Journal of Ayurveda & Holistic Medicine

www.jahm.co.in

eISSN-2321-1563

ORIGINAL RESEARCH ARTICLE -EXPERIMENTAL OPEN ACCESS

BIOACTIVE PHYTOCHEMICALS AS INHIBITORS AGAINST DENGUE VIRUS PROTEIN NS-5 METHYLTRANSFERASE: *IN SILICO* MOLECULAR DOCKING APPROACH

ARVIND KUMAR SHAKYA

Date of Submission: 15-04-2023; Date of revision: 19-04-2023; Date of acceptance: 25-04-2023

ABSTRACT:

Background: Dengue fever has emerged globally as a major public health concern since the last decade due to its high morbidity and mortalities. Phytochemicals play an important role as antivirus inhibitors against virus induced diseases. This study aims to an *in silico* identification of phytochemicals as potential inhibitors against dengue viral protein NS5 methyltransferase (NS5-MTase), an enzyme critical to dengue RNA replication (PDB ID 5E9Q). It is an attractive drug target for new antiviral medicines. **Materials and Methods:** The bioinformatics tool-AutoDock1.5.6 was used for prediction of binding interactions between ligands and the receptor protein. Lipnki's rule of five was also used to assess the drug-Likeness properties of ligands. Discovery studio software was used for visualisation of 2D and 3D interactions. The five phytochemicals selected for this study were rutin, curcumin, D-camphor, quercetin and amentoflavone. **Results:** The five phytochemicals were docked with the NS-5 MTase and exhibited hydrogen bonding interactions at the binding pocket of receptor protein. Among the phytochemicals *i.e*. rutin (-4.59 kcal/mol), curcumin (-5.20 kcal/mol), D-camphore (-5.21 kcal/mol), quercetin (-5.41 kcal/mol), amentoflavone displayed the highest docking score -6.05 kcal/mol with the most hydrogen bonds (nine) at the active site of the target protein. Hydrogen bonding interactions between phytochemical compounds and NS5-MTase's active binding pocket suggested that phytochemicals may block substrate binding or disrupt its catalytic mechanism in order to inhibit enzyme activity of NS-5 MTase. All phytochemicals favour drug-likeness properties except rutin and amentoflavone. **Conclusion:** The docking results suggested that all five phytochemicals were successfully interacted with the binding residues of NS-5MTase and indicated the binding affinity with the NS5-methyltransferase by forming hydrogen bonds. Amentoflavone was most potential antiviral NS-5 methyltransferase inhibitor which have high docking score -6.05 kcal/mol among other phytochemicals. However, this study needs an experimental validation of these phytochemicals for the development of dengue antiviral therapies.

Key words: Phytochemicals, NS-5 Methyltransferase, Molecular Docking, Docking Score

[1]**Assistant Professor, Discipline of Biochemistry, School of Sciences, Indira Gandhi National Open University, New Delhi, INDIA**

Corresponding Email id: arvind.kumar@ignou.ac.in **Access this article online:** www.jahm.co.in

INTRODUCTION

Dengue fever is a global health issue that affects millions of people every year. According to the World Health Organization (WHO), an estimated 390 million dengue infections occur worldwide each year, and around half of the world's population is at risk of infection[1]. According to the National centre for vector born diseases control (NCVBDC), 2023, India, there were about 1,93,245 cases of dengue fever reported in the 2021 year and approximate 1,10,473 cases in 2022[2] . Hence, dengue fever is a major public health concern because it can cause severe illness and death, particularly in children and young adults. In addition to the direct impact on human health, dengue fever also has significant economic consequences, including healthcare costs and lost productivity. Dengue virus (DENV) is a mosquito-borne virus belonging to the Flaviviridae family. It is responsible for causing dengue fever, a viral infection that can range from mild to severe and can be fatal[3]. The genome of dengue virus is a single-stranded RNA molecule that is approximately 11 kilobases in length. Dengue virus genome is composed of three structural proteins, including capsid (C), precursor membrane (prM), and envelope (E), as well as seven non-structural proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Non-structural 5 (NS5) protein is a AdoMet-dependent mRNA methyltransferase (MTase) responsible for adding a 5'-methyl cap structure to the viral RNA. It is essential for the stability and translation of the RNA. The AdoMet-dependent MTase domain of NS5 has a characteristic fold known as the "SAM (S-adenosylmethionine) domain", which contains a central six-stranded beta-sheet flanked by several alpha-helices. The SAM domain is responsible for binding to the cofactor S-adenosylmethionine (AdoMet), which donates a methyl group for the formation of the methyl cap structure [4]. The MTase domain of NS5 also contains a binding site for the RNA substrate, which is recognized by a conserved sequence motif known as the K-D-K-E motif. This motif interacts with the phosphate backbone of the RNA, positioning it for catalysis by the AdoMet-dependent MTase domain. Overall, the AdoMet-dependent MTase domain of NS5 plays a crucial role in the replication of the flavivirus RNA genome, and is an attractive target for the development of antiviral drugs[5] . Hence, it is considered as a target protein for prevention of dengu virus growth [6] . This genetic diversity makes it challenging to develop effective vaccines and antiviral therapies against dengue virus infection. Currently, there is no effective medicine and vaccine for the treatment of dengue viral fever.

Phytochemicals are naturally occurring compounds found in plants, and they have a range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Several research studies have reported that phytochemicals have antiviral activity against various viruses, including influenza virus, human immunodeficiency virus (HIV), herpes simplex virus (HSV), and hepatitis C virus (HCV), among others. Some examples of phytochemicals with antiviral activity include flavonoids, terpenoids, alkaloids, and polyphenols [7-10] . Our study used a molecular docking approach to identify antiviral phytocompounds such as rutin, D-camphor, curcumin, quercetin, amentoflavone against dengue virus protein NS5-emthyltransferase using molecular docking approach. Rutin is a flavonoid, which is a type of phytocompound with antioxidant and anti-inflammatory and antiviral properties [11-12]. Camphor is a white, crystalline substance with a strong, aromatic odor. It is derived from the wood of camphor trees (*Cinnamomum camphora*) and can also be synthesized from turpentine oil. In traditional medicine, camphor has been used for its analgesic, antispasmodic, and anti-inflammatory properties[13-14].

Quercetin is a type of flavonoid responsible for providing color to fruits, vegetables, and flowers. It is found in a variety of foods, including apples, onions, berries, citrus fruits, and leafy greens. It is known for its antioxidant properties, which means it can help protect cells in the body from damage caused by free radicals. Research has also suggested that quercetin may have other health benefits, such as improving exercise performance, reducing allergy symptoms, and promoting healthy blood pressure levels [15-16] .

Curcumin is a naturally occurring phytocompound found in the spice turmeric, which is commonly used in Indian and Southeast Asian cuisine. It is responsible for the bright yellow color of turmeric and has been used for centuries in Ayurvedic and traditional Chinese medicine to treat a variety of health conditions [17] . It is a group of plant-based compounds known for their antioxidant and anti-inflammatory properties [18] . Studies have shown that curcumin may have a range of health benefits, including reducing inflammation, improving brain function, and lowering the risk of heart disease and certain types of viral diseases[19].

Amentoflavone is a naturally occurring biflavonoid compound found in various plants[20], including *Ginkgo biloba*, *Hypericum perforatum* (St. John's Wort), cannabis *and selaginella bryopteris* [21] . One of the most notable properties of amentoflavone is its antioxidant activity, which makes it a potentially valuable compound for protecting

against oxidative stress and reducing inflammation. Additionally, it has been shown to have antiviral, antibacterial, and antitumor properties, and may have potential therapeutic applications in the treatment of cancer, viral infections, and other diseases [22-23] .

Molecular docking is the popular bioinformatics method used in computer-aided drug designing (CADD) [24]. One key application of bioinformatics in drug discovery is the use of molecular docking technique to predict the interaction between drugs and their target proteins[25-26]. Therefore, the aim of the present study was to identify binding affinity of phytochemicals as a antiviral compounds against dengue virus NS-5 Methyltransferase protein.

MATERIALS AND METHODS

Selection and preparation of receptor protein

X-ray crystal structure of dengue 2- NS5 Methyl transferase (MTase) was retrieved from Protein Databank (http://www.rcsb.org/) in PDB format (PDB Id: 5E9Q; resolution 1.79 Å). This protein contains two chains: A and C with bound native ligands (S-adenosyl-L-homocysteine-SAM and 5KY and 5KY [27] . The protein was initially optimised by removing water molecules, hetroatoms and chain C using the discovery studio and the chain A was considered as receptor protein for docking study.

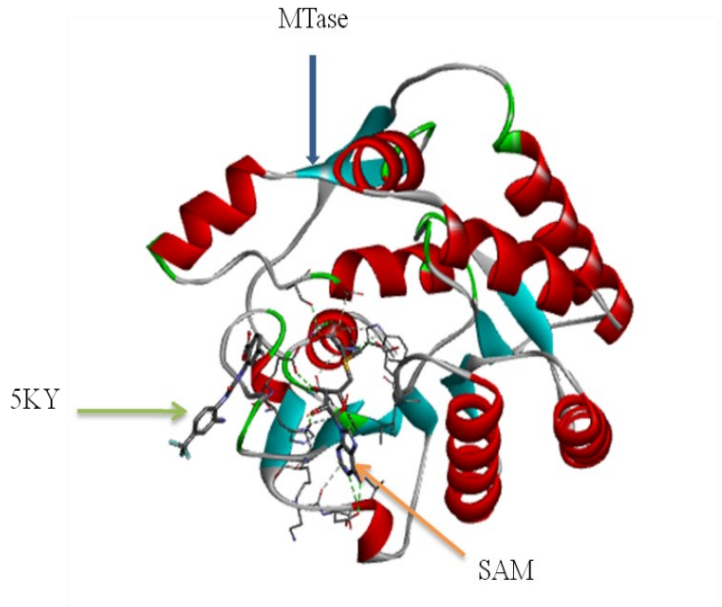
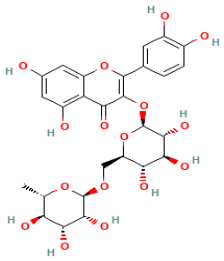
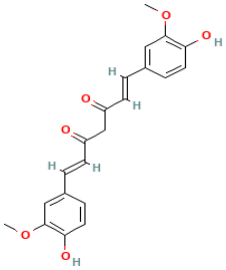
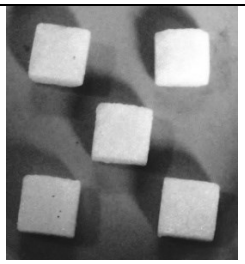
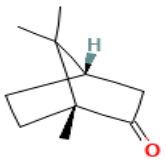
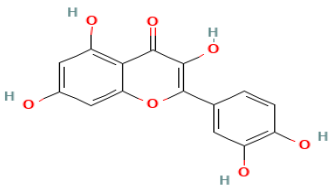
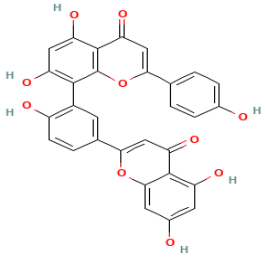


Fig.1 Crystal structure of DENV NS5 monomer. The SAM bound with MTase domain is shown in cyan color (PDB ID: 5E9Q).

Ligand preparation

The chemical structures of ligands - rutin, D-camphor, quercetin, curcumin, amentoflavone, were retrieved in sdf (structure data file) from the Pubchem database https://pubchem.ncbi.nlm.nih.gov/ (Table 1) and then, sdf of all ligands was converted to the .pdb format using the OpenBabel software [28].

Table 1: List of phytochemicals (ligands)

Name of ligands with PubChem CID	Natural sources	Molecular structure	Pubchem Url link
Rutin (5280805)	 Black grapes		https://pubchem.ncbi.nlm.nih.gov/compound/5280805
Curcumin (969516)	 Haldi		https://pubchem.ncbi.nlm.nih.gov/compound/969516
D-Camphor (230921)			https://pubchem.ncbi.nlm.nih.gov/compound/159055
Quercetin (5280343)	 Apple		https://pubchem.ncbi.nlm.nih.gov/compound/5280343
Amentoflavone (5281600)	 *Selaginella Bryopteris*		https://pubchem.ncbi.nlm.nih.gov/compound/5281600

Active site prediction of protein (5E9Q)

Active site coordinates of NS-5 MTase was determined by the discovery studio software 2.0 and the active site residue in chain A was VAL55, SER56, GLY 58, CYS82, ARG84, **GLY86**, THR 104, LYS105, GLY106, **HIS 110**, **GLU111**, **ASP131**, **ASP146**, VAL132, PHE 133, ILE147 which were bound to the native ligands-SAM through non-covalent interactions such as hydrogen bond, hydrophobic,Vander- walls, carbon-hydrogen bonds (Fig.2). These all active residues were set into the grid box to allow the ligand interaction within the region specify in the binding pocket ofNS-5 MTase protein.

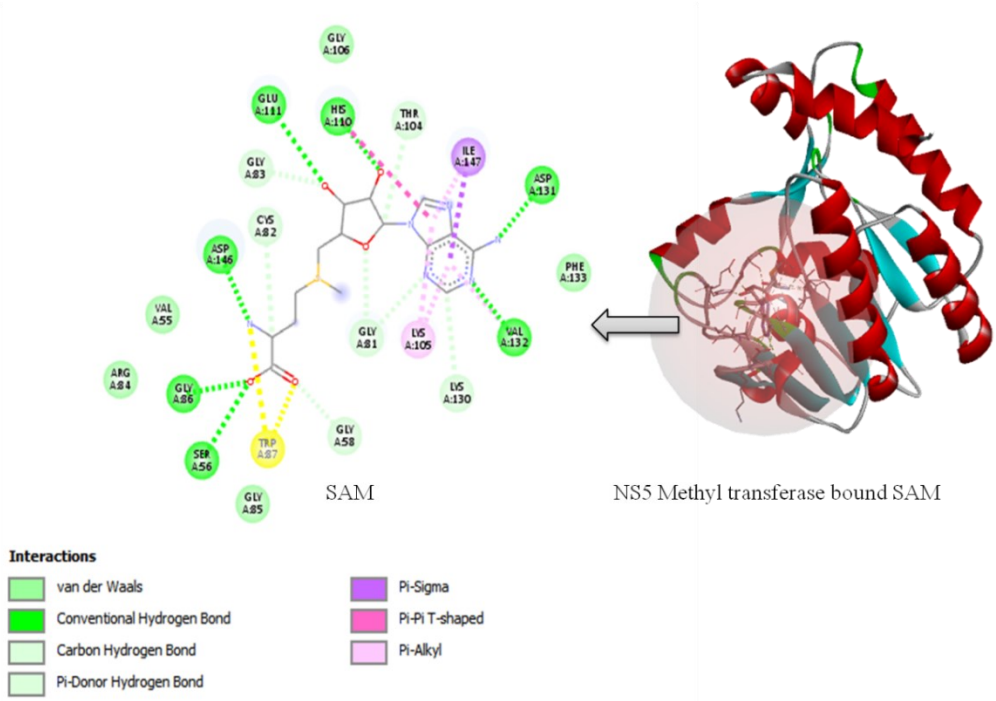


Fig. 2 Tertiary structure of DENV2-NS5 MTase with predicted binding sites.

Molecular docking study

AutoDockTools-1.5.6 was used to predict binding affinity between ligands and the target receptor protein, NS5MTase[29]. Before docking, water molecules and native ligands were removed from the A chain of PDB of 5e9q (NS5 MTase) and then polar hydrogen atoms and kollman charges were added and pdb was saved in .pdbqt. Torsion root was set for the pdb file of ligands and then saved it in .pdbqt format. The grid box centre was set with size 40x40x40 with 0.35Å and it was defined at X=32.02; Y=41.81; Z= -6.094. After generating autogrid (.dlg), the docking parameters were set (.dpf) and then run in two times through a Lamarckian genetic algorithm. Docking energy was analysed using AutoDock tools to assess the most ideal possible docking with ligands. BIOVIA-discovery studio 2021 was utilized to visualise

the 2D and 3D interactions of docked complex. Surface view of docked complex was prepared from the PyMol software. The docking results were expressed in docking score (kcal/mol).

Assessment of Drug-likeness properties of ligands: The Lipinski rule is widely used in drug discovery and has been shown to be a useful tool for predicting the drug-likeness of small molecules. However, it should be noted that the rule is not a strict guideline and there are exceptions to the rule.

The Lipinski rule, also known as the Rule of Five, is a set of rules used to evaluate the drug-likeness of a molecule based on its chemical and physical properties [30]. The Lipinski rule states that a molecule is likely to be orally active and have good pharmacokinetic properties if it meets the following criteria:

- Molecular weight (MW) should be less than or equal to 500 g/mol.
- The number of hydrogen bond donors (HBD) should be less than or equal to 5.
- The number of hydrogen bond acceptors (HBA) should be less than or equal to 10.
- The octanol-water partition coefficient (LogP) should be less than or equal to 5.

Drug-likeness of phytochemicals was predicted by online server of molinsprotion (https://www.molinspiration.com/).

RESULTS AND DISCUSSION

Docking study

Docking study of ligand-protein interaction was assessed by docking score and hydrogen bonding. In the present study, five (05) phytochemicals have been evaluated for their binding affinity with theNS-5 methyltransferase. Docking score is a measure of the quality of the predicted binding affinity between ligand and protein receptor in a molecular docking simulation. A higher docking score indicates a stronger predicted binding affinity between ligands and the receptor [31]. NS5- Methyltransferase of dengue viral protein was commonly used for prediction of potential drug candidates using the molecular docking approach [32].

The binding score and interactive amino acid residues and H-bonds of docked complex were represented in Table 2.

Table 2: Docking results and binding interactions between ligand molecules and the NS-5 Methyltransferase

Molecule name	Docking score (kcal/mol)	Receptor PDB ID	Interacting amino acid residues	Number of conventional H-bonds
Rutin	- 4.59	5e9q	GLY109, GLU111, LYS180,	7

			LYS130, GLY 148	
Curcumin	-5.20	5e9q	LYS130, VAL 132, GLY148, HIS 110	4
D-Camphor	-5.21	5e9q	GLY148	1
Quercetin	-5.41	5e9q	ARG84, LYS105, GLY148, ASP146	4
Amentoflavone	-6.05	5e9q	LYS 61, CYS 82, GLY86, LYS105, ASP131, SER150, GLU149, SER150, GLU216	9

2D and 3D presentation of docked complex of phytocompounds *i.e*. rutin, cucurmin, camphor, quercetin and amentoflavone with the catalytic site of NS5-methyltransferase were shown in Fig.3-7 respectively. The binding affinities of the ligands in the binding pocket of receptor protein can be explained by number of hydrogen bonds which predicts stability of docked complex with the receptor protein.

Rutin is a flavonoid glycoside made up of the flavonol quercetin and the disaccharide rutinose. It is also called rutoside. Rutin is made up of two aromatic rings and a number of hydroxyl groups. A benzene ring and a pyran ring are the two aromatic rings in rutin[33] . The benzene ring is attached to the pyran ring at position 2. Benzene and pyran rings are attached to the hydroxyl groups of rutin. In the present study, the hydroxyl groups of rutin act as proton donor and interact with amino acid residues of protein binding pocket of NS-5 Methyltransferase. Fig. 3a&b showed the hydrogen bonding interactions between rutin and NS-5 MTase and this docked complex formed seven H-bonds with the amino acid residues i.e. GLY109, **GLU111**, LYS180, LYS130, GLY148 present in the receptor binding pocket (NS-5 MTase) (Fig. 4c). Docking score of rutin was -4.59 kcal/mol.

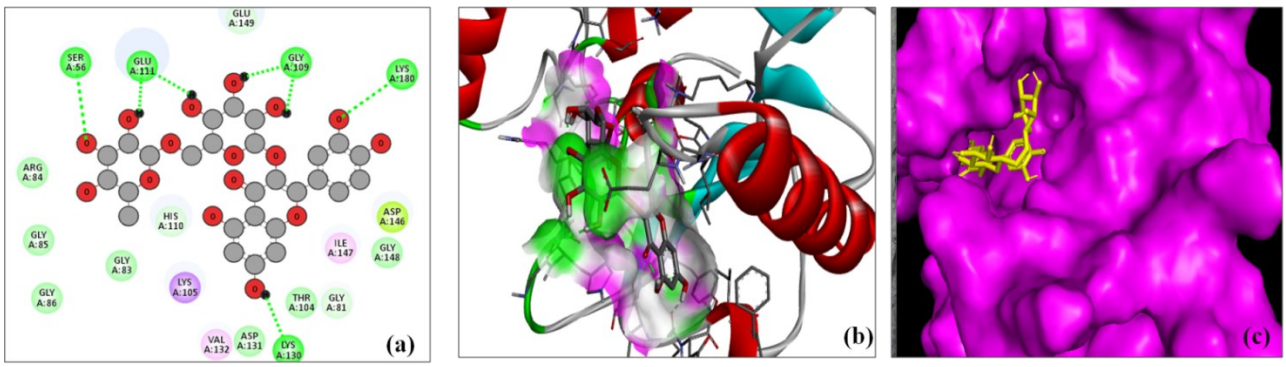


Fig. 3: Binding interactions between rutin and NS-5MTase. (a) 2D binding interaction (b) 3D view (c) Surface view of the docked complex.

The curcumin consists of two aromatic rings connected by a seven-carbon linker. The two aromatic rings are both phenyl rings, with one being substituted with two methoxy groups (-OCH_3) and the other with two hydroxyl groups (-OH) [34]. These aromatic rings with hydroxyl groups serves as functional group which play important role in biological activity. Fig. 4 a&b exhibits the hydrogen bonding interactions (2D and 3D views) of docked complex of curcumin and receptor protein respectively. The curcumin aromatic rings successfully docked with the binding pocket of NS-5 MTase and form four H-bonding interaction with active amino acid residues LYS130, VAL 132, GLY148, **HIS 110** and the dock score of -5.20 kcal/mol was obtained. Surface view of docked complex is shown in Fig. 4c.

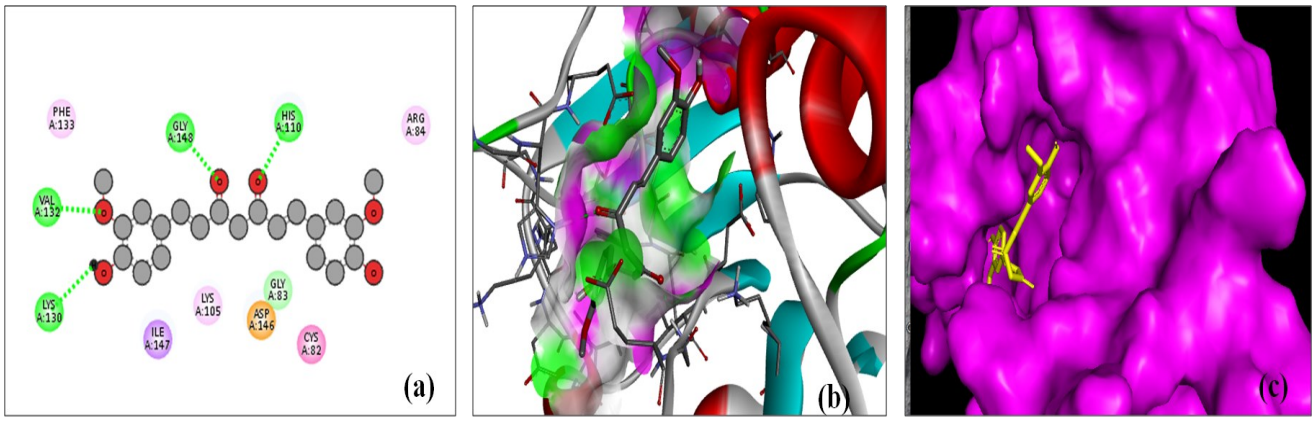


Fig. 4 Binding interactions between curcumin and NS-5MTase. (a) 2D binding interaction and 3D view (c) Surface view of the docked complex.

The camphor comprises of a bicyclic system with a fused cyclohexanone ring and a fused cycloheptene ring which contains a ketone group (C=O) [35]. Camphor does not contain any hydroxyl group (-OH) in its chemical structure. Camphor also showed interaction with the single amino acid GLY148 of the docking pocket having docking energy -5.21 kcal/mol (Fig.5).

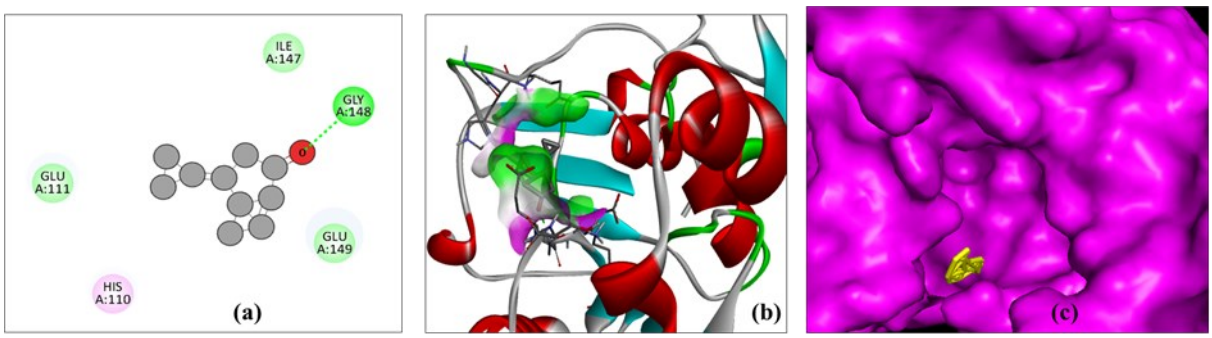


Fig. 5: Binding interactions between camphor and NS-5MTase. (a) 2D binding interaction (b) 3D view (c) Surface view of the docked complex.

Quercetin is a flavonoid with three rings: two aromatic and one heterocyclic. The two aromatic rings are benzene and pyran rings, which are comparable to the rings found in rutin [36]. Our findings show that quercetin docked with binding pocket amino acids by interacting with ARG84, LYS105, GLY148, and ASP146 (Fig.6). With a binding affinity of -5.41 kcal/mol, it creates four H-bonds.

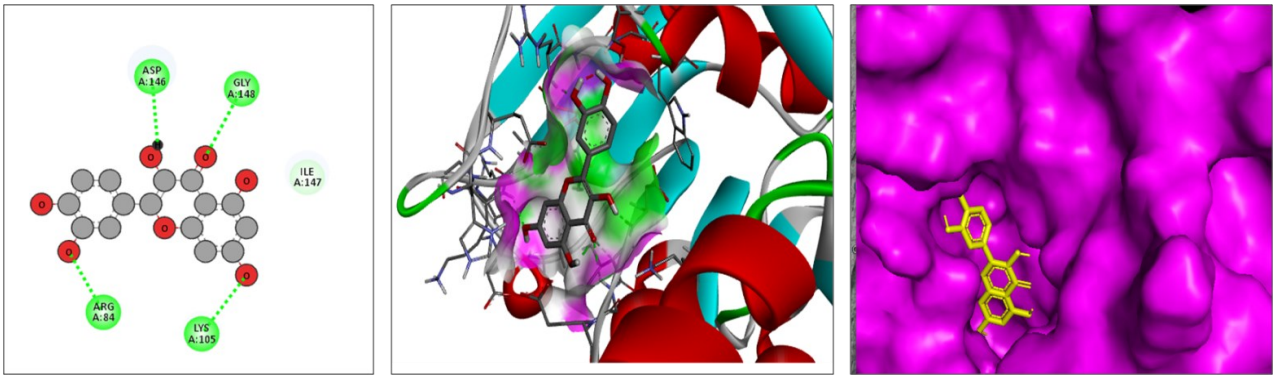


Fig. 6: Binding interactions between Quercetin and NS-5MTase. (a) 2D binding interaction (b) 3D view (c) Surface view of the docked complex.

The amentoflavone is a biflavonoid consisting of two apigenin units linked by a benzene ring and a cyclohexene ring linker [37]. Each apigenin unit is made up of a flavanone ring with a hydroxyl group (-OH) at position 5 and an aromatic ring with hydroxyl groups (-OH) at positions 7 and 4' [38]. Amentoflavone's chemical structure has six hydroxyl groups, four of which are placed on the aromatic rings of the apigenin units and two on the linker. According to the results of the docking investigation, amentoflavone interacted with active amino acid residue *i.e*. LYS61, CYS82, GLY86, LYS105, ASP131, SER150, GLU149, SER150, GLU216. Fig.7 depicts the docked complex of amentoflavone with the receptor protein (NS-5 MTase).The amentoflavone structure generates nine hydrogen bonds in the binding pocket, with a binding affinity of -6.05 kcal/mol due to the presence of hydroxyl groups.

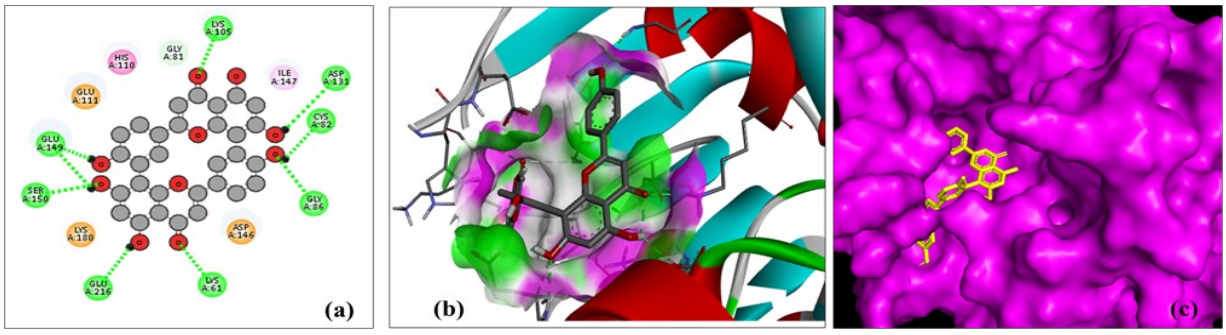


Fig. 7: Binding interactions between Amentoflavone and NS-5MTase. (a) 2D binding interactions (b) 3D view (c) Surface view of the docked complex.

Therefore, based on the docking results, the amino acid residues of NS-5 MTase binding pocket were interacted and successfully docked with the phytochemicals rutin (GLU 111), curcumin (HIS 110), quercetin (ASP146) and amentoflavone (GLY86, ASP 131) besides the D-camphor, and these interactive amino acid residues are also present within the binding pocket of native ligands (SAM) attached with receptor protein NS-5 MTase. Thus, the docking results suggest that these phytochemicals could block the activity of NS-5 MTase and therefore could be considered as potential inhibitors against dengue virus.

Lipinski rule of five

Lipinski rule of five is commonly used for prediction of suitable drug candidate based on its molecular weight, number of rotatable bonds, number of hydrogen bonds acceptor, number hydrogen bonds donor and logP [39] . The phytocompounds - cucurmin, camphor, quercetin shows the favourable Lipinski rule except rutin and amentoflavone which have Lipinski's violation (Table 3).

Table 3: Drug-likeness properties of phytochemicals

Compounds	Lipinski Rule of five	LogP ≤4.15	Molecular Weight g/mol ≤500	H-bond acceptor ≤10	H bond donor ≤5	Rotable bonds ≤10	Lipinski violation ≤1
1.	Rutin	-1.06	610.52	16	10	6	2
2.	Curcumin	2.30	368.38	6	2	8	0
3.	D-Camphor	2.16	152.24	1	0	0	0
4.	Quercetin	1.68	302.24	7	5	1	0
5.	Amentoflavone	5.16	538.46	10	6	3	2

CONCLUSION:

From these results, it can conclude that all studied phytochemicals were successfully docked at the active site of the target receptor NS5 Methyltransferase due to formation hydrogen bonding interactions. The rutin, quercetin, amentoflavone have showed maximum hydrogen bonding with the migh be have strongest inhibitory activity was - 4.59 kacl/mol, -5.20 kacl/mol, -5.21 kacl/mol, -5.41 kacl/mol, -6.05 kacl/mol respectively. Amentoflavone showed the strongest affinity in the binding site of the NS-5 Methyltransferase with maximum hydrogen bonding (8) followed by rutin (7). The docking score indicates that the amentoflavone could

have a greater inhibitory potential against NS-5 MTase followed by other docked phytochemicals. However, this study needs further validation through experimental analysis.

REFERENCES:

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI (2013). The global distribution and burden of dengue. *Nature*, 496(7446): p. 504–507.
2. National Center for Vector Borne Diseases Control (NCVBDC), Ministry of Health & Family Welfare, Government of India (2023). https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715
3. Brady OJ and Hay SI (2020). The global expansion of dengue: how *Aedes aegypti* mosquitoes enabled the first pandemic arbovirus**.** *Annu Rev Entomol.* **65**: 191-208.
4. Shimizu H, Saito A, Mikuni J, Nakayama EE, Koyama H, Honma T, et al. (2019). Discovery of a small molecule inhibitor targeting dengue virus NS5 RNA-dependent RNA polymerase. PLoS Negl Trop Dis. 13(11): e0007894. https://doi.org/10.1371/journal.pntd.0007894.
5. Benmansour F, Trist I, Coutard B, Decroly E, Querat G, Brancale A, Barral K (2017). Discovery of novel dengue virus NS5 methyltransferase non-nucleoside inhibitors by fragment-based drug design. Eur J Med Chem. 5;125:865-880. doi: 10.1016/j.ejmech.2016.10.007. Epub 2016 Oct 5. PMID: 27750202.
6. Lim SP, Noble CG, Shi PY (2015). The dengue virus NS5 protein as a target for drug discovery. Antiviral Res. 119:57-67. doi: 10.1016/j.antiviral.2015.04.010. Epub 2015 Apr 24. PMID: 25912817.
7. Ghildiyal, R., Prakash, V., Chaudhary, V. K., Gupta, V., & Gabrani, R. (2020). Phytochemicals as Antiviral Agents: Recent Updates. *Plant-derived Bioactives: Production, Properties and Therapeutic Applications*, 279–295. https://doi.org/10.1007/978-981-15-1761-7_12
8. Min N, Leong PT, RCH L, Khuan JSE, Chu JJH (2018). A flavonoid compound library screen revealed potent antiviral activity of plant-derived flavonoids on human enterovirus A71 replication. *Antiviral Res.* 150:60–68. doi: 10.1016/j.antiviral.2017.12.003.
9. Kaushik S, Kaushik S, Sharma V, Yadav JP (2018). Antiviral and therapeutic uses of medicinal plants and their derivatives against dengue viruses. *Pharmacogn Rev.* 12(24):177. doi: 10.4103/phrev.phrev_2_18. [CrossRef] [Google Scholar]
10. Anusuya S, Gromiha MM. Structural basis of flavonoids as dengue polymerase

inhibitors: insights from QSAR and docking studies (2019). *J Biomol Struct Dyn.* 37(1):104–115. doi: 10.1080/07391102.2017.1419146

11. Ganeshpurkar A , Saluja AK. (2017). The Pharmacological Potential of Rutin. *Saudi Pharmaceutical Journal*. 25, 2; 149-164.
12. Beatriz Gullón, Thelmo A. Lú-Chau, María Teresa Moreira, Juan M. Lema, Gemma Eibes (2017). Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology*. 67, 220-235,
13. Assia Belhassan, Hanane Zaki, Samir Chtita, Marwa Alaqarbeh, Nada Alsakhen, Mohamed Benlyas, Tahar Lakhlifi, Mohammed Bouachrine (2021). Camphor, Artemisinin and Sumac Phytochemicals as inhibitors against COVID-19: *Computational approach, Computers in Biology and Medicine*. 136, 104758.
14. Oreshko VV, Kovaleva KS, Mordvinova ED, Yarovaya OI, Gatilov YV, Shcherbakov DN, Bormotov NI, Serova OA, Shishkina LN, Salakhutdinov NF (2022). Synthesis and Antiviral Properties of Camphor-Derived Iminothiazolidine-4-Ones and 2,3-Dihydrothiazoles. *Molecules.* 25;27(15):4761.
15. Di Petrillo A, Orrù G, Fais A, Fantini MC (2022). Quercetin and its derivates as antiviral potentials: A comprehensive review. Phytother Res. 36(1):266-278. doi: 10.1002/ptr.7309.
16. Lakhanpal P, Rai DK (2007). Quercetin: A versatile flavonoid. *Int J Med Update.* ;2:22–37.
17. Hewlings SJ, Kalman DS (2017). Curcumin: A Review of Its Effects on Human Health. *Foods*. 6(10):92. https://doi.org/10.3390/foods6100092.
18. Rattis BAC, Ramos SG and Celes MRN (2021). Curcumin as a Potential Treatment for COVID-19. Front. Pharmacol. 12:675287. doi: 10.3389/fphar.2021.675287
19. Rathore S, Mukim M, Sharma P et.al (2020). Curcumin: a review for health benefits. *International Journal of Research and Review*. 7(1): 273-290.
20. Shu-Mei Yue and Wen-Yi Kang (2011). Lowering blood lipid and hepatoprotective activity of amentoflavone from *Selaginella tamariscina* in vivo*. J. of Medicinal Plants Research*. 5(14), 3007-3014, 18 .
21. Verma M, Gangwar M, SahaiM. et al. (2015). Antimicrobial Activity of Phytochemicals Isolated from *Selaginella bryopteris*. Chem Nat Compd 51, 341–345. https://doi.org/10.1007/s10600-015-1277-9
22. Xiong X, Tang N, Lai X, Zhang J, Wen W, Li X, Li A, Wu Y and Liu Z (2021). Insights Into Amentoflavone: A Natural Multifunctional Biflavonoid. *Front. Pharmacol.* 12:768708. doi: 10.3389/fphar.2021.768708

23. Dey, D., Hossain, R., Biswas, P., Paul, P., Islam, M. A., Ema, T. I., Gain, B. K., Hasan, M. M., Bibi, S., Islam, M. T., Rahman, M. A., & Kim, B. (2022). Amentoflavone derivatives significantly act towards the main protease (3CLPRO/MPRO) of SARS-CoV-2: in silico admet profiling, molecular docking, molecular dynamics simulation, network pharmacology. *Molecular diversity*, 1–15. Advance online publication. https://doi.org/10.1007/s11030-022-10459-9
24. Xia X (2017). Bioinformatics and Drug Discovery. *Curr Top Med Chem* . 17(15):1709-1726. doi: 10.2174/1568026617666161116143440. PMID: 27848897; PMCID: PMC5421137.
25. Pagadala NS, Syed K, Tuszynski J (2017). Software for molecular docking: a review. Biophys Rev. 9(2):91-102.
26. De Ruyck J, Brysbaert G, Blossey R, Lensink M (2016). Molecular docking as a popular tool in drug design, an in silico travel. *Adv Appl Bioinform Chem*.;9:1-11. https://doi.org/10.2147/AABC.S105289 .
27. Fatiha Benmansour, Iuni Trist, Bruno Coutard, Etienne Decroly, Gilles Querat, Andrea Brancale, Karine Barral (2017). Discovery of novel dengue virus NS5 methyltransferase non-nucleoside inhibitors by fragment-based drug design, European Journal of Medicinal Chemistry. 125; 865-880
28. O'Boyle, N.M., Banck, M., James, C.A. *et al* (2011). Open Babel: An open chemical toolbox. *J Cheminform* **3**, 33. https://doi.org/10.1186/1758-2946-3-33
29. Morris GM Huey R, Lindstrom W, SannerMF, Belew RK, Goodsell DS and Olson AJ (2009). Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Comput. Chem., 30: 2785-2791.
30. O'Hagan, S., Swainston, N., Handl, J. et al. A 'rule of 0.5' for the metabolite-likeness of approved pharmaceutical drugs. Metabolomics 11, 323–339 (2015). https://doi.org/10.1007/s11306-014-0733-z
31. Ying TC, Ibrahim Z, Abd Rahman MB, Tejo BA (**2018)**. Structure-based design of peptide inhibitors for protein arginine deiminase type iv (PAD4). *Encycl. Bioinform. Comput. Biol. ABC Bioinform.*, *1*, 729–740.
32. Chaudhary S, Kumar A, Srivastava V(2017). In Silico Molecular Docking of Antiviral drugs against NS5- Methyltransferase of dengue virus . *International Journal of Scientific and Innovative Research*. 2017; 5(1) : 41-44.
33. Panhwar, Qadeer and Memon, Shahabuddin (2014). "Synthesis, characterisation, and antioxidant study of Cr(III)-rutin complex" *Chemical Papers*, 68 (5); 614 623. https://doi.org/10.2478/s11696-013-0494-6.

34. Priyadarsini, Kavirayani Indira (2014). "The Chemistry of Curcumin: From Extraction to Therapeutic Agent" *Molecules* .19 (12): 20091-20112. https://doi.org/10.3390/molecules191220091.
35. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 2537, https://pubchem.ncbi.nlm.nih.gov/compound/Camphor.
36. Muhammet Ay, Adhithiya Charli, Huajun Jin, Vellareddy Anantharam, Arthi Kanthasamy, Anumantha G. Kanthasamy, (2021) - Quercetin, Editor(s): Ramesh C. Gupta, Rajiv Lall, Ajay Srivastava, Nutraceuticals (Second Edition), Chapter 43, Academic Press, 749-755.
37. Hyun Jun Jung, Keunnam Park, In-Seon Lee, Hyun Soo Kim, Soo-Hwan Yeo, Eun-Rhan Woo, Dong Gun Lee, S (2007).-Phase Accumulation of Candida albicans by Anticandidal Effect of Amentoflavone Isolated from *Selaginella tamariscina*, *Biological and Pharmaceutical Bulletin*. 30 (10):1969-1971,
38. Shamsudin, Nur Farisya, Qamar Uddin Ahmed, Syed Mahmood, Syed Adnan Ali Shah, Alfi Khatib, Sayeed Mukhtar, Meshari A. Alsharif, Humaira Parveen, and Zainul Amiruddin Zakaria (2022). "Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation" *Molecules* 27 (4): 1149. https://doi.org/10.3390/molecules27041149.
39. Vinod G. Ugale, Harun M. Patel, Sanjay J. Surana (2017). Molecular modeling studies of quinoline derivatives as VEGFR-2 tyrosine kinase inhibitors using pharmacophore based 3D QSAR and docking approach. *Arabian Journal of Chemistry*. 10 (2): S1980-S20032.

CITE THIS ARTICLE AS

Arvind Kumar Shakya. Bioactive Phytochemicals as Inhibitors against Dengue virus protein NS-5 Methyltransferase: *In Silico* Molecular Docking Approach. *J of Ayurveda and Hol Med (JAHM)*. 2023;11(3):253-267

Conflict of interest: None

Source of support: None