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Article

An *in silico* investigation of phytochemicals as potential inhibitors against non-structural protein 1 from dengue virus 4

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Dengue fever has emerged as a big threat to human health since the last decade owing to high morbidity with considerable mortalities. The proposed study aims at the *in silico* investigation of the inhibitory action against DENV4-NS1 of phytochemicals from two local medicinal plants of Pakistan. Non-Structural Protein 1 of Dengue Virus 4 (DENV4-NS1) is known to be involved in the replication and maturation of viron in the host cells. A total of 129 phytochemicals (50 from *Tanacetum parthenium* and 79 from *Silybum marianum*) were selected for this study. The tertiary structure of DENV4-NS1 was predicted based on homology modelling using Modeller 9.18 and the structural stability was evaluated using molecular dynamics simulations. Absorption, distribution, metabolism, excretion and toxicity (ADMET) along with the drug-likeness was also predicted for these phytochemicals using SwissADME and PreADMET servers. The results of ADMET and drug-likeness predictions exhibited that 54 phytochemicals i.e. 25 from *Tanacetum parthenium* and 29 from *Silybum marianum* showed effective druglikeness. These phytochemicals were docked against DENV4-NS1 using AutoDock Vina and 18 most suitable phytochemicals with binding affinities ≤ -6.0 kcal/mol were selected as potential inhibitors for DENV4-NS1. Proposed study also exploits the novel inhibitory action of Jaceidin, Centaureidin, Artecanin, Secotanaparthenolide, Artematin, Schizolaenone B, Isopomiferin, 6, 8-Diprenyleriodictyol, and Anthraxin against dengue virus. It is concluded that the screened 18 phytochemicals have strong inhibition potential against Dengue Virus 4.

Keywords: DENV4-NS1, Phytochemicals, Molecular Docking, Molecular Dynamics Simulations, Druglikeness

INTRODUCTION

Dengue Virus which is known to cause dengue fever belongs to the class flaviviral and genus flavivirus has four serotypes i.e. Dengue Virus 1 (DENV-1) to 4 (DENV-4) (Searo, 2011). DENV-4 is quite prevalent in tropical and subtropical areas of the world, commonly Brazil as it is observed to spread rapidly throughout the several Brazilian states since 2010. It is strongly associated with serious cases of Dengue Shock Syndrome (DSS) and Dengue hemorrhagic fever (DHF) (Temporão *et al*., 2011).

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Dengue virus is comprised of a single strand RNA which encodes ten proteins including three structural and seven nonstructural proteins. The structural proteins are membrane protein (M), capsid protein (C), and envelope protein (E). The remaining seven proteins are nonstructural proteins named as Non-structural protein 1 (NS1), Non-structural protein 2A (NS2A), Non-structural protein 2B (NS2B), Non-structural protein 3 (NS3), Non-structural protein 4A (NS4A), Non-structural protein 4B (NS4B) and Non-structural protein 5 (NS5) (Lindenbach and Rice, 2001). Nonstructural proteins are hydrophobic in nature and play a pivotal role in virus proliferation. The nonstructural proteins are only expressed in infected host cells. NS1 is considered to be the foundation of viral replication (Whitehead *et al*., 2007).

NS1 protein plays an important role in various stages of the life cycle of dengue virus 8-9. NS1 is secreted by infected mammalian cells and is released into the blood which leads towards disease. NS1 protein transports to the cell surface where it is further released into extracellular milieu as a hexameric form. The proper processing of NS1 requires sequence in the C-terminus of the envelope glycoprotein. It is synthesized and moved to the endoplasmic reticulum where it is modified into homodimer with partial hydrophobicity (Avirutnan *et al*., 2010). Non-Structural Protein 1 of Dengue Virus 4 (DENV4-NS1) is known to be involved in the replication and maturation of viron in the host cells. It is comprised of 352 amino acids, having 46 to 50 kDa glycoprotein present in the cell membrane and is secreted in the blood to infect the cell (Scaturro *et al*., 2015).

Discovery of drugs against viruses is always an attractive area of research. It is considered that to prevent viral infection, its replication would have to be ceased at early stages of reproduction, growth, and development. Plants are an important natural source for the discovery of drugs against viruses, comprising of different groups of phytochemicals (secondary metabolites). Some major groups are alkaloids, flavonoids, monoterpenes, and sesquiterpenes. Different studies have been conducted to analyze the medicinal potentials of these phytochemicals against various diseases (Mirza *et al*., 2016).

The *in silico* characterization of DENV4-NS1 and its interaction with the phytochemicals from *Tanacetum parthenium* and *Silybum marianum* is targeted in this study. *Tanacetum parthenium* is a fever-reducing plant which belongs to Asteraceae found in gardens and along roadsides. It has been proved biologically active compound, used for the treatment of arthritis, asthma, constipation, fever, headache and inflammatory conditions (Chavez, Chavez, 1999). The widely studied chemical constituents in *Tanacetum parthenium* are sesquiterpene lactones, and flavonoids (Pareek *et al*., 2011). *Silybum marianum*, commonly known as milk thistle or blessed thistle, is also a member of Asteraceae and is locally inhabited in plant of Mediterranean regions, North Africa and Australia. The phytochemicals of *Silybum marianum* has importance due to its medicinal point of view, especially Betaine, Isosilibinin, Silibinin, Silidianin, Silychristin, and Silymarin (Hussain *et al*., 2010; Qaddir *et al*., 2017). The most important chemical Silymarin which is the further combination of seven compounds (Polyak *et al*., 2013). The current study focuses on the inhibitory action of phytochemicals from these two plants against dengue virus. The drug-likeness properties of these phytochemicals were also determined in this study for their efficient use in the control of the spread of disease caused by Dengue virus 4.

MATERIAL AND METHODS

The methodology was comprised of several techniques and phases. An overview is being provided in the flowchart (Figure 1).

Tanacetum parthenium and *Silybum marianum* are medicinal plants locally produced in Pakistan. Phytochemicals of these plants are known to have an inhibitory effect against many viral diseases (Pareek *et al*., 2011; Polyak *et al*., 2013). The proposed study emphasized on the antiviral behaviour of these two plants against Non-Structural Protein 1 of Dengue Virus 4. About 50 phytochemicals (secondary metabolites) were selected from *Tanacetum parthenium* and 79 were selected from *Silybum marianum* to analyze their antiviral activity against DENV-4. Among these 129 phytochemicals, there were 91 flavonoids (79 from *Silybum marianum* and 12 from *Tanacetum parthenium*) 10 sesquiterpene from *Tanacetum parthenium*, and 28 monoterpenes which were also from *Tanacetum parthenium*.

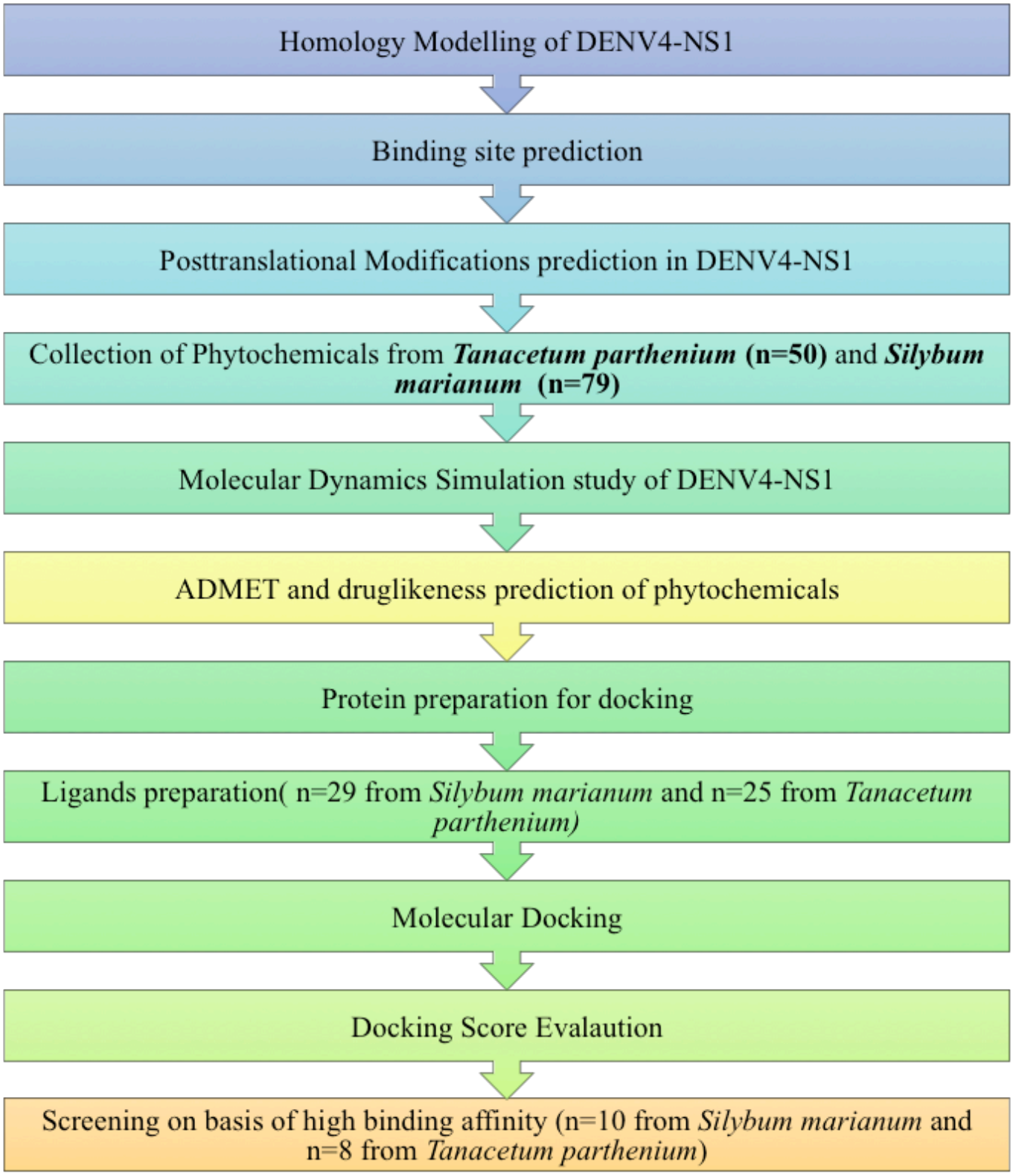


FIGURE 1 - Flowchart of the methodology.

Collection of Phytochemicals

Homology modelling and binding site prediction

The homology modelling of DENV4-NS1 was performed due to the unavailability of its tertiary structure in Protein Data Bank. With the help of Basic Local Alignment Search Tool (BLAST), similar homologous structures were found and top 4 structures with the highest similarity (Protein Databank Identifiers: 4O6B, 5IY3, 4OIG and 4OII) were selected as the template for the homology modelling (Johnson *et al*., 2008). Homology modelling was performed using Modeller 9.17. Python language based scripts were used for the profile building, comparison, two-dimensional alignment, and modelling. Homology modelling generated 5 models and these models were evaluated on the basis of Discrete Optimized Protein Energy (DOPE) score. The model with the lowest DOPE was selected for further studies as per. The binding sites were predicted using MetaPocket Server (Huang, 2009).

Posttranslational Modifications

PTM including phosphorylation, glycosylation, ubiquitination, methylation, acetylation, and palmitoylation were performed on predicted model using NetPhos, BioCUCKOO server by the CUCKOO workgroup and Yin O Yang server (Gupta, Brunak, 2001; Blom *et al*., 2004).

Molecular Dynamics Simulation

Thermodynamics and structure dynamics of DENV4-NS1 were studied with the help of Groningen Machine for chemical simulation (GROMACS) v 5.0 (Abraham *et al*., 2015). Primarily, the optimized potential for liquid simulation force field was applied on all atoms (OPLS-AA). Neutralization of solvent was done by the addition of counter ions Na+ and Cl- using Verlet cut-off scheme. The whole system was subjected to energy minimization (EM) by the use of the steepest descent algorithm, having 50000 steps. Equilibrations of the whole setup were performed involving the constant Number, Volume and Temperature (NVT) and Constant Number, Pressure and Temperature (NPT). During the whole process, pressure and temperature remained constant i.e. 1atm and 300K respectively. The time duration for both equilibrations was 100ps, while the Particle Mesh Ewald force field was used with cubic interpolation operation. Linear Constraint Solver (LINCS) was used for the modification of hydrogen bonds. Production molecular dynamics (MD) simulation was performed for 1ns and the results of MD simulation were analyzed by the aid of RMSD and gyration in the backbone of the enzyme. Visual molecular dynamics (VMD) was used to view the molecular dynamics simulation results (Humphrey *et al*., 1996).

ADMET and Drug-likeness Prediction

Absorption, Distribution, Metabolism, Excretion and Toxicity of the phytochemicals from Silybum marianum and *Tanacetum parthenium* was calculated using SwissADME server and PreADMET server (Lee *et al*., 2003; Daina *et al*., 2017). The program also helped in predicting the pharmacokinetics, drug-likeness and medicinal chemistry friendliness of all the phytochemicals. The prediction was made using the molecular structure files of all the molecules. Physically significant descriptors and pharmaceutically relevant properties associated with the ligand molecules were calculated. Phytochemicals were being assessed for the properties such as molecular weight being < 500 Daltons with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5. The Gastrointestinal Absorption and Brain Penetration was predicted and represented using Boiled-Egg model (Daina, Zoete, 2016).

Molecular Docking

The chemical structures of phytochemicals were retrieved from PubChem compound database. Molecular Docking of phytochemicals against DENV4-NS1 protein was performed using AutoDock Tools and AutoDock Vina (Morris *et al*., 2009; Trott and Olson, 2010).

Receptor preparation for docking was done using Autodock Tools by adding hydrogen bonds and designing of a grid box. Ligands were also prepared with the same module, including torsion adjustments and modifications. Ligands were docked with the predicted model of DENV4-NS1 using AutoDock Vina and were evaluated on the basis of binding affinity.

RESULTS AND DISCUSSION

Structure of DENV4-NS1 and binding sites

Based upon the primary structure of the DENV4-NS1, it has shown maximum similarity (73%) with DENV2- NS1. Among the five generated models by Modeller 9.17, the model with the lowest DOPE energy i.e. -29910.83 was selected for further studies (Figure 2). A Ramachandran plot was generated for the predicted model using RAMPAGE tool (Lovell *et al*., 2002). As per the analysis of possible conformations of ϕ and ψ angles for individual amino acid residues in DENV4-NS1, it was observed that 89.1% residues were in the favoured region and 10.0% residues were in the allowed region while only 0.9% residues were in outlier region (Figure 3). The predicted model of DENV4-NS1 comprised of 4 α-helices and 12 β-sheets while 2 α-helices and 17 β-sheets are found in DENV2-NS1 (Akey *et al*., 2014). The superimposition of both tertiary structures illustrated that both structures have Template Match Score (TM-Score) and RMSD of 0.53 and 2.2Å, respectively. The binding sites in both protein models are highly conserved i.e. comprising of Val5, Lys14, Phe20, Ala187 and Lys189 (Figure 2).

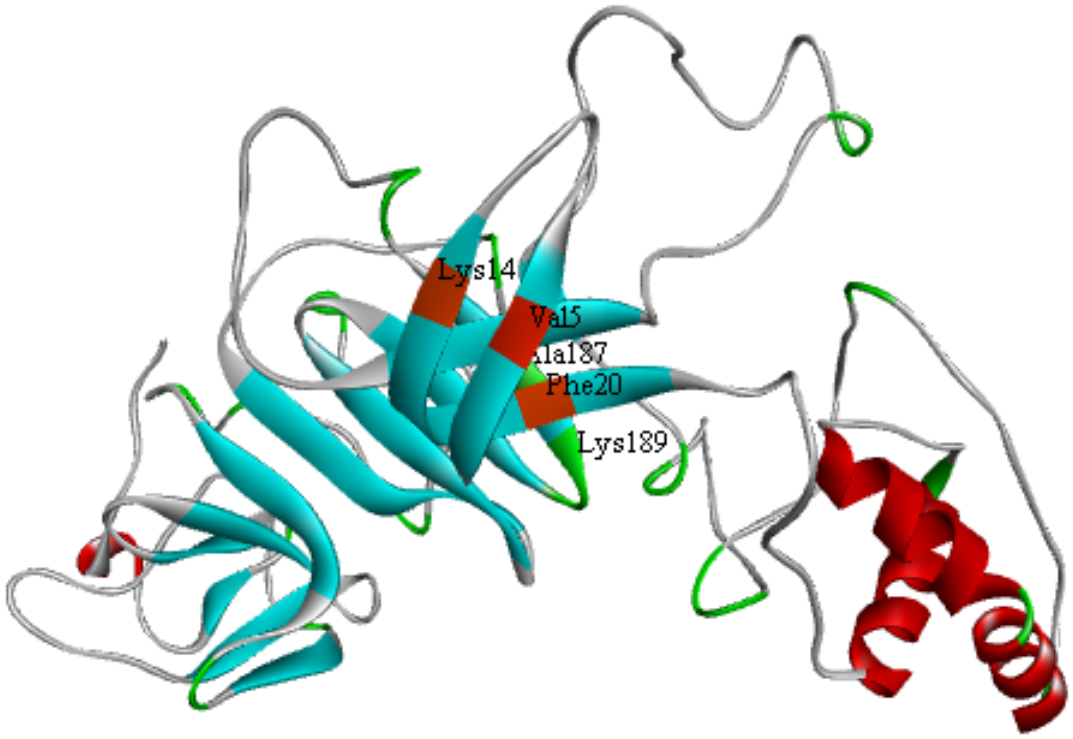


FIGURE 2 - Tertiary structure of DENV4-NS1 with predicted binding sites.

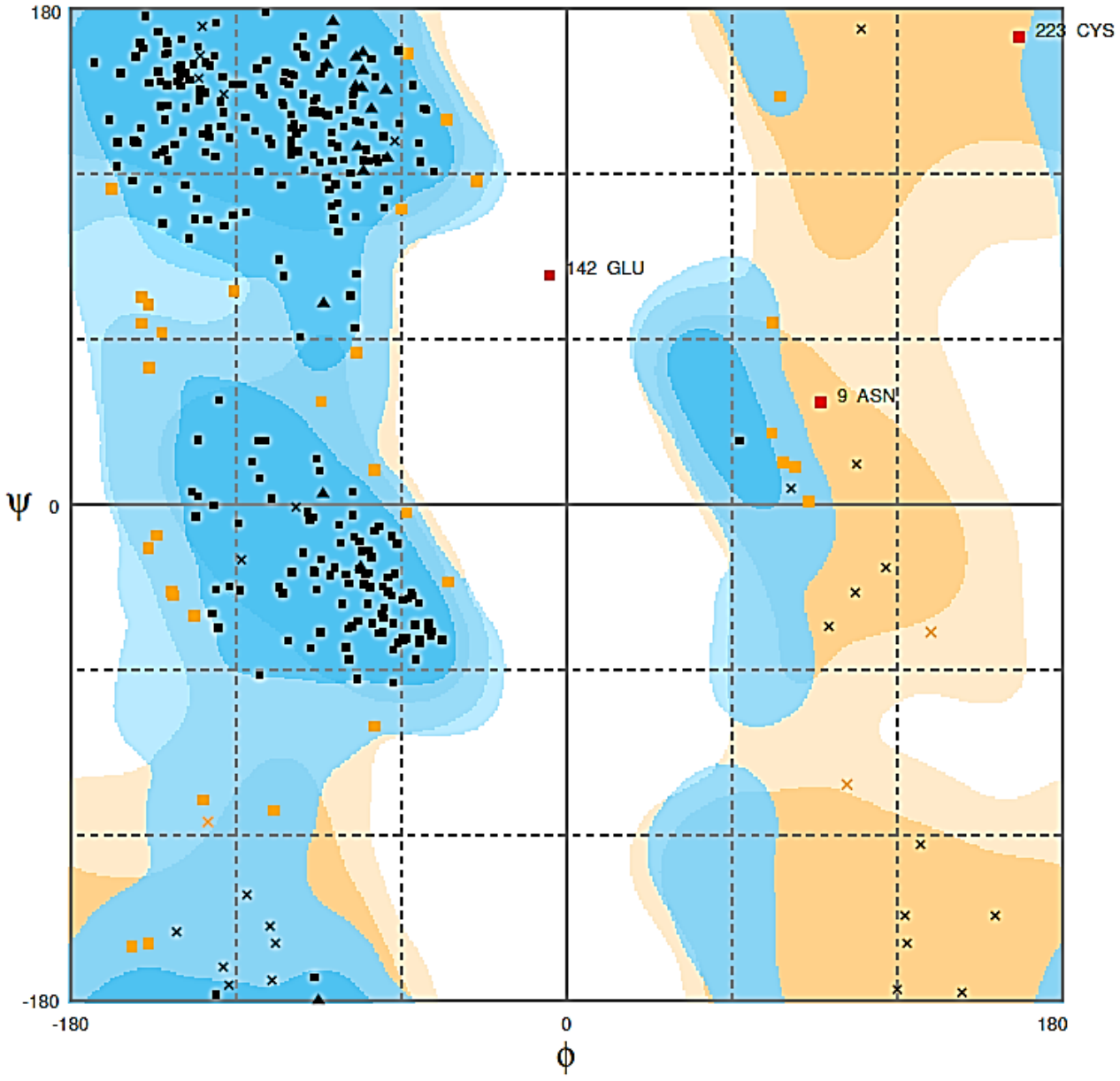


FIGURE 3 - Ramachandran plot of predicted DENV4-NS1.

Posttranslational Modifications

A total of 98 posttranslational modifications were predicted in the DENV4-NS1 model including 62 Phosphorylations, 11 Ubiquitinations, 11 Sumoylation, 7 glycosylation and 3 Palmitoylations. *O*-(beta)-GlcNAc sites were also observed on 3 different sites. Ubiquitination can occur on Lys14 and Lys189, which are binding pocket residues if exposed to the exterior environment. Small ubiquitin-like modifier (SUMO) plays major roles in a reversible posttranslational modification process (SUMOylation) which actually monitor a wide range of cellular processes for numerous viruses during infection. The SUMOylation pathway is a key regulator of the DENV life cycle. It is also known to be involved in the regulation of replication in dengue virus (Su *et al*., 2016).

Molecular Dynamics and Simulation

Thermodynamics properties of the predicted model including temperature and total energy values are used

to analyze the quality of the 3D structure of the protein. The structure of DENV4-NS1 obtained after dynamics simulations were compared with the crude predicted model without the simulation studies and were evaluated through TM-Score by superimposing both structures. The TM-Score value was observed to be 0.82 at 300K while RMSD was 1.8. The compactness of structure was analyzed by unsteady gyration (Rg) radius which showed that protein is not folded under the stable configurations (Figure 4).

The RMSD graph illustrated the instability of DENV4-NS1 structure under the high temperature, representing the mesophilic nature of protein as instability of the structure increased with increasing temperature (Figure 5).

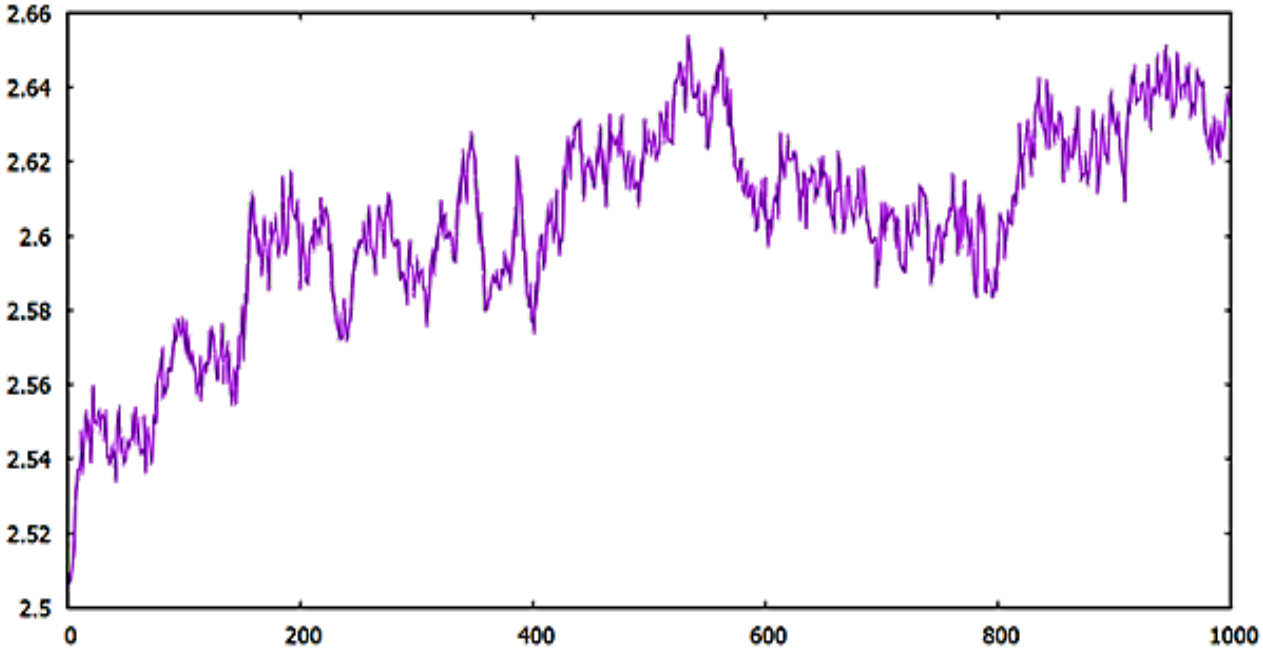


FIGURE 4 - Gyration plot of DENV4-NS1 at 300K.

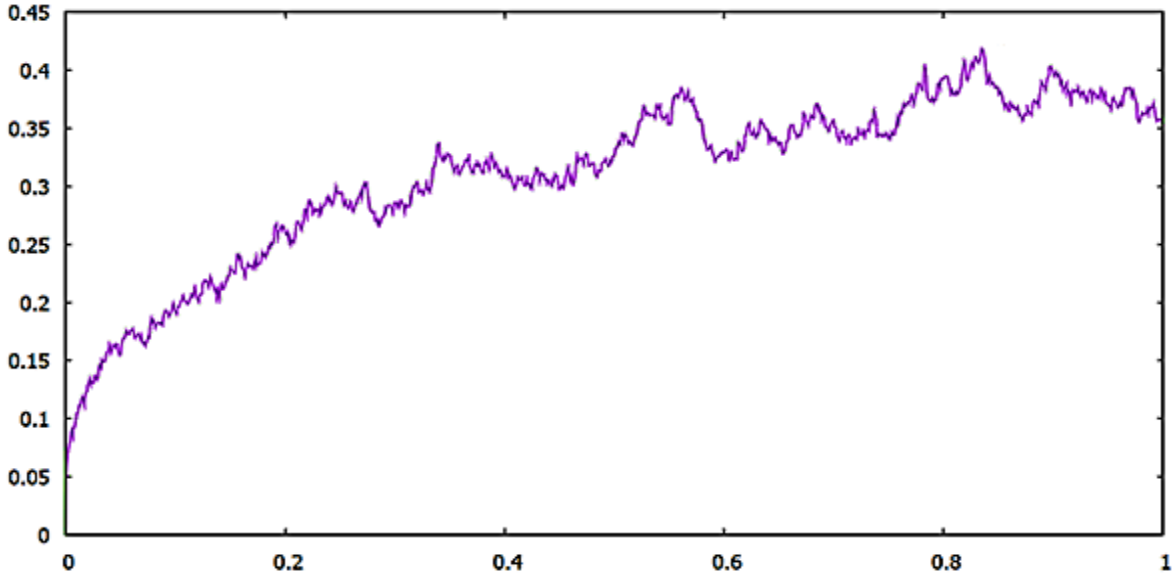


FIGURE 5 - RMSD plot of DENV4-NS1 at 300K.

Drug suitability and their virtual screening

ADMET and drug-likeness prediction were performed for 129 phytochemicals (secondary metabolites). The results of the ADMET and drug-likeness helped in evaluating the phytochemicals for their use as potential drugs in the dengue fever. A total of 54 phytochemicals i.e. 29 from Silybum marianum and 25 from *Tanacetum parthenium* were predicted be suitable on the basis of Lipinski's rule of 5.

The phytochemicals from *Silybum marianum* are flavonoids and have low solubility in water as compared to the few phytochemicals from Tanacetum parthenium, having sesquiterpene nature. Moreover, it was observed that gastrointestinal absorption was high in all the phytochemicals from both plants. Therefore these can be used effectively as drugs against human dengue virus 4.

The most suitable drugs, which were also docked with high binding affinities, were further filtered on the basis of the Blood-Brain Barrier (BBB) penetration behaviour. The BBB is a mechanism which does not usually allow the drugs reaching the brain and central nervous system and it is preferred that a drug should not usually approach the central nervous system (Tran, 2011). Non-penetrating drugs were considered as final candidates.

Docking with phytochemicals

DENV4-NS1 was subjected to docking with 54 phytochemicals which showed effective ADMET properties. Among these 54 phytochemicals, 18 (10 from Silybum marianum and 8 from Tanacetum parthenium) docked successfully at the binding site of DENV4-NS1 with high binding affinities. These are shown occupying binding site of DENV4-NS1 protein and their ADMET properties are also mentioned (Table I-II). High binding affinities show that these chemicals are tightly bound to the active site of DENV4-NS1 (Table III). Inhibition of viral proteins by phytochemicals would result in reduced viral replication, hence control the spread of dengue fever (N Powers and N Setzer, 2016).

TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

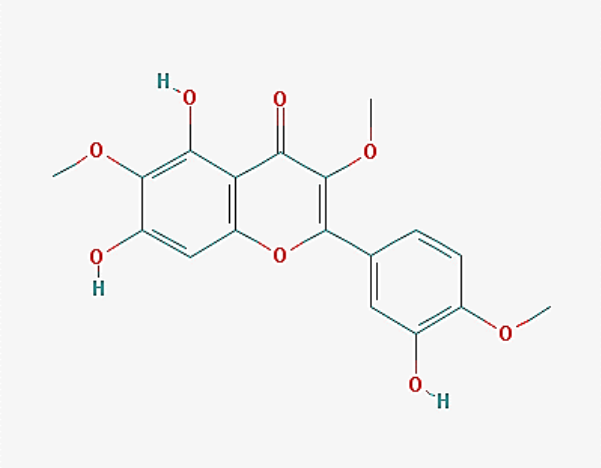
Name of phytochemical	Chemical formula	Structural formula
	Tanacetum parthenium	
Centaureidin	$C_{18}H_{16}O_8$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

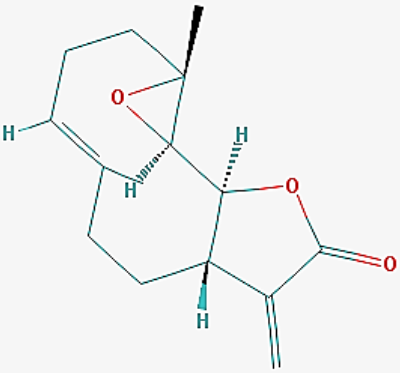
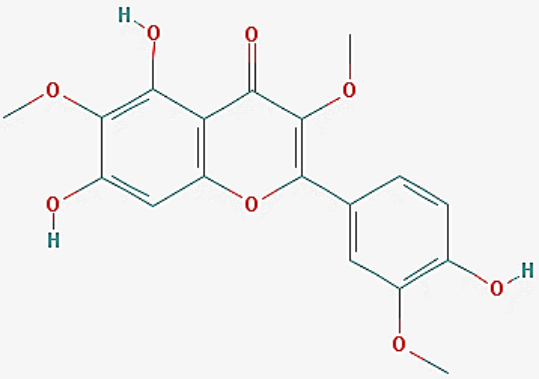
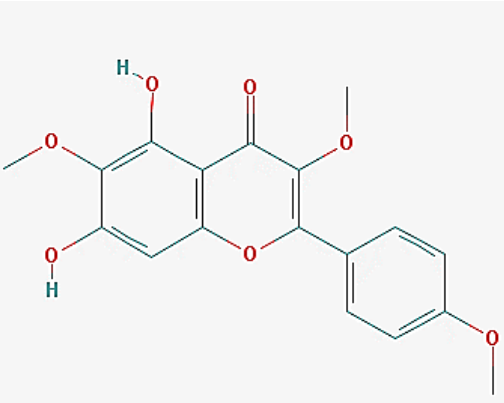
Name of phytochemical	Chemical formula	Structural formula
Parthenolide	$C_{15}H_{20}O_3$	
Jaceidin	$C_{18}H_{16}O_8$	
Santin	$C_{18}H_{16}O_7$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

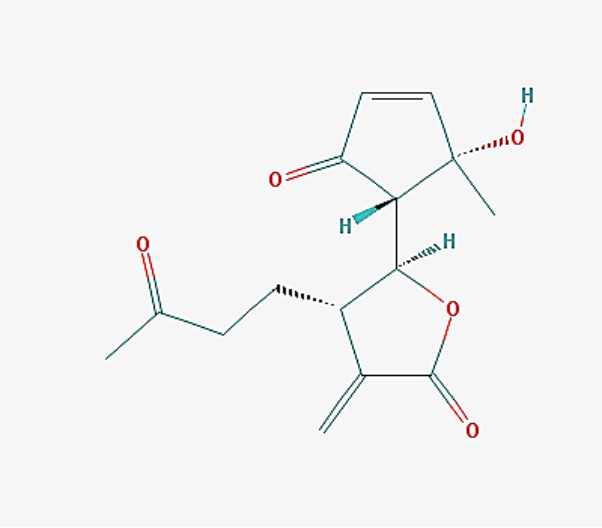
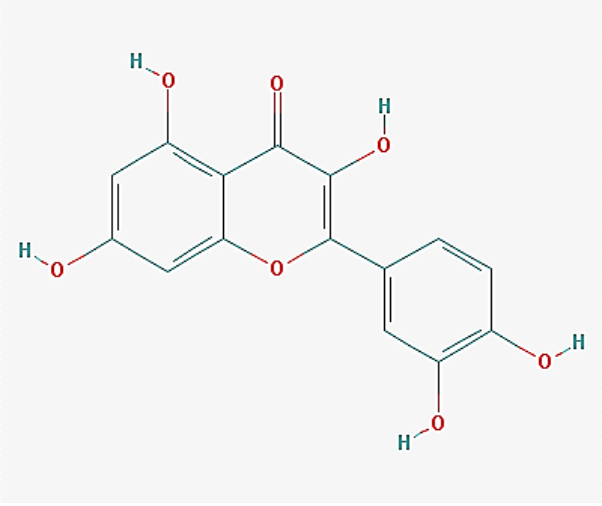
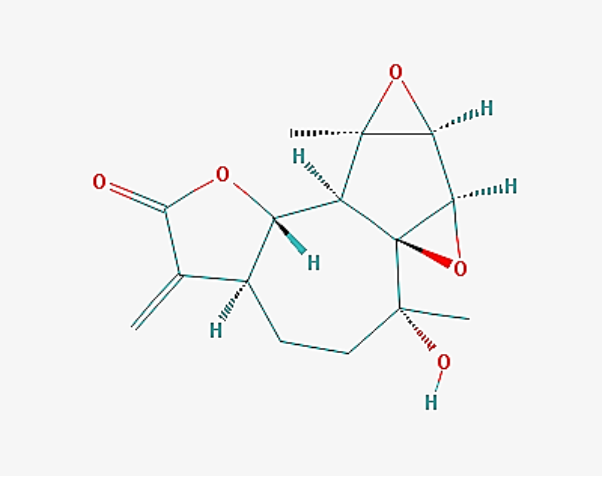
Name of phytochemical	Chemical formula	Structural formula
Secotanaparthenolide B	$C_{15}H_{18}O_5$	
Quercetin	$C_{15}H_{10}O_7$	
Artecanin	$C_{15}H_{18}O_5$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

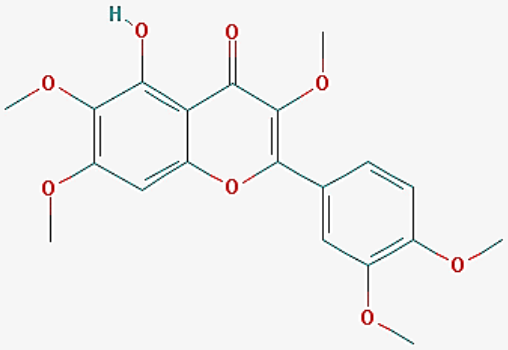
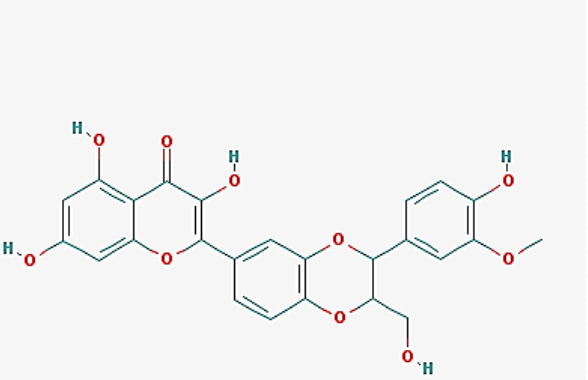
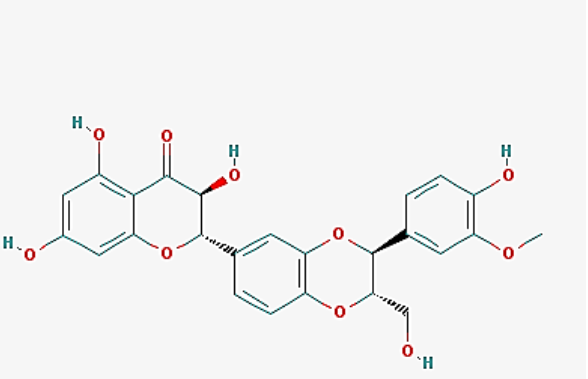
Name of phytochemical	Chemical formula	Structural formula
Artematin	$C_{20}H_{20}O_8$	
Silybum marianum		
2,3-dehydrosilybin	$C_{25}H_{20}O_{10}$	
Silybin	$C_{25}H_{22}O_{10}$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

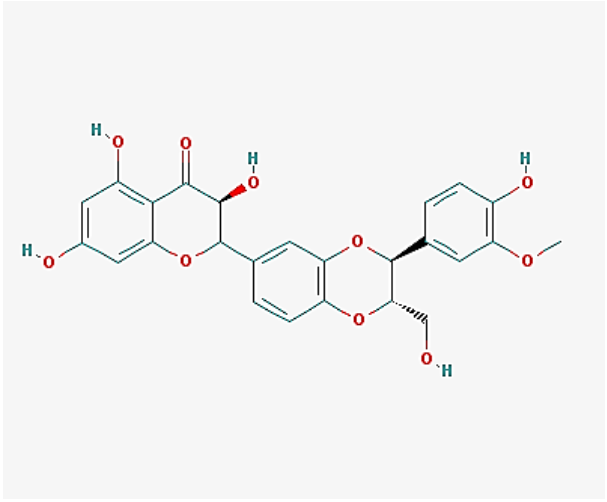
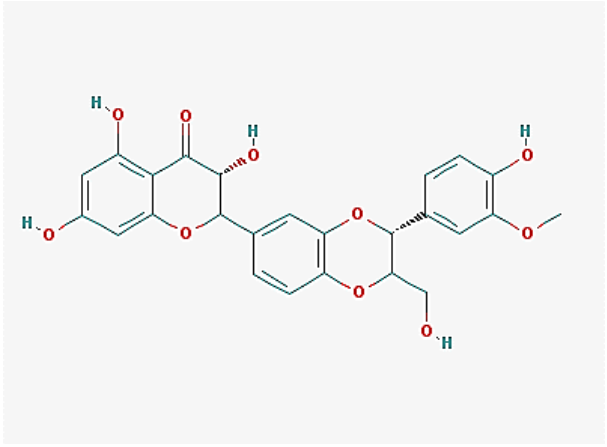
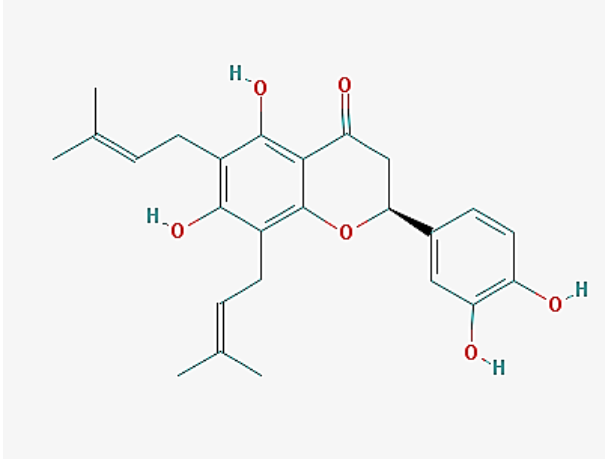
Name of phytochemical	Chemical formula	Structural formula
Silymarin	$C_{25}H_{22}O_{10}$	
Flavobion	$C_{25}H_{22}O_{10}$	
6,8-Diprenyleriodictyol	$C_{25}H_{28}O_6$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

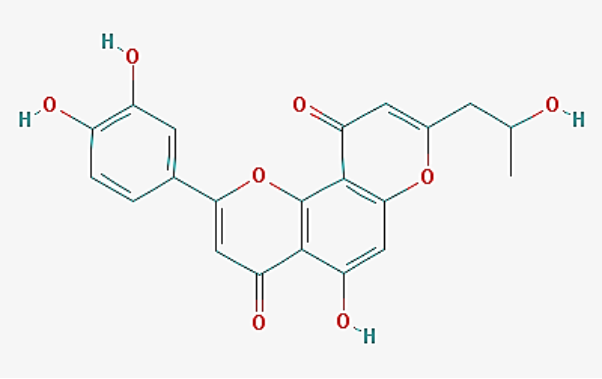
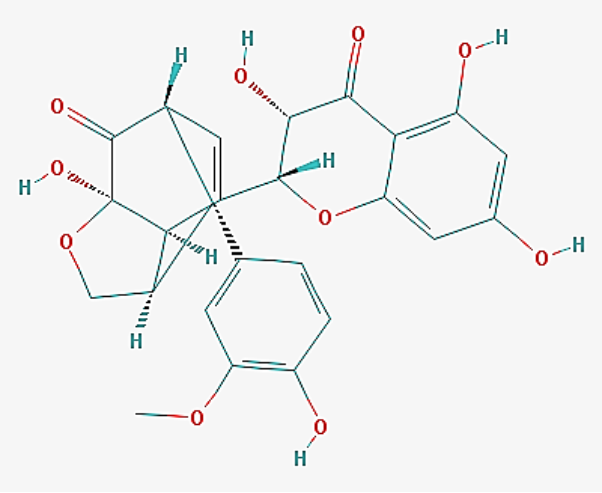
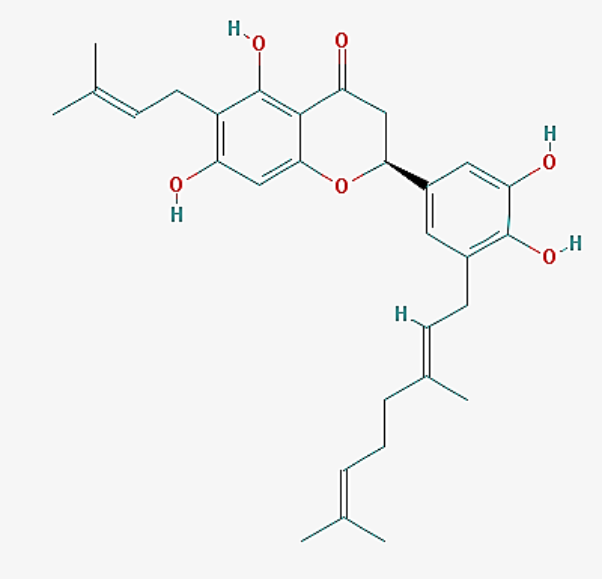
Name of phytochemical	Chemical formula	Structural formula
Anthraxin	$C_{21}H_{16}O_8$	
Silydianin A	$C_{25}H_{22}O_{10}$	
Schizolaenone B	$C_{30}H_{36}O_6$	

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TABLE I - Phytochemicals from *Tanacetum parthenium* and *Silybum marianum*

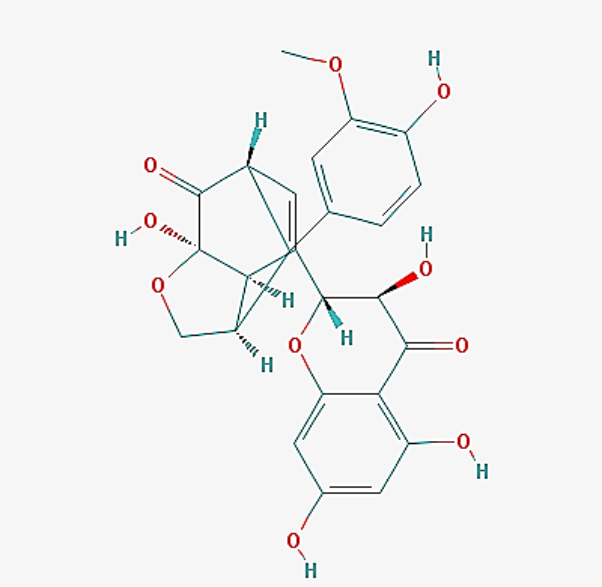
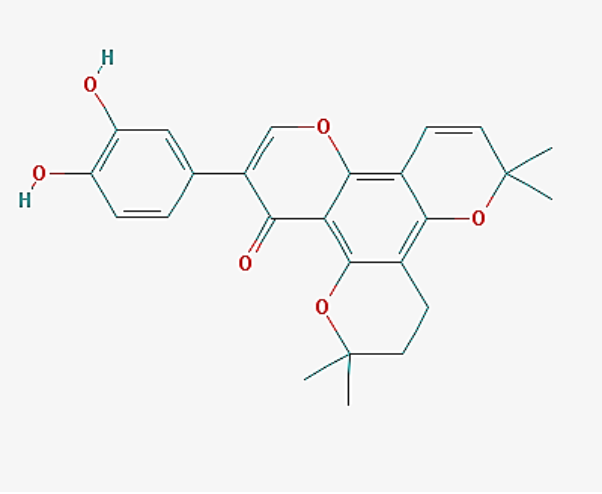
Name of phytochemical	Chemical formula	Structural formula
Silydianin B	$C_{25}H_{22}O_{10}$	
Isopomiferin	$C_{25}H_{24}O_6$	

TABLE II - Results of Drug likeness from both plants

Molecule	ESOL Log S	ESOL Class	GI absorption	BBB permeant	Lipinski violations	Toxicity	Carcinogenecity
			Tanacetum parthenium				
Artecanin	-3.1	Soluble	High	No	0	Non Toxic	Non carcinogenic
Artementin	-4.15	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Centaureidin	-5.5	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Parthenolide	-4.44	Moderately soluble	High	No	1	Non Toxic	Non carcinogenic
Jaceidin	-2.29	Soluble	High	No	0	Non Toxic	Non carcinogenic
Quricetin	-4	Soluble	High	No	0	Non Toxic	Non carcinogenic
Santin	-4.89	Moderately soluble	High	No	1	Non Toxic	Non carcinogenic
Secotanaparthenolide B	-1.92	Soluble	High	No	0	Non Toxic	Non carcinogenic
			Silybum marianum				
2,3-dehydrosilybin	-4.99	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Silybin A	-4.14	Soluble	High	No	0	Non Toxic	Non carcinogenic
Silymarin	-4.14	Soluble	High	No	0	Non Toxic	Non carcinogenic
Flavobion	-4.14	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
6,8-Diprenyleriodictyol	-6.14	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Anthraxin	-4.2	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Silydianin A	-3.39	Soluble	High	No	0	Non Toxic	Non carcinogenic
Schizolaenone B	-7.5	Moderately soluble	High	No	0	Non Toxic	Non carcinogenic
Silydianin B	-3.39	Soluble	High	No	0	Non Toxic	Non carcinogenic
Isopomiferin	-3.4	Soluble	High	No	0	Non Toxic	Non carcinogenic

TABLE III - Docking of phytochemicals from Tanacetum parthenium and Silybum marianum with DENV4-NS1

Phytochemical name	Binding sites (DENV4-NS1)	Binding Affinities (kcal/mol)	Ki value (μM)
	Tanacetum parthenium		
Centaureidin	Asn_{9}, Lys_{14}, Phe_{20}, Ala_{187}, Lys_{189}, Lys_{192}	-6.4	20.117
Parthenolide	Val_{5}, $Asn_{9,}$ Lys_{189}, Lys_{192}	-8.6	0.489
Jaceidin	$Ser_{7,}$ $Trp_{8,}$ Asn_{9} $Gly_{18,}$ Phe_{20}, Val_{21}, $Ala_{187,}$ Lys_{189}, Lys_{192}	-7.0	7.299
Santin	$Val_{5,}$ $Trp_{8,}$ $Asn_{9,}$ $Gly_{18,}$ $Ile_{19,}$ Lys_{189}	-7.4	3.713
Secotanaparthenolide B	$Val_{5,}$ $Trp_{8,}$ $Asn_{9,}$ $Lys_{14,}$ $Ile_{19,}$ Lys_{189}	-7.4	3.713
Quercetin	$Val_{5,}$ $Trp_{8,}$ $Asn_{9,}$ $Lys_{189,}$ Lys_{192}	-8.0	1.347
Artecanin	$Ser_{7,}$ $Val_{21,}$ $Ala_{187,}$ $Lys_{189,}$ Lys_{192}	-7.4	3.713
Artematin	$Val_{5,}$ $Trp_{8,}$ Lys_{189}	-6.4	20.117
	Silybum marianum		
2,3-Dehydrosilybin	Asn_{9}, Ser_{17}, $Ile_{19,}$ Phe_{20}, $Ala_{187,}$ Lys_{189}, Lys_{192}	-7.8	1.889
Silybin A	Met_{2}, $Val_{5,}$ Asn_{9}, Phe_{20}, Val_{21}, Lys_{192}	-7.7	2.237
Silymarin	$Ile_{19,}$ Phe_{20}, Ala_{187}, Lys_{189}, Lys_{192}	-7.6	2.648
Flavobion	Ser_{7}, $Trp_{8,}$ Asn_{9}, $Lys_{14,}$ Ile_{19}, Phe_{20}, Val_{21}, Ala_{187}, Lys_{189}, Lys_{192}	-7.7	2.237
6,8-Diprenyleriodictyol	Trp_{8}, Asn_{9}, Lys_{14}, Lys_{189}, Lys_{192}, Trp_{201}	-7.8	1.889
Anthraxin	Val_{5}, Ser_{7}, $Lys_{14,}$ Gly_{18}, Phe_{20}, Val_{21}, Lys_{189}	-7.7	2.237
Silydianin A	Val_{5}, Trp_{8}, Asn_{9}, Ala_{187}, Lys_{189},	-8.6	0.489
Schizolaenone B	Val_{5}, Trp_{8}, Asn_{9}, Ala_{187}, Lys_{189},	-7.7	2.237
Silydianin A	Val_{5}, Trp_{8}, Asn_{9}, Ala_{187}, Lys_{192}	-8.0	1.347
Isopomiferin	Val_{21}, Asp_{23}, Ala_{187}, Lys_{189}, Lys_{192}, Trp_{201}, Glu_{263}	-8.7	0.413

*Docking of **Tanacetum parthenium** phytochemicals against DENV4 - NS1*

A total of 25 phytochemicals of T. parthenium were used for docking with DENV4 - NS1. The binding affinities of these phytochemicals ranged from -4.8 to -8.7 kJ/mol. A threshold of -6.0 kcal/mol was used for screening the compounds and out of the 25, 8 phytochemicals passed this threshold by making interactions with Val5, Ser7, Trp8, Asn9 Gly18, Phe20, Val21, Ala187, Lys189, and Lys192 residues of the binding pocket.

Parthenolide made interactions with Val5, Asn9, Lys189, Lys192 having binding affinity -8.6 kcal/mol (Ki = 0.489 μM). Quercetin having a binding affinity -8.0 kcal/mol (Ki = 1.347 μM) inhibited DEN4-NS1 by making interactions with Val5, Trp8, Asn9, Lys189 and Lys192. Santin and Secotanaparthenolide B showed binding affinity -7.4 kcal/mol (Ki = 3.713μM) and made interactions with Val5, Trp8, Asn9, Gly18, Ile19 and Lys189 residues of the binding pocket. Artecanin also docked with a binding affinity -7.4 kcal/mol (Ki = 3.713μM) and made interactions with Ser7, Val21, Ala187, Lys189 and Lys192. Jaceidin docked with a binding affinity -7.0 kcal/mol (Ki = 7.299 μM) at binding pocket, making interactions with Ser7, Trp8, Asn9 Gly18, Phe20, Val21, Ala187, Lys189 and Lys192. Centaureidin and Artematin docked with a binding affinity -6.4 kcal/mol (Ki = 20.117 μM) by making interactions with Asn9, Lys14, Phe20, Ala187, Lys189 and Lys192 residues of the binding pocket. The proposed study has highlighted novel inhibition potential of phytochemicals from *Tanacetum parthenium* against dengue virus which are Jaceidin, Centaureidin, Artecanin, Secotanaparthenolide, and Artematin. The application of these phytochemicals against dengue virus is not reported previously in any study.

Antiplasmodial and antiproliferative effects of Centaureidin and Jaceidin have been reported (Pareek *et al*., 2011). Both were also analyzed against chromosomal breakage in mitogen-induced human lymphocytes (Aljancix *et al*., 2010). The mitotic blocking activity by closely related flavonols i.e. Santin, Jaceidin and Centaureidin obtained from *Tanacetum parthenium* have also been reported (Long *et al*., 2003).

Sesquiterpene lactones like Parthenolide, 3-beta-hydroxyparthenolide, Secotanaparthenolide, canin and Artecanin present in the extract of *Tanacetum parthenium* have been reported to inhibit the secretion of granular contents from platelets and neutrophils (Marles *et al*., 1992). Artematin, which is a flavonoid, is known for the reduction of plasma and vascular ACE activity *in vitro* (de Souza *et al*., 2011). Antioxidant activity, hepatoprotective activity, antibacterial activity, lipoxygenase inhibition and antiproliferative activity have also been reported for Artematin (Csupor-Löffler *et al*., 2009; Choudhary *et al*., 2009; Michielin *et al*., 2009; Sridevi *et al*., 2012). Besides the novel antiviral agents, various phytochemicals with already reported antiviral activity have been docked successfully such as Costunulide, Parthenolide, and Quercetin. Costunolide, which is present in *Tanacetum parthenium* as well as in different medicinal plants such as *Saussurea lappa* and *Laurus nobilis*, has been analyzed against various types of cancer including Leukaemia, liver cancer, ovarian cancer, prostatic cancer and bladder cancer (Choi *et al*., 2009; Butturini *et al*., 2011; Liu *et al*., 2011; Kim *et al*., 2012).

Parthenolide, a sesquiterpene lactone, is an important phytochemical in *Tanacetum parthenium* which docked with high binding affinity against DENV4-NS1 (-8.6kcal/mol). It is reported against Epstein-Barr virus, migraines and rheumatoid arthritis for hundreds of years. Several studies have reported it performing inhibition of cell proliferation and DNA synthesis, and antitumor activity in various cancer cell lines (Kapadia *et al*., 2002; Pareek *et al*., 2011).

Quercetin is known to play an important role in the maintenance of physically and mentally human health as it reduces the risk of different infections due to its unique biological properties. Quercetin has been used for the treatment of cancer, oxidative damage and infections due to virus and bacteria (Markham, 1989). Quercetin has been observed to binding tightly with DENV4-NS1 having an excellent binding affinity (-8.0kcal/mol). Previously, it has also been reported to show strong inhibition potential against another non-structural protein namely NS2B/NS3 protease from DENV2 (Senthilvel *et al*., 2013).

Docking of *Silybum marianum* phytochemicals with DENV4-NS1

By analyzing ADMET properties of phytochemicals from *Silybum marianum*, 29 phytochemicals showed effective properties and these were docked against DENV4-NS1. A threshold of -6.0 kcal/mol was used for screening the compounds and out of the 29, 10

phytochemicals passed this threshold. Val5, Ser7, Trp8, Asn9, Gly18, Phe20, Val21, Ala187, Lys189, and Lys192 were involved in binding of these compounds.

Isopomiferin docked with highest binding affinity -8.7 kcal/mol (Ki = 0.413 μM), inhibiting the protein by making interactions with Val21, Asp23, Ala187, Lys189, Lys192, Trp201, Glu263 residues of the binding pocket. Silydianin A and Silydianin B made interactions with Val5, Trp8, Asn9, Ala187 and Lys192 residues of the binding pocket. Silydianin A docked with binding affinity -8.6 kcal/mol (Ki = 0.489 μM) while Silydianin B docked with binding affinity -8.0 kcal/mol (Ki = 1.347 μM). 2, 3-dehydrosilybin made interactions with the binding pocket residues Asn9, Ser17, Ile19, Phe20, Ala187, Lys189 and Lys192, docking with binding affinity -7.8 kcal/mol (Ki = 1.889 μM). Similarly, 6,8-Diprenyleriodictyol docked with binding affinity -7.8 kcal/mol (Ki = 1.889 μM) by making interactions with Trp8, Asn9, Lys14, Lys189, Lys192 and Trp201. Silybin A and Flavobion docked with binding affinity -7.7 kcal/mol (Ki = 2.237 μM). Silybin made interactions with Met2, Val5, Asn9, Phe20, Val21 and Lys192 while Flavobion interacted with Ser7, Trp8, Asn9, Lys14, Ile19, Phe20, Val21, Ala187, Lys189 and Lys192 residues of the binding pocket. Similarly, Anthraxin and Schizolaenone B docked with binding affinity -7.7 kcal/mol (Ki = 2.237 μM). Anthraxin interacted with Val5, Ser7, Lys14, Gly18, Phe20, Val21 and Lys189 residues while Schizolaenone B made interactions with Val5, Trp8, Asn9, Ala187, Lys189 residues of the binding pocket. Silymarin made interactions with Ile19, Phe20, Ala187, Lys189 and Lys192 residues of binding pocket with binding affinity -7.6 kcal/mol (Ki = 2.648 μM). The proposed study presents the novel inhibitory activities of phytochemicals from Silybum marianum i.e. Schizolaenone B, Isopomiferin, 6, 8-Diprenyleriodictyol, and Anthraxin against dengue virus.

Schizolaenone B has been analyzed against the A2780 human ovarian cancer cell line while Isopomiferin has been reported for having antioxidant properties (Murphy *et al*., 2005; Diopan *et al*., 2008).

Antiproliferative properties of 6,8-diprenyleriodictyol for leukaemia and solid cancer cells have been reported (Keute *et al*., 2011). Other activities such as antimicrobial activity and antioxidant activity have also been reported (Dufall *et al*., 2003Dzoyem *et al*., 2013). Anthraxin has been used for Immunological vaccination showing best results against various viral disease (Shlyakovm, 1994).

The phytochemicals of *Silybum marianum* showed the potent inhibitory effect against DENV4-NS1. Silymarin, a well-studied phytochemical of *Silybum marianum* was docked effectively against DENV4-NS1 and showed good binding affinity. Studies show that Silymarin has an inhibitory effect against acute and chronic viral hepatitis liver disease, hepatotoxicity and liver cancer (Agarwal *et al*., 2006; Wagoner *et al*., 2010; Freedman *et al*., 2011). Different studies analyzed that silymarin extracted from Silybum marianum inhibits HCV in both, *in vitro* and *in vivo*, by inhibiting viron entry, viral transcription and translation, and production of infectious virus (Anthony and Saleh, 2013). Silymarin shows anti-inflammatory, anti-fibrotic, and antioxidant activities. Silymarin has been observed to inhibit the post-entry steps of CHIKV infection significantly (Lani *et al*., 2015).

This study was based on computer-aided drug development against dengue 4 virus. Targeting the in silico approach to drug discovery, the molecular dynamics simulations help in the study of the motions of biological macromolecules such as proteins and nucleic acids. Atomistic computer simulations of therapeutic proteins/enzyme help in the discovery and development of drug through the identification of binding sites using various traditional methodologies of virtual screening, and prediction of binding energies of ligand/protein complex. Posttranslational modifications (PTM) of a protein are used for covalent modification of the protein by non-protein molecule. PTM is the process which is used as a post-process of biosynthesis when a genome is transformed into a proteome. The drugs are actually the inhibitors for a specific enzyme involved in an onset of the specific pathological condition. The docking of such inhibitors with the enzymes elucidate the mechanism of inhibition along with the specificity and efficiency of that inhibitor. Around 40% of drugs which are discovered through in silico molecular docking fail in clinical trials if the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties are not analyzed in these approaches. These failures can lead to finding new drugs that can be costly. Accurate prediction of ADMET properties can help in overcoming these failures and can be inexpensive. The *in silico* approach for drug discovery helps in finding drugs for diseases instead of the experimental work by docking mechanism. Drug development methods, purposed in this study can be helpful for further studies *in vitro* and *in vivo* analyses against dengue viral diseases. In the proposed study, 18 phytochemicals from both plants were identified as potential inhibitors against dengue virus 4. These novel inhibitors include Jaceidin, Centaureidin, Artecanin,

Secotanaparthenolide and Artematin from Tanacetum parthenium while Schizolaenone B, Isopomiferin, 6, 8-Diprenyleriodictyol, and Anthraxin from Silybum marianum. It is concluded that the screened 18 compounds from both of the plants can be used as therapeutic agents against dengue fever, specifically from Dengue Virus 4.

Most of the phytochemicals have been reported to be used in humans for the treatment of various diseases. Such studies support our hypothesis that these chemicals are not only good curative agents against dengue virus but also are biologically safe for human use, so the development of these phytochemicals as potential drugs for dengue virus would be therapeutically and economically beneficial.

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