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Understanding Antiviral activity of Flavonoids against Dengue Virus by using Computational Approaches

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ABSTRACT
The viral illness dengue fever has a substantial influence on more than 100 nations worldwide. Mosquitoes, notably the *Aedes aegypti* mosquito and, to a lesser extent, the *Aedes albopictus* mosquito, are the main carriers of the virus. The dengue virus (DENV), which has four different DENV serotypes, is the agent that causes dengue. There are currently no antivirals or medications that can effectively treat dengue, despite the fact that it is one of the most fatal disease that affects people all over the world. No vaccine or drug with long-lasting effects has been developed to treat dengue fever till date. Flavonoids, are a group of chemical compounds with various phenolic structures, and are found in a wide variety of foods, including fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine. Flavonoids offer various organic treatments that are well renowned for treating a variety of diseases. The objective of the current research was to identify a flavonoid as an eco-friendly alternative to conventional antivirals against dengue. Baicalein, fisetin, quercetin, silymarin are potentially strong antiviral that are also environmentally benign and interestingly they interact with several envelop proteins of various viruses. So, in the current study, we used computational techniques to examine the relationship between these flavonoids with the dengue virus envelop protein. The findings of the current study demonstrate that fisetin has an extremely high affinity for the dengue envelop proteins. Furthermore, because it was naturally obtained, it's deterioration has little impact on the ecosystem in the area. As a result, it can be used as a potent, environmentally safe, and broad-spectrum antiviral to treat the illness.
Keywords: Dengue, Dengue virus (DENV), flavonoid, baicalein, fisetin, quercetin, silymarin, molecular docking, molecular dynamics simulations.

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INTRODUCTION

Dengue is among the most typical illnesses brought on by mosquitoes. Its prevalence is widespread in numerous tropical and subtropical regions across the globe, and it is rapidly extending its reach to other nations where the Aedes aegypti and Aedes albopictus mosquitoes, responsible for its transmission, are present [1, 2]. The flavivirus that causes dengue infection is known as the dengue virus (DENV). The four distinct DENV genotypes are DENV-1, DENV-2, DENV-3, and DENV-4. From a mild febrile infection to severe dengue hemorrhagic fever and dengue shock syndrome, all four genotypes can cause a range of illnesses. Dengue is thought to kill 50 million individuals annually worldwide [3]. At present, there is no approved vaccine for dengue, and the development of such a vaccine has presented significant challenges primarily due to the complex nature of immune responses associated with dengue infection. Extensive data indicate a strong correlation between the quantity of dengue virus (DENV) present in the bloodstream during the viremic phase and the severity of dengue illness. This implies that the viral load during the viremic phase serves as a crucial factor influencing the development and progression of severe dengue symptoms. The intricate relationship between viral load and disease severity underscores the importance of accurately measuring and monitoring DENV levels in order to better understand and predict the clinical outcomes of dengue infections. Such insights are instrumental in guiding research efforts towards the development of effective vaccines that can address the challenges posed by dengue [4]. Hence, if the administration of effective antiviral treatments leads to a reduction in viral load, there is a potential decrease in the risk of severe dengue complications such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome

(DSS). By successfully lowering the viral load, the likelihood of experiencing severe manifestations and complications associated with dengue can be mitigated.

Plants and their derived compounds remain a valuable resource in the search for new antiviral drugs, primarily due to their expected minimal side effects and wide availability in nature. Several research studies have demonstrated the antiviral effects of specific phytochemicals, particularly various flavonoids, against different viruses [5–10]. Flavonoids, which are phenolic compounds with relatively low molecular weight, are present in a variety of plant species. These flavonoids can be found in the fruits, roots, nuts, seeds, bark, stems, and flowers of plants. Bioflavonoids, polyphenolic plant chemicals with a variety of unique biological benefits, including antiviral capabilities [11–13]. According to several researches, flavonoids including fisetin, quercetin, silymarin have anti-dengue properties [14, 15]. The root of the plant *Scutellaria baicalensis* is widely used to extract the flavonoid known as baicalein ($C_{15}H_{10}O_5$) [16]. It is a vital traditional Chinese medicine and a member of the Labiatae family. Inflammation, hypertension, hyperlipidemia, and a number of viral diseases have all been treated using its roots as medication.

Although all four dengue serotypes possess the potential for toxicity, there exist certain connections between these serotypes and the severity of the resulting illnesses. In other words, different serotypes of the dengue virus may lead to varying degrees of disease severity in individuals. These associations suggest that specific serotypes may have a greater propensity to cause more severe symptoms or complications compared to others. Understanding these associations is crucial for the effective management and treatment of dengue cases, as it allows healthcare professionals to better predict and respond to the potential outcomes associated with each serotype. Extensive research has provided evidence that primary infections with dengue serotypes 1 or 3 tend to result in more severe illness compared to infections caused by serotypes 2 or 4. Individuals who experience their initial encounter with dengue virus serotype 1 or 3 are more likely to exhibit a higher risk of developing severe symptoms and complications. This indicates that these specific serotypes have a greater propensity to elicit a robust immune response, which can lead to more pronounced clinical manifestations of the disease. Conversely, infections with serotypes 2 or 4 generally exhibit milder symptoms and a lower likelihood of severe outcomes [17]. However, serotype 2 infection has been connected to severe dengue infections, including DSS [18]. An envelope and a nucleocapsid make up a dengue virus particle. Three structural proteins (envelope E), three nonstructural proteins (capsid C), and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, and NS4A) make up a dengue virion [19].

In the present work we focus on serotype 1 and 3 because they cause severe infection as compared to other two serotypes. The envelop protein of dengue virus serotypes 1 and 3 was retrieved for the current investigation. To comprehend the topology of the protein structure, the structural landscape of the envelop protein was examined. In order to understand the protein and ligand binding pattern, we docked the envelop proteins of both the serotypes with baicalein, fisetin, quercetin and silymarin. The research aimed to unravel the intricate binding patterns between proteins and ligands, particularly in the context of dengue virus. To achieve this, the envelope proteins of different serotypes were subjected to docking analysis with four specific ligands: baicalein, fisetin, quercetin, and silymarin. Through a comprehensive assessment of the complementary fit and energy dynamics between the proteins and ligands, the researchers sought to gain a deep understanding of their interaction. Crucially, identifying the amino acids that are vital for protein-ligand binding was of utmost importance. By extracting this crucial information, it is possible to lay the foundation for the development of dengue drugs with a solid structural basis. Such a study holds the potential to contribute significantly to the advancement of therapeutics targeting dengue virus.

MATERIAL AND METHODS

a) DENV Sequence Analysis and Molecule Preparation: The three-dimensional structures of the envelop proteins of DENV1 (3irc) and DENV3 (3vvt) proteins were retrieved from the Protein Data Bank (PDB) [20]. In the Discovery studio 3.5 programme (available for download at https://discover. 3ds. com/discovery studio visualizer), the CHARMM27 force field was used to reduce the E protein shape. ProtParam was used to calculate the primary structural properties of DENV1 and DENV3 [21].The structure of baicalein, fisetin, quercetin, silymarin was downloaded from the pubChem website (https:// pubchem.ncbi.nlm.nih.gov/) [22].

b) Tabulation of the topography of DENV1 and DENV3: To preserve the functional integrity of proteins, it is crucial to understand their geometric and topological properties, including surface features and internal structures. The Computed Atlas of Surface Topography of Proteins (CASTp) is an online resource accessible via a web server that facilitates the identification, characterization, and measurement of specific geometric properties of proteins. By utilizing CASTp, a comprehensive and accurate assessment of the protein landscape can be obtained [23]. Additionally, the visualization of protein structures was carried out using PyMOL, a widely employed software tool in structural biology. PyMOL, constructed using Python, is

a cross-platform molecular graphics software extensively utilized for three-dimensional visualization of macromolecules [24]. Its versatile functionality has enabled diverse applications such as macromolecular analysis, homology modeling, protein-ligand docking, pharmacophore modeling, and molecular dynamics simulations, facilitating a deeper understanding of protein structures and their interactions. Hence the topology of DENV1 and DENV3 was analyzed and tabulated using CASTp and the results were visualized using PyMOL.

c) Molecular docking and simulation: The software CB dock is used to carry out the molecular docking after automatically identifying the binding sites, determining the size and centre, and setting the docking box size for the query ligand. Large-scale benchmarks show that cavity-focused docking can increase the hit rate and precision of blind docking. CB-Dock may expedite the docking process and improve accuracy by predicting the binding sites of target proteins using our curvature-based cavity detection technique (CurPocket) [25]. Individual docking between DENV1 and DENV3 was conducted using the ligand baicalein, fisetin, quercetin, and silymarin, the results were carefully analyzed. In order to check the overall energy landscape of the protein-ligand complexes, the docked complexes were used as input to SwissDock server [26, 27]. In order to have an in-depth analysis into the binding cavity of best docked conformation and assessing which amino acids are involved to interaction, we performed a LigPlot analysis of the docked complex [28, 29].

RESULTS AND DISCUSSION

a) Sequence Analysis of DENV1 and DENV3 and molecule preparation: We examined the DENV1 and DENV3 sequences for the study using ProtParam. The parameters have been compiled (Tables 1). The FASTA sequence and PDB structure for DENV1 and DENV3 were downloaded from the Protein Data Bank (PDB). We looked at the protein structure and prepared it for further analysis. The 2D structure of quercetin (the ligand) was obtained from the PubChem service at https://pubchem.ncbi.nlm.nih.gov/. It had a unique PubChem ID (5281605). The SMILES Translator was then used to convert the structure into a 2D representation [30] (https://cactus.nci.nih.gov/translate/).

b) Tabulation of the topography of dengue proteins: The CASTp server completely disassembled all of the atoms in DENV1 and DENV3 It provided details on the surface pockets, internal cavities, and cross channels of the protein. It also identified the precise volumes and regions of the binding cavity. Both the molecular surface model (Connolly's surface) and the solvent accessible surface model (Richards' surface) were used in the analytical derivation of these metrics. It has been shown that the binding pocket of DENV1 has a volume of 562.379 by 3 inches and a surface area of 597.770 by 2 inches. The binding pocket of DENV3 has a volume of 494.349 by 3 inches and a surface area of 431.770 by 2 inches. The binding cavities were seen using PyMol.

c) Molecular docking and simulation: CB-Dock was used to do the docking procedure rather than just binding proteins blindly throughout their full surface. Finding probable binding sites is therefore the first stage (cavity detection). Since ligand binding sites are frequently bigger cavities, the programme selects a few of the top cavities for additional research based on cavity size (Cavity sorting). The docking box size is then adjusted after determining the docking centre. These parameters (Centre and Size) are required when using AutoDock Vina for molecular docking. After the docking procedure is finished (Dock and Rerank), the bound postures are re-ranked based on the docking score. The query ligand may bind most effectively at the matching site, and the first conformation is thought to represent the ideal binding posture.

Considering both the Vina scores and the **ΔG** values, the best inhibitor against DENV1 appears to be Fisetin, as it has the lowest Vina score (-5.6) and a relatively low **ΔG** value (-8.34). For DENV3, the best inhibitor seems to be Baicalein, as it has the lowest Vina score (-6.5) and a relatively low **ΔG** value (-8.28). To identify an inhibitor that is potentially effective against both DENV1 and DENV3, we can compare the Vina scores and **ΔG** values of the inhibitors for both viruses. Among the provided inhibitors (Baicalein, Fisetin, Quercetin, and Silymarin), Fisetin shows relatively strong performance for both DENV1 and DENV3. It has the lowest Vina score (-5.6) for DENV1 and the second lowest Vina score (-6.7) for DENV3. In terms of **ΔG** values, Fisetin has the lowest value (-8.34) for DENV1 and the second lowest value (-7.56) for DENV3. Based on these observations, Fisetin appears to be a promising candidate as it exhibits relatively strong binding affinity for both DENV1 and DENV3. However, it's important to note that the choice of the best inhibitor may also depend on other factors and additional research would be needed to determine the most suitable inhibitor for effectively targeting DENV1 and DENV3. However, further studies and experimental validations are necessary to confirm its efficacy and suitability as a broad-spectrum inhibitor against both viruses.

On viewing the surface view of proteins, fisetin fits into the most likely cavities of DENV1 and DENV3, which are located towards the inner side of the protein, according to a bird's-eye observation of the docked stance from the surface (Figure1 and Figure3). Visualising the ligand in a ball and stick form and DENV1 and

DENV3 in cartoon perspective allows for a more thorough examination of the same orientation (Figure1 and Figure3). An observation of this kind reveals that the ligand is sandwiched inside the cavity and is able to appropriately connect with the cavity. The ligand moiety and the DENV1 and DENV3 neighbouring amino acids create a close interaction in this configuration and play the most important function in anchoring ligand inside the binding cavity, according to a comprehensive look into the envelop protein's binding cavity. The amino acids Met301, Asn355, Leu351,Ser 338, Arg350, Phe 337, Gly349, Ile 335, Thr339 of DENV1 while amino acids Ser616, Phe615, Leu629, Thr617, Gly627, Ile613 of DENV3 play crucial role of binding with fisetin (Figure 2 and 4).

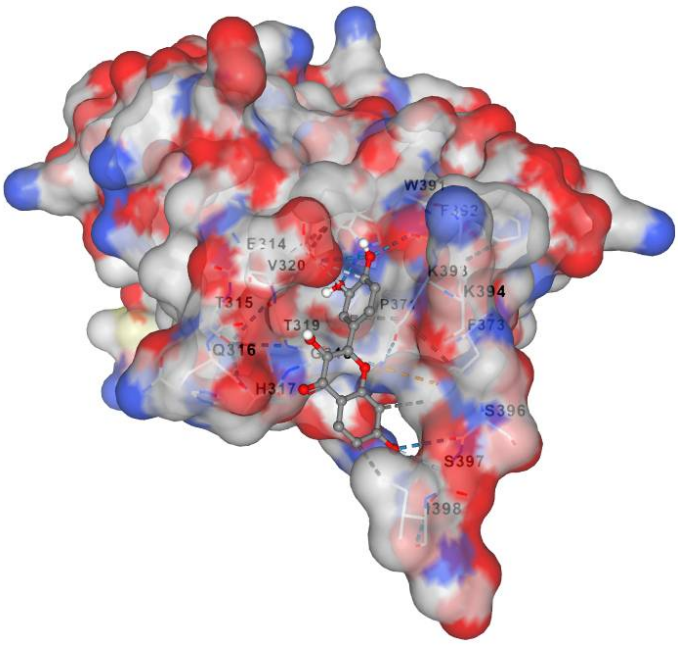

Figure 1: Docked structure of DENV1- fisetin generated by CB dock.

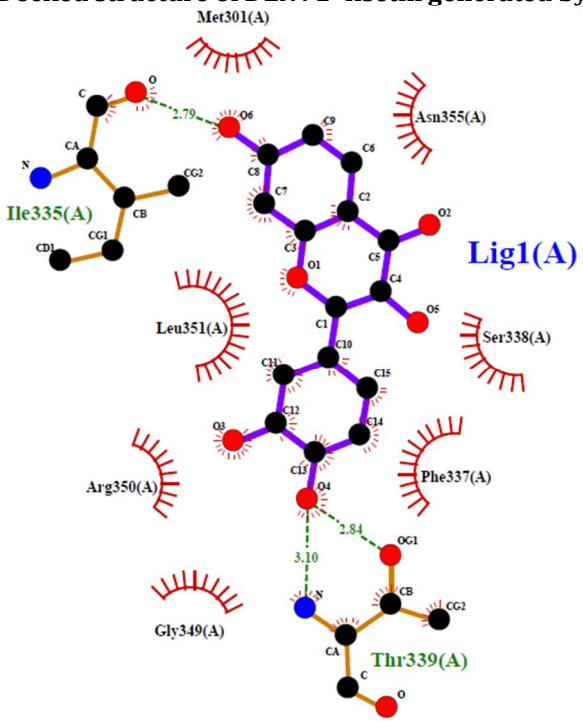

Figure 2: Ligplot analysis of DENV1 and fisetin

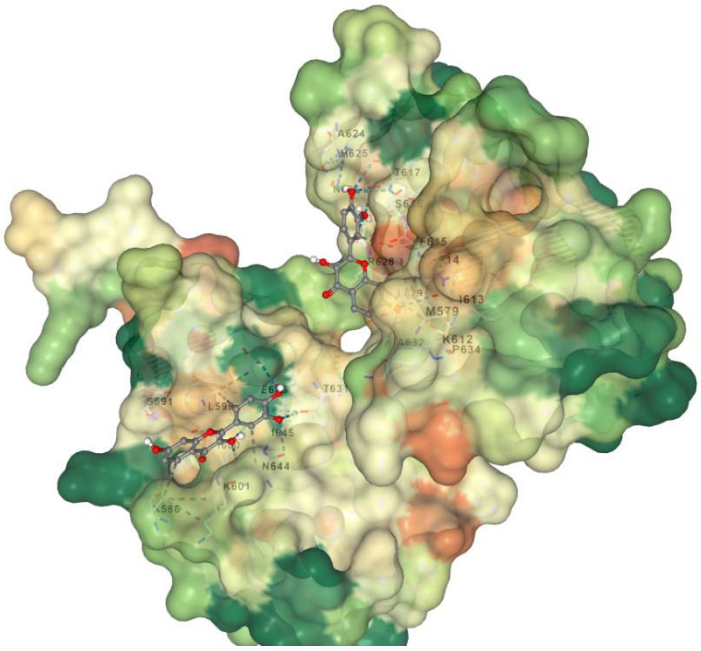

Figure 3: Docked structure of DENV3- fisetin generated by CB dock.

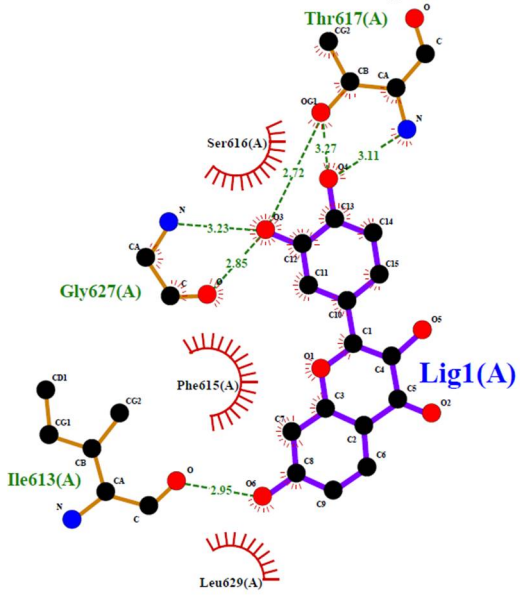

Figure 4: Ligplot analysis of DENV3 and fisetin

Sr No.	Primary structure properties	DENV1	DENV3
1.	Number of amino acids	108	107
2.	Molecular Weight	11682.41	11607.29
3.	Theoretical pI	6.58	7.94
4.	Total positively charged residues	13	13
5.	Total negatively charged residues	13	14
6.	Aliphatic index	73.98	79.25
7.	Grand average of hydropathicity	-0.306	-0.405

Table 1: The primary structure analysis of envelop protein DENV1 and DENV3

Sr No	Flavonoid inhibitor	DENV1	DENV3

		Vina Score	ΔG	Vina Score	ΔG
1.	Baicalein	-5.8	-7.27	-6.5	-8.28
2.	Fisetin	-5.6	-8.34	-6.7	-7.56
3.	Quercetin	-5.8	-7.16	-6.7	-7.54
4.	Silymarin	-6.4	-7.10	-7.3	-7.63

Table 2: Vina scores generated from CB Dock and ΔG generated from SwissDock for Baicalein, Fisetin, Quercetin, and Silymarin against DENV1 and DENV3

Conclusion

Our research revealed that fisetin, which may interact with DENV structural proteins, might bind to DENV-1 and DENV-3 and decrease viral contagiousness. These interactions might prevent DENV from attaching to its cellular receptors, including heparan sulphate, nLc-4Cer, DC-SIGN, mannose receptor, HSP70/HSP90, and GRP7823. In order to investigate the *in silico* mode of action of fisetin against the DENV E protein, molecular docking was employed. The DENV E protein is renowned for facilitating viral attachment and entry into host cells. Fisetin can bind to envelop protein the DENV1 and DENV3 create strong H-bonds. As a result of direct interactions with viral surface proteins, fisetin can decrease the infectiousness of DENV and hence limit viral attachment and penetration. Further research is required to determine the molecular mechanism by which this flavonoids target DENV and whether DENV is capable of developing a resistance to them.

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