respectively. This result opens up the possibility of applying these compounds in the inhibition of chikungunya viral protein.

Key words: Binding affinity using Autodock, Chikungunya virus capsid protein, Molecular docking of flavonoids, Biochanin A, Chrysin, Fisetin and Naringenin

INTRODUCTION

Chikungunya is a viral disease that threatens human health worldwide nearly in 55 different countries. The Chikungunya (CHIKV) virus was first diagnosed in 1952 in southern Tanzania.[1] It spread from Africa to Asia, Islands of Indian Ocean, Europe, Australia and America. In 2008 National institute of allergy and infection diseases, USA has listed Chikungunya in the category C priority pathogen group. It is primarily transmitted to humans by two mosquito species namely *Aegypti* and *Albopictus*. The main symptoms of this viral infection are fever and severe joint pain alongside headache and vomiting. More severe infection is caused to newborn children, neonatal infants, and adults of more than 65 years of age, people with high blood pressure, diabetes and heart patients. It is also reported that if a person is infected once, that person is protected from the future infections occurring through Chikungunya virus[2] through the development of antibodies. At present there are no vaccines or effective drugs are available to prevent or inhibit the Chikungunya virus.

CHIKV belongs to *Alphaviruses* which is one of two genera in the family of *Togaviridae.* Alphaviruses are enveloped, positive-sense single-stranded RNA viruses that are transmitted by mosquitoes. The genus alphavirus contains 29 members of viruses that infect a variety of animals such as humans, horses, rodents and fish. Other members of this genus include Ross River virus (RRV), Sindbis virus (SINV), Semiliki Forest virus (SFV), Western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV). They are further classified as Old World and New World viruses based on the mechanism of shutting the host transcription off, mortality rate and disease appearance.[3] The Old World viruses such as SINV, CHIKV and SFV, etc. utilize the nsp2 protein to suppress the host cell transcription, have a low mortality rate and cause arthralgia. The New World viruses such as VEEV, WEEV, etc. utilize their capsid protein (CP) to reduce the host cell transcription, have a high mortality rate and cause encephalitis.[4,5]

The CHIKV CP shares ~93% sequence homology with Semliki Forest virus capsid protein. CHIKV CP crystallizes as a dimer in the asymmetric unit whereas it is a monomer in solution form. The Structure of CHIKV CP exhibits a chymotrypsin-like protease fold with a conserved hydrophobic pocket for interaction with the cytoplasmic tail of E2 (cdE2) which is similar to the capsid protein of other alphaviruses. Dioxane, Picolinic acid, Mandelic acid and Ethyl 3-aminobenzoate were docked against CHIKV CP hydrophobic pocket.[3]

Studies on the crystal structure of capsid protease in complex with dioxane from Sindbis and Aura virus[6-8] and studies of alphavirus inhibition by heterocyclic compounds such as

dioxane derivatives and piperazine derivatives have been carried out.[9-10] A structural study on CHIKV CP by docking the Dioxane, Picolinic acid, Mandelic acid and Ethyl 3-aminobenzoate has revealed that the hydrophobic pocket of CP is a potential antiviral drug target.[11]

There is no specific antiviral drug treatment for Chikungunya as of now. Treatment is directed primarily at relieving the symptoms, including the joint pain using antipyretics, optimal analgesics and fluids. There is no commercial Chikungunya vaccine so far and much research is put in its way to create a vaccine.[12] There are no approved antiviral treatments currently available for CHIKV.[13] Currently, CHIKV is treated symptomatically, usually with non-steroidal anti-inflammatory drugs or steroids, bed rest, and fluids. A combination of mild exercises and movements can decrease the stiffness and morning arthralgia, whereas heavy exercises may worsen rheumatic symptoms. In vitro testing suggests several drugs effective against CHIKV which include Chloroquine, Ribavirin, α-Interferon, Arbidol, Favipiravir, and Furin inhibitors. However, there is no specific antiviral treatment against CHIKV infection.[14] Meclofenamic acid in combination with Ribavirin exhibited significant anti-CHIKV activity by impairing viral replication *in vitro* and *in vivo*.[15]

Several studies have been undertaken to estimate the efficacy of ayurvedic medicines in treating CHIKV. The studies reveal that a large number of ayurvedic medicines are being prescribed in Kerala including, Vettumaran gulika, Sudarsanam gulika, Amritarishta. Amruthotharam kashayam, Amruthadiguggulu, Kwatha churna, Vilvadi gulika Rasnaeranda Vanathulasi patra, and Dhanvantaram gutika in the treatment of the disease are found to be efficient. Medicinal plants such as *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Chromolaena odorata* were recommended for CHIKV especially for pain relief.[16] Phytochemicals present in the medicinal plants are responsible for the biological activity of the plant extracts.

Flavonoids are naturally occurring phytochemicals widely found in plants and are responsible for a wide variety of biological activities such as anti-oxidant, anti-inflammatory, anti-ulcer, anti-viral, anti-cancer, anti-diabetic, antimicrobial and immunomodulatory functions.[17,18] More than 6000 compounds have been identified as flavonoids and classified into flavonols, flavanones, isoflavones, flavones, and anthocyanidins. The effect of Flavonoids has been studied against a variety of DNA and RNA viruses. In general, flavonoids exhibit several mechanisms through which they regulate the biological function. Flavonoids can block attachment and entry of viruses into host cells, interfere with various stages of viral replication processes or translation and polyprotein processing to prevent the release of the viruses to infect other cells. Different flavonoids have been found to inhibit the wide variety of viruses through various mechanisms of action. Flavonoids can be prophylactic inhibitors, therapeutic inhibitors otherwise indirect inhibitors by interaction with the immune system. Due to the wide range of biological activities exhibited by flavonoids, they have become molecules of interest for natural drug discovery research.[19]

Molecular docking is one of the widely used methods in structure-based drug design. Docking is a method used to predict proper orientation between two different molecules to form a stable complex when the molecules are bound to each other. Preferred orientation is in turn used to predict the strength of the binding affinity between two molecules using scoring functions.[20] Molecular docking plans to reach an optimized conformation for both protein and ligand so that the free energy of the overall system is minimized. Molecular recognition plays an important role in promoting enzyme-substrate interaction, drug-protein interaction and the drug-nucleic acid interaction. The general principles which govern the nature of the interactions are H-bonding, hydrophobic interaction, van der Waals interaction, etc. between the ligand and the protein target which provides the base for the design of specific potential drug which is responsible for the given therapeutic effect.[21]

In the present study, we have selected 19 flavonoids from the herbal formulations used for Chikungunya in Kerala which have natural abundance and wide range of infectious activity. All the selected Flavonoids are docked at the binding site of the Chikungunya viral capsid protein to find out the potential flavonoids, which can inhibit the Chikungunya viral disease by binding to its capsid protein and can be used as a lead compound in the development of a new anti-viral drug.

EXPERIMENTAL

Materials and Methods

Softwares. Softwares used were Autodock 4.2 (Scripps Research Institute), Discovery studio 4.0 client (Accelrys) and DruLiTo (NIPER).

Ligand generation. The two-dimensional (2D) chemical structures of the selected flavonoids were obtained from PubChem data bank and energy minimized using MMFF94, then saved as pdb file. The 2D structures of 19 selected flavonoids are shown in *Fig.* 1.

Protein preparation. Protein target was the Crystal

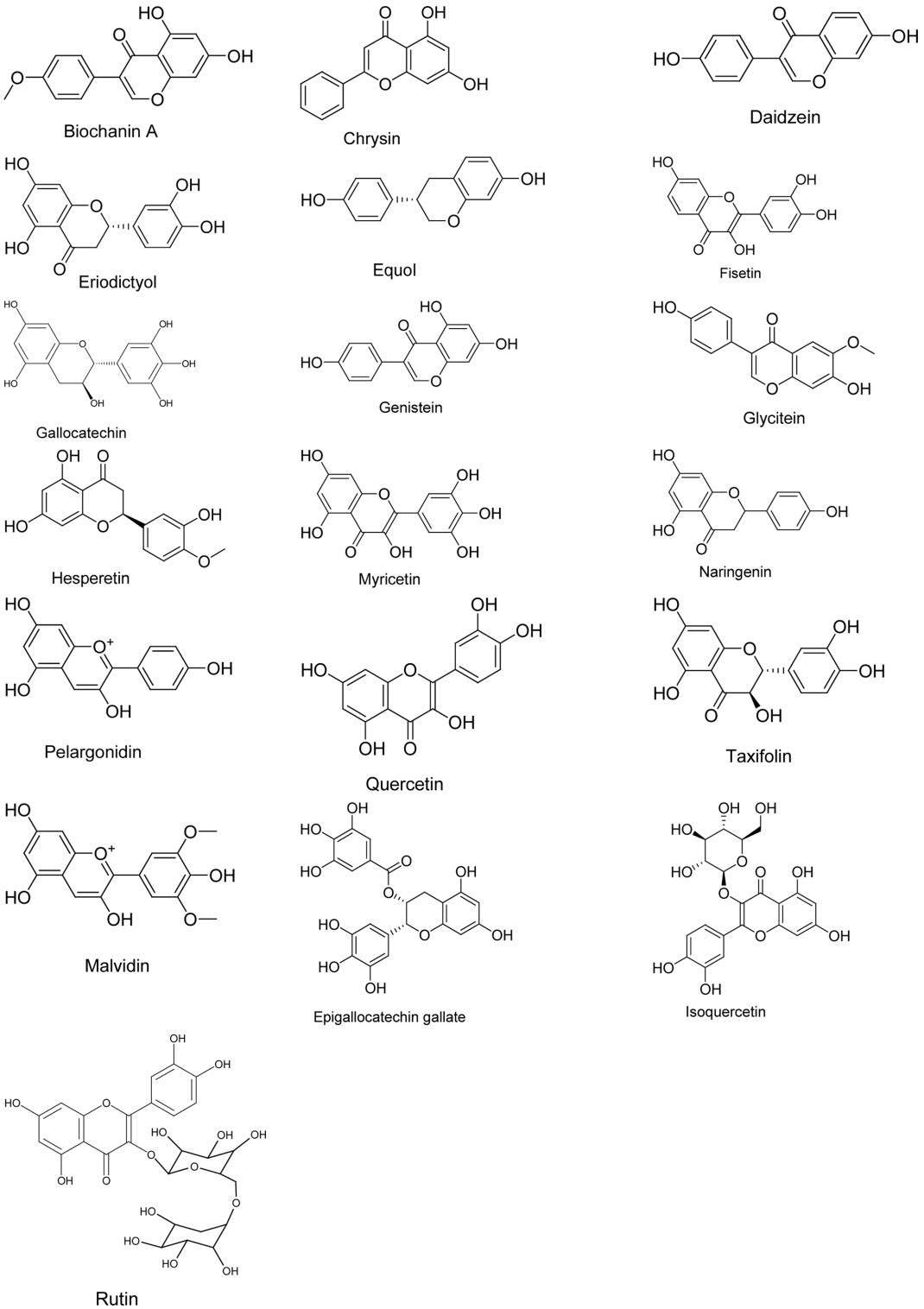

***Figure* 1.** List of Selected Naturally occurring flavonoids structures.

structure of Chikungunya virus capsid protein (PDB code: 5h23; resolution: 2.20 Å; R-value free: 0.261; R-value work: 0.176) downloaded from Protein Data Bank (http://www.rcsb.org). As preliminary conditioning, the protein is checked for errors and possible interactions of water molecules present in the crystal structure were analyzed

(H-bonding and Hydrophobic interactions). After analysis, it is decided to delete water molecules from the crystal structure and natural ligand present in it using Discovery studio 4.0 client. The three-dimensional structure of the protein along with the natural ligands is shown in *Fig*. 2.

Drug-likeness. The drug-likeness properties of selected flavonoids were calculated by DruLiTo software using Lipinski's rule.

Molecular docking. Binding mode and interaction of capsid protein 5H23 of CHIKV with selected flavonoids were performed using AutoDock4.2 software.

Docking was performed to obtain a population of possible conformations and orientations for the ligands, in our case the flavonoids at the binding site.[22] The protein code for CHIKV is 5h23 with a resolution of 2.2 Å. The protein chain of CHIKV is constituted by 261 amino acids in chain A and 261 amino acids in chain B. Since the two chains are identical in their secondary structure, only Chain A was chosen for docking study. The protein contains natural ligand such as GOL, PEG, EDO, and BGO. The protein was loaded in Autodock4.2 software, the polar hydrogen atoms, Kollman and Gasteiger charges were added to amino acid residues of the protein structure and converted to PDBQT format that is free from water molecules and natural ligands.[23]

For flexible docking, all the bonds of ligands were set to be rotatable and for rigid docking, all the bonds of ligands were set to be rigid i.e. non-rotatable. The docking site on the protein target was set using a grid box with the dimensions of X: 126 Å, Y: 126 Å, Z: 126 Å. The best conformation was chosen with the lowest docked binding energy after the docking search was completed. Ten runs with AutoDock4.2 were performed in all cases for each ligand structure, and for each runs the best pose was saved.

Intermolecular Interaction

The interactions of complex CHIKV protein and ligand conformations, including hydrogen bonds and hydrophobic interactions were analyzed using Discovery studio 4.0 clients.

RESULTS AND DISCUSSION

Drug-likeness Property

Drug-likeness is used as one of the sort out method in the virtual screening of chemical databases with the purpose to screen in those molecules which have the property similar to that of known drugs.[24] Lipinski's rule of five is used as a principle filter for screening the chemical compounds.

Where, MW-Molecular weight, HBD-No. of hydrogen bond donors, HBA-No. of hydrogen bond acceptor.

Lipinski's rule of five filters was used to calculate the drug-likeness properties of flavonoids. If the molecule in

***Table* 1.** The drug-likeness properties of flavonoids

Name with ID	MW	logP	HBA	HBD
Value to be	≤ 500	≤ 5	≤ 10	≤ 5
Biochanin (1)	284.07	1.364	5	2
Chrysin (2)	254.06	1.852	4	2
Daidezin (3)	254.06	1.124	4	2
Epigallocatechin gallate (4)	458.08	2.984	11	8
Equol (5)	242.09	1.182	3	2
Eriodictyl (6)	288.06	1.138	6	4
Fisetin (7)	286.05	1.915	6	4
Gallocatechin (8)	306.07	1.2	7	6
Genistein (9)	270.05	1.043	5	3
Glycetein (10)	284.07	1.364	5	2
Hesperetin (11)	302.08	1.03	6	3
Isoquercetin (12)	464.1	0.099	12	8
Malvidin (13)	331.08	2.099	6	4
Myricetin (14)	318.04	2.182	8	6
Narinenin (15)	272.07	0.79	5	3
Palargonidin (16)	271.06	1.619	4	4
Quercetin (17)	302.04	1.834	7	5
Rutin (18)	610.15	-1.027	16	11
Taxifolin (19)	304.06	0.803	7	5

***Table* 2.** Binding affinity of selected flavonoids With CHIKV protein

Sl. No	Name of the Flavonoids with Mol ID	Binding energy of Rigid Docking (Kcal/mol)	Binding energy of Flexible docking (Kcal/mol)
	Biochanin (1)	- 6.05	- 7.3
	Chrysin (2)	-6.49	-8.09
	Daidzein (3)	- 6.16	- 7.21
	Epigallocatechin gallate (4)	- 4.53	- 5.13
	Equol (5)	- 6.12	- 7.28
	Eriodictyl (6)	- 6.88	- 6.78
	Fisetin (7)	- 6.56	- 8.01
	Gallocatechin (8)	- 4.85	- 6.71
	Genistein (9)	-6.04	-6.96
	Glycetein (10)	- 6.1	-7.01
	Hesperetin (11)	-5.87	-6.59
	Isoquercetin (12)	- 5.24	-6.59
	Malvidin (13)	-4.91	-6.7
	Myricetin (14)	-5.46	-6.58
	Narinenin (15)	-6.8	-7.6
	Palargonidin (16)	-5.96	-6.45
	Quercetin (17)	-7.04	-6.56
	Rutin (18)	-3.42	-3.0
	Taxifolin (19)	-7.1	-6.32
	BOG (Natural Ligand)	-3.5	-3.4
	EDO (Natural Ligand)	- 2.58	-2.7
	PEG (Natural Ligand)	- 2.49	-3.15
	GOL (Natural Ligand)	-3.07	- 3.42
	Chloroquine	-5.6	-4.8
	Favipiravir	-4.8	-5.6

analysis violates any one of the rules, it loses drug-like properties. In the present study, the 19 flavonoids are checked for the rules, five flavonoids, Epigallocatechin gallate, Gallocatechin, Isoquercetin, Myricetin and rutin showed some violation. All other flavonoids show drug-like properties. The selected flavonoids along with their drug-likeness properties are listed in *Table* 1.

Molecular Docking

Docking was performed to obtain the best fit ligand at the binding site. Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure-based drug design process. The parameters chosen for the docking can be evaluated by the docking tool's ability to reproduce the binding mode of a ligand against protein when the structure of the ligand-protein complex is known. The ten different orientations of the selected 19 flavonoids to the receptor CHIKV protein were carried out. The binding affinities of the selected flavonoids with the CHIKV protein are presented in *Table* 2.

The natural ligands (GOL, PEG, EDO and BGO) of CHIKV CP contains 2 to 4 hydroxyl groups. The natural ligands are very small compared to the active site cavity. The flavonoids occupy higher molecular volume in the active site cavity than natural ligands that may be the reason for its higher binding energy with CHIKV protein. Standard antiviral drugs such as Chloroquine and Favipiravir shows higher binding energy than natural ligands and less than most of the flavonoids due to its moderate molecular volume.

The flexible docking showed good binding affinity than that of rigid docking towards the CHIKV protein. It is because flexible ligands have rotatable bonds that can rotate and form a more stable and effective conformer. In rigid docking, it is not possible because the rotatable bonds are converted to non-rotatable bonds. Hence flexible ligand shows better binding affinity than rigid ligands. Quercetin, Taxifolin and Rutin showed higher binding energy in rigid docking compared to flexible docking. In some cases, high flexibility may lead to more unrealistic

***Table* 3.** Hydrogen bonding interactions of selected flavonoids with CHIKV protein (5h23) by rigid docking

MOL ID	Atom of Compound Involving Interaction	Amino acid Residue Involving Interaction	Atom of Amino acid Residue Involving Interaction	Distance (A°)
1	O	ASP248	NH	1.72
	H	GLU184	O	2.15
	O	ASP248	NH	2.07
2	-	-	-	-
3	H	VAL243	O	2.01
4	O	ASP248	NH	1.94
	O	ASN246	H	1.99
	H	GLU181	O	1.55
5	-	-	-	-
6	O	CYS113	NH	2.00
	O	ARG215	H	1.99
	O	ASP112	NH	2.67
	O	ILE114	NH	2.24
7	O	ASP112	NH	1.68
	O	ARG215	H	1.79
	H	ILE114	O	2.17
8	O	ASP248	NH	2.07
	O	GLU184	NH	2.12
	H	ASP248	O	2.09
9	O	ASN111	H	2.17
	O	ARG215	H	2.O1
	O	HIS190	NH	1.96
10	H	VAL243	O	2.00
11	O	LYS155	H	2.17
12	O	ASN246	H	1.98
	O	ASP248	H	1.76
	O	LYS247	H	3.03
13	O	ARG156	H	1.95
	H	PHE154	O	2.43
	H	GLU163	O	1.86
	H	GLY142	O	2.11
14	H	GLU181	O	1.88
	H	HIS180	O	1.80
	O	LYS182	H	2.19
	H	TYR196	O	1.91
15	H	GLU116	O	1.99
	O	ASP112	H	1.68
	H	CYS113	O	1.99
	O	ILE114	H	2.25
16	O	ASP132	H	2.17
17	O	ASP112	H	1.69
	H	GLU116	O	2.03
	H	ILE114	O	2.12
	O	CYS 113	H	2.01
	O	ARG215	H	1.90
18	H	LYS159	O	1.80
	H	TYR254	O	1.88
	O	LYS252	H	2.13
19	H	GLU116	O	2.10
	O	ARG215	H	2.09
	H	ILE114	O	1.96
	O	ILE114	H	2.24
	O	ASP112	H	1.69
	O	CYS113	H	2.03

***Table* 4.** Hydrogen bonding interactions of selected flavonoids with CHIKV protein (5h23) by flexible docking

MOL ID	Atom of Compound Involving Interaction	Amino acid Residue Involving Interaction	Atom of Amino acid Residue Involving Interaction	Distance (A°)
1	O	LYS155	H	1.93
2	H	ASP132	O	1.73
	O	ASP132	H	2.08
3	H	VAL243	O	1.89
4	H	GLU181	O	1.90
	O	LYS247	H	1.96
	O	ASP248	H	2.89
5	H	VAL243	O	2.14
6	H	GLU116	O	2.19
	O	HIS190	H	2.19
	O	TYR126	H	2.17
	H	PRO216	O	1.82
7	O	ASP132	H	1.91
	H	ASP132	O	1.90
	H	VAL243	O	1.94
8	O	LYS182	H	1.77
	H	HIS180	O	1.86
	H	GLU184	O	2.02
	H	GLU184	O	2.09
	H	GLU181	O	1.84
9	-	-	-	-
10	H	VAL243	O	1.82
11	O	TYR216	H	2.00
	O	HIS190	H	2.24
12	H	LYS252	O	2.10
	H	LYS252	O	2.07
	O	LYS252	H	2.02
	O	LYS252	H	1.95
	H	THR254	O	2.08
	O	THR254	H	1.98
13	H	ASP248	O	1.98
	O	ASP248	H	1.89
	H	LYS182	O	2.20
	O	GLU184	H	2.20
14	H	PRO216	O	1.80
	O	TYR126	H	2.13
	H	Glu116	O	2.06
15	H	ASP132	O	1.95
16	H	ASP248	O	2.03
	O	ASP248	H	1.93
	H	LYS182	O	1.99
17	O	ILE144	H	1.90
	O	ARG156	H	1.84
	O	ARG156	H	2.24
	O	GLY142	H	2.00
	H	GLU163	O	1.85
18	O	ARG156	H	1.77
	H	GLU163	O	2.33
	H	GLU163	O	1.83
	H	GLY142	O	1.82
	H	ALA138	O	2.17
19	H	THR124	O	1.93
	O	GLU260	H	2.12
	O	LYS141	H	2.00
	H	HIS139	O	2.09

conformers which bind to the non-active site. Considering these points, a rigorous analysis has been made and resulted in four best binding energy modes of flavonoids, Chrysin (-8.09 kcal/mol), Fisetin (-8.01 kcal/mol), Naringenin (-7.6 kcal/mol) and Biochanin A (7.3 kcal/mol). Chrysin, Fisetin and Naringenin have common Hydrogen bonding with ASP132 residue.

The computational study is supported by the following experimental findings. The molecular docking results coincide with reported DFT studies of Chrysin, Fisetin and Naringenin in which Chrysin showed low energy gap.[25] Chrysin exerts a strong inhibitory effect on Enterovirus 71 replication.[26] Fisetin shows a strong inhibitory effect on the dengue virus.[27] Naringenin inhibits chikungunya virus replication by *invitro*.[28] Almost all the selected flavonoids are more active than the natural ligands.

Intermolecular Interactions. The weak intermolecular interactions such as hydrogen bonding and hydrophobic interactions are key players in stabilizing energetically-favored ligands in an open conformational environment of protein structure. The hydrogen bonding and hydrophobic interactions of the best orientations of the selected flavonoids in rigid docking and flexible docking methods are presented in *Table* 3 and *Table* 4 respectively.

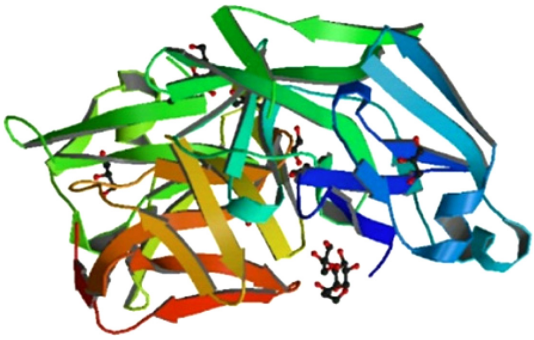

***Figure* 2.** 3-D structure of chikungunya protein.

Results of molecular docking of Mandelic acid (MDA) and Ethyl 3-aminobenzoate (EAB) suggested that these two molecules have a good binding affinity for the hydrophobic pocket of CHIKV CP consists of VAL130, VAL250, TRP245, LYS133, PHE178, ASP132, GLY1311 and MET135. All the active flavonoids bind to the same hydrophobic pockets and form similar interactions. MDA had hydrogen bonding with ASP132 similarly best active flavonoids such as Chrysin, Fisetin and Naringenin also show hydrogen

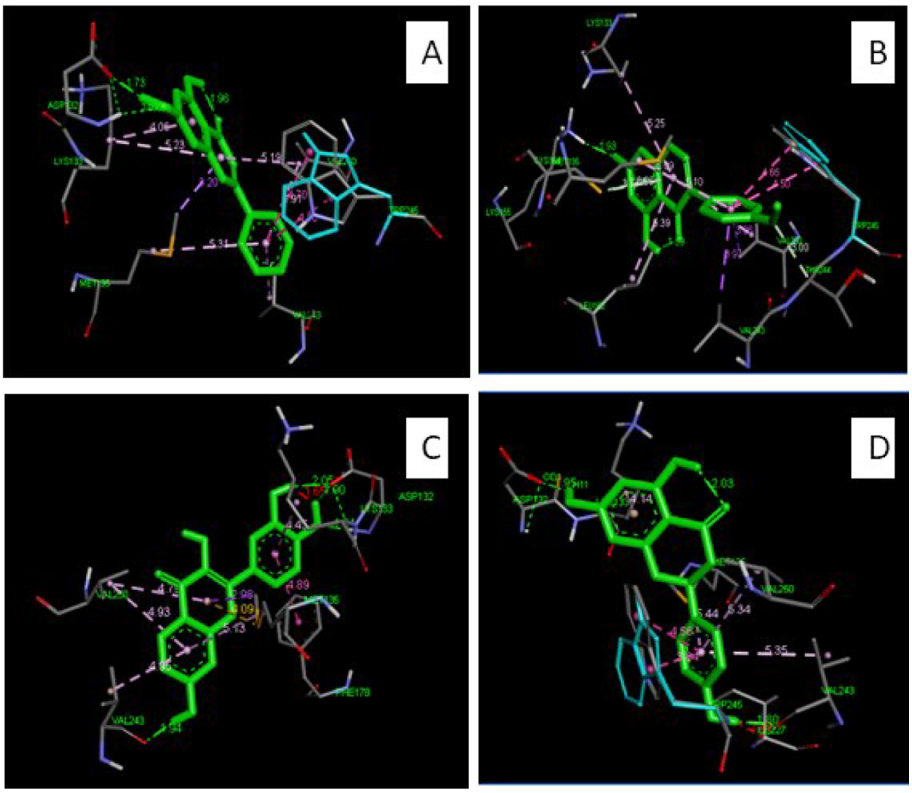

***Figure* 3.** Highest binding affinity and the most active flavonoids are shown in the above figure; (A) Chrysin, (B) Biochanin A, (C) Fisetin and (D) Naringenin.

***Table* 5.** Hydrophobic interactions of selected flavonoids with CHIKV protein

MOL ID	Rigid docking	Flexible docking
1	PRO183 (4.88), LYS182(5.48, 2.90)	CYS164(3.73,3.96), LEU162(5.39), LYS133(5.25), MET135(5.09,5.10), VAL250(4.95,3.96), VAL243(3.97), TRP245(4.50, 4.66)
2	VAL250(5.06,3.88,5.25), CYS164(4.63), MET135(4.82, 5.00), VAL243(3.85), LEU162(5.34), ILE227(5.37), TRP245(5.14, 4.66)	LYS133(4.06,5.23), MET135(5.31, 3.20), VAL243(4.91), VAL250(4.91,5.19), TRP245(4.16, 4.70)
3	ILE227(5.47),VAL243(4.97), MET135(4.03,4.81), VAL250(5.35,3.57,5.40), LYS133(5.22), TRY245(4.56), ILE227(5.47)	LYS133(4.77), CYS164(5.01,5.40), MET135(3.81,5.42,4.00), LEU162 (5.41), VAL243(5.01), TRP245(4.63,4.02), VAL250(3.99, 4.99)
4	TRY196(5.65), LYS182(5.14,3.05), PRO183(4.73, 4.19)	ASN246(3.15), LYS182(4.97,3.61), PRO183(4.44), GLU181(4.35)
5	VAL250(4.83,4.01,4.81),TRP245(5.27),MET135(5.21,5.21), VAL243(3.70)	LEU162(5.40), MET135(5.03,5.31), VAL243(5.18), TRP245(4.07,4.79,5.15), VAL250(5.42, 3.90, 4.57)
6	ILE114(3.24), CYS113(5.36), TYR126(4.98), HIS190 (5.81)	ILE114(5.17, 4.72), TYR126(4.93), PRO216(4.68), ARG215(4.88)
7	CYS113(5.34), ILE114(3.48,3.22, 3.88), TYR126(4.75), HIS190(5.93)	LYS133(4.45), PHE178(4.89), MET135(2.98,5.13), VAL250(4.79, 4.93), VAL243(4.95)
8	PRO183(5.18)	LYS182(5.13), LYS247(3.67)
9	HIS190(4.82,4.80), ARG215(4.81,4.93), PRO216(4.70), ILE114(4.70,3.54,3.07), TYR126(5.50)	VAL250(5.16,4.41,5.04), TRP245(4.46,3.88), CYS164(4.81), LYS133(5.42), MET135(3.51,5.40), VAL243(5.25)
10	LYS133(5.25), CYS164(4.75), MET135(3.77), LEU162 (5.35), VAL243(4.86), VAL250(4.71, 4.87)	TYR126(4.83), ILE114(4.82), HIS190(4.38), PRO216(3.57)
11	VAL250(5.44,3.57,5.45), TRP245(4.52), MET135(5.46, 4.79), LYS133(5.14), ILE227(5.49)	VAL243(4.88), LEU162(5.40), MET135(5.37,3.93), CYS164(5.18), CYS133(4.94), TRP245(4.76,4.15), VAL250(5.00, 3.80, 5.40)
12	PHE154(4.99,5.15)	LYS182(5.40) PRO183(4.87)
13	LYS247(5.08,2.86,5.10), ASP248(3.26)	TYR126(4.94), ILE114(5.25,3.64,4.82), ARG215(4.87), PRO216(4.94), HIS190(4.20, 4.58)
14	ILE114(3.24),CYS113(5.43), TYR126(4.65), HIS190 (5.80)	LYS133(4.14), MET135(5.44), VAL243(5.35), VAL250(5.34), TRP245(3.84, 4.56)
15	MET135(4.69),VAL243(5.20), VAL250(4.86), LYS133 (4.99, 4.32)	LYS182(3.15, 5.35), PRO183(5.00)
16	CYS113(5.41), ILE114(3.30,3.98, 3.53), HIS190(5.90), TYR126(4.80)	PHE154(4.68,5.96), ILE144(5.46), LYS141(3.84)
17	CYS113(5.39), ILE114(3.18), TYR126(4.92), HIS190(5.81)	-
18	PRO183(4.75, 4.74, 3.79)	LYS159(5.45, 4.74, 4.79), LYS252(5.39)
19	TYR251(3.38), ILE253(3.89,4.48)	ALA150(5.24)

bonding[3] with ASP132 (*Fig*. 3). The hydrophobic interactions are listed in *Table* 5.

CONCLUSION

The rigid and flexible docking of 19 natural flavonoids against the chikungunya protein was performed using Autodock 4.2 software. The rigid and flexible ligands bind in different binding packets in the target protein. Normally flexible ligand shows higher binding energy but in some cases, flexibility reduces the binding energy. It may be due to the formation of different conformers which may bind in the non-active site. The present study concludes that Chrysin, Fisetin, Naringenin and Biochanin A are found to be most active against chikungunya viral protein. The results indicate that molecular modeling is a valuable tool for predicting the biological activity of flavonoids. The analysis of the docking result allowed us to know the efficiency of the flavonoids to control Chikungunya. In future, molecular dynamics and Pharmacophore mapping may tune the inhibitors for Chikungunya.

Acknowledgments. The authors are thankful to Research Centre and the Management of Dnyanprassarak Mandal's College for their encouragement and support in carrying out the work. Publication cost of this paper was supported

by the Korean Chemical Society.

Supporting Information. *Table* S1 showing the drug likeness properties using MDDR-like rule, Veber Rule, Ghose Filter, BBB Rule and CMC-50 like rule. *Fig*. S1 showing the Hydrogen bonding and hydrophobic interaction of flavonoids with active site amino acids of CHIKV protein.

REFERENCES

1. Lumsden, W. H. R. *Trans. R. Soc. Trop. Med. Hyg*. **1955**, *49*, 33.
2. Rahman, S.; Suchana, S.; Rashid, S.; Pave, O. A Review Article on Chikungunya Virus. *World J. Pharm. Res.* **2017**, *6*, 100.
3. Sharma, R.; Kesari, P.; Kumar, P.; Tomar, S. *Virology.* **2018**, *515*, 223.
4. Garmashova, N.; Gorchakov, R.; Volkova, E.; Paessler, S.; Frolova, E.; Frolov, I. *Virol. J.* **2007**, *81*, 2472.
5. Hahn, C. S.; Lustig, S.; Strauss, E. G.; Strauss, J. H. *PNAS.* **1988**, *85*, 5997.
6. Aggarwal, M.; Tapas, S.; Preeti.; Siwach, A.; Kumar, P.; Kuhn, R. J.; Tomar, S. *PLOS ONE.* **2012**, *7*, e51288.
7. Choi, H. K.; Lee, S.; Zhang, Y. P.; McKinney, B. R.; Wengler, G.; Rossmann, M. G.; Kuhn, R. J. *J. Mol. Biol.* **1996**, *262*, 151.
8. Lee, S.; Kuhn, R. J.; Rossmann, M. G. *Proteins.* **1998**, *33*, 311.
9. Kim, H. Y.; Patkar, C.; Warrier, R.; Kuhn, R.; Cushman, M. *Bioorganic Med. Chem. Lett.* **2005**, *15*, 3207.
10. Aggarwal, M.; Kaur, R.; Saha, A.; Mudgal, R.; Yadav, R.; Dash, P. K.; Parida, M.; Kumar, P.; Tomar, S. *Antivir. Res.* **2017**, *146*, 102.
11. Sharma, R.; Fatma, B.; Saha, A.; Bajpai, S.; Sistla, S.; Dash, P. K.; Parida, M.; Kumar, P.; Tomar, S. *Virol.* **2016**, *498*, 265.
12. Staples, J. E.; Breiman, R. F.; Powers, A. M. *Clin. Infect. Dis.* **2009**, *49*, 942.
13. Wang, J.; Zhang, T.; Du, J.; Cui, S.; Yang, F.; Jin, Q. *PLOS ONE*. **2014**, *9*, e89668.
14. Thiberville, S. D.; Moyen, N.; Dupuis-Maguiraga, L.; Nougairede, A.; Gould, E. A.; Roques, P.; de Lamballerie, X. *Antivir. Res.* **2013**, *99*, 345.
15. Rothan, H. A.; Bahrani, H.; Abdulrahman, A. Y.; Mohamed, Z.; Teoh, T. C.; Othman, S.; Rashid, N. N.; Rahman, N. A.; Yusof, R. *Antivir. Res.* **2016**, *127*, 50.
16. Dilip, C.; Saraswathi, R.; Krishnan, P.; Azeem, A.; Raseena; azeez, A.; Ramya; jose, J. *Asian Pac. J. Trop. Med.* **2010**, *3*, 443.
17. Samanta, A.; Das, G.; Das, S. K. Roles of Flavonoids in Plants. *Int. J. PharmSciTech.* **2011**, *6*, 12.
18. Panche, A. N.; Diwan, A. D.; Chandra, S. R. *J. Nutr. Sci.* **2016**, *5*, e47.
19. Lalani, S.; Poh, C. L. *Viruses* **2020**, *12*, 184.
20. Mukesh, B.; Rakesh, K. *Int. J. Res. Ayurveda Pharm.* **2011**, *2*, 1746.
21. Gaba, M.; Gaba, P.; Singh, S.; Gupta, G. D. *Int. J. Drug Dev. Res.* **2010**, *2*, 219.
22. Daisy, C.; Asha, R. N.; Kumar, G. S. S.; Vadivel, E.; Bhuvanesh, N.; Nayagam, B. R. D. *J. Mol. Struct.* **2020**, *1222*, 128894.
23. Sanner, M. F. *J. Mol. Graph. Model.* **1999**, *17*, 57.
24. Brüstle, M.; Beck, B.; Schindler, T.; King, W.; Mitchell, T.; Clark, T. *J. Med. Chem.* **2002**, *45*, 3345.
25. Bitew, M.; Desalegn, T.; Demissie, T. B.; Belayneh, A.; Endale, M.; Eswaramoorthy, R. *PLOS ONE.* **2021**, *16*, e0260853.
26. Wang, E.; Volkova, E.; Adams, A. P.; Forrester, N.; Xiao, S. Y.; Frolov, I.; Weaver, S. C. *Vaccine* **2008**, *26*, 5030.
27. Jasso-Miranda, C.; Herrera-Camacho, I.; Flores-Mendoza, L. K.; Dominguez, F.; Vallejo-Ruiz, V.; Sanchez-Burgos, G. G.; Pando-Robles, V.; Santos-Lopez, G.; Reyes-Leyva, J. *Infect Drug Resist.* **2019**, *12*, 1833.
28. Ahmadi, A.; Hassandarvish, P.; Lani, R.; Yadollahi, P.; Jokar, A.; Bakar, S. A.; Zandi, K. *RSC Adv.* **2016**, *6*, 69421.