CURRENT SCIENCE AND TECHNOLOGY
E-ISSN: 2785-8804
VOLUME 3, ISSUE 1, 2023, 9 – 15
DOI: https://doi.org/10.15282/cst.v3i1.9338

RESEARCH ARTICLE

In-silico Studies of Usnic Acid against DENV-3 Methyltransferase

M. Roney[1,2], A. Dubey[3,4], A. M. Huq[2,5] and M. F. F. Mohd Aluwi[1,2*]

[1]Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, 26300 Kuantan, Pahang Darul Makmur, Malaysia
[2]Centre for Bio-Aromatic Research, Universiti Malaysia Pahang, 26300 Kuantan, Pahang Darul Makmur, Malaysia
[3]Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, 600077, Tamil Nadu, India
[4]Department of Computational Chemistry and Drug Discovery Division, Quanta Calculus, Greater Noida, 201310, Uttar Pradesh, India
[5]Department of Pharmacy, School of Medicine, University of Asia Pacific 74/A, Green Road, Dhaka 1205, Bangladesh

ABSTRACT - There is currently no antiviral medication for dengue, a highly fatal tropical infectious disease spread by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. The most conserved of the four Dengue serotypes and an essential element in viral replication, Dengue NS5 MTase is a promising therapeutic target. Applying in-silico techniques such as molecular docking, pharmacokinetics, and pharmacophore analysis, we intend to discover novel inhibitors against Dengue NS5 MTase from Usnic acid. In the end, the docking results indicated that usnic acid had satisfactory docking values of -9.3 kcal/mol. We were able to confirm that the usnic acid had higher potential scores in docking and bound amino acids than the reference compound during our in-silico evaluation. Molecular docking, pharmacokinetics, and pharmacophore evaluations revealed that usnic acid has high pharmacological potential. Additionally, we anticipate that the testing in vitro and in vivo of usnic acid would indicate potential medicinal benefits.

ARTICLE HISTORY
Received : 12th May 2022
Revised : 9th Feb 2023
Accepted : 14th Feb 2023
Published : 15th June 2023

KEYWORDS
Usnic acid
In-silico
Anti-dengue
Docking
Pharmacophore

1.0 INTRODUCTION

Over four billion people are in danger from dengue, a mosquito-borne virus illness carried by *Aedes aegypti* and *Aedes albopictus* that is now present in over 128 tropical and subtropical regions. Every year, the dengue virus (DENV) affects 390 million individuals and causes about 20,000 fatalities [1]. According to Jarerattanachat et al., this might result in hospitalization and life-threatening symptoms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. Dengue virus (DENV) has four serotypes (DENV-1, DENV-2, DENV-3, and DENV4) and is a member of the Flaviviradae family. Each serotype contains 65-70% of the same genome sequence [1, 3]. Prevention efforts and methods must be increased in light of these depressing numbers. Unfortunately, supportive care and symptomatic therapy are still the only options available for treating dengue today. Except for Dengvaxia, which the FDA has licensedlicensed for use solely in seropositive patients, there is no anti-dengue medication or vaccine.

The DENV genome is 10–11 KB in size and codes for a polyprotein that contains three structural and seven nonstructural proteins [4]. Core/capsid protein, membrane-associated protein, and envelope protein are the three proteins that make up structural proteins. Seven proteins are classified as nonstructural proteins: NS, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. They are arranged in the following order: 5-CprM (M) -E-NS1- NS2A-NS2B-NS3-NS4A-NS4B-NS5-3. According to Qamar et al., nonstructural proteins are crucial for viral replication and other cellular processes, while structural proteins are crucial for the structural organization of viruses and their entry into host cells [5].

The NS5 protein is the biggest among structural and non-structural proteins and is crucial for viral replication [2]. The RNA-dependent RNA-polymerase (RdRp) is located close to the N-terminal area of the NS5 protein, while the methyltransferase (MTase) is located close to the C-terminal region [6]. The RNA capping site, which is a Guanosine 5′-Triphosphate (GTP) binding pocket, and the AdoMet binding site are the two active sites that make up the MTase domain. S-Adenosyl methionine (SAM or AdoMet), a small molecule methylating agent, is the natural ligand at the AdoMet binding site. The ribonucleotide of the viral RNA chain that is located at the RNA capping site can get a methyl group from SAM. When the methyl group is lost after methylation, SAM is changed to S-Adenosyl-l-homocysteine (SAH or AdoHcy) [2]. All four Dengue serotypes and all Flaviviruses share the NS5 protein, which makes it an attractive target for the development of broad-spectrum antiviral drugs [7]. Many organizations have experimentally or virtually screened numerous chemical databases against the enzyme due to the interest in NS5 MTase as a therapeutic target.

***CORRESPONDING AUTHOR | M. F. F. Mohd Aluwi** | ✉ fasihi@umpsa.edu.my

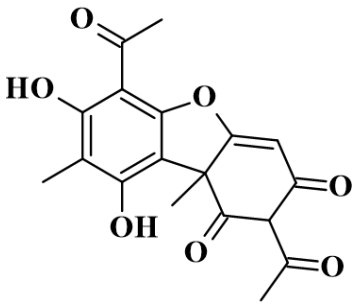

Figure 1. 2D Structure of Usnic Acid (UA)

Usnic acid (UA), a dibenzofuran that was first discovered in lichens, is a member of the secondary metabolites and exhibits a wide range of biological activities [8]. It possesses antiviral [9], anti-inflammatory [10], antitumor [11], antimitotic [12], insecticidal [13], antineoplastic [14], antibacterial [15], fungicidal [16] and antimycotic [12] agent. In addition, UA has a strong larvicidal activity against *A. aegypti* [17]. We used a variety of in-silico techniques, including as molecular docking, and pharmacokinetic and pharmacophore investigations to evaluate the antiviral efficacy of UA against DENV-3 MTase based on the antiviral activity and larvicidal activity against *A. aegypti*.

2.0 METHODS AND MATERIAL

2.1 Ligand Selection and Preparation

Based on the antiviral and high larvicidal activity against *A. aegypti* and anti-viral activity, we selected the UA as a ligand to docked with the target protein. The structures of UA and the reference compound (Quercetin) were constructed using the ChemSketch software and saved in .mol format.

2.2 Target Protein Selection and Preparation

The crystal structure of DENV-3 methyltransferase was selected from the literature [2] and downloaded in PDB format (PDB ID: 4R8S) from the RCSB Protein Data Bank [18].

2.3 Active Site Prediction

This site exploration is crucial to the docking process since it aids in locating the protein's ligand binding pocket. The produced protein was used for the analysis. The protein has a huge number of binding sites, but without this analysis, not all of them could be recognised as good docking sites. Therefore, a suitable location was chosen utilizing the DrugRep online tool for the docking of the phytochemicals with this protein after a comprehensive investigation [19].

2.4 Molecular Docking

The above-described methodologies were used to determine the molecular docking analysis using the online CB-Dock (cavity-detection-guided blind docking) application [20]. The interaction between UA and the DENV-3 methyltransferase (PDB ID: 4R8S) [18] was examined. Using the ChemSketch program, the structure of the compound and the reference drug were created and saved in .mol format. The 3D structure of the target protein was obtained from the Protein Data Bank and saved in .pdb format.

2.5 Pharmacokinetics Analysis

The online tool pkCSM software was used to forecast the characteristics for absorption, distribution, metabolism, and toxicity of the selected compound and the reference compound [21]. Furthermore, using the online platform Molinspiration (https://www.molinspiration.com), the drug-like qualities were evaluated of the compounds [22]. In this software, the SMILES or SD file structures of the compounds are needed for preparation; knowledge of the active site or binding mechanism is not necessary.

2.6 E-Pharmacophore Analysis

The energetic (e)-pharmacophore technique now incorporates both structure- and ligand-based approaches. The pharmacophore sites of UA, such as hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), positively ionizable (P), negatively ionizable (N), and aromatic ring (R), were identified using the phase v 3.4 module in Schrödinger [23, 24].

3.0 RESULTS AND DISCUSSION

3.1 Active Site prediction

The proper putative binding pocket in the target molecules is identified at this crucial stage of the docking process. One site might be utilized to a potential ligand binding pocket despite the protein having a huge number of binding sites

[24]. Nearly five drug-grade pockets were found in this binding pocket detection to be acceptable ligand binding sites. Later, a single docking point was selected based on measurements for the site values (Figure 2 and Table 1).

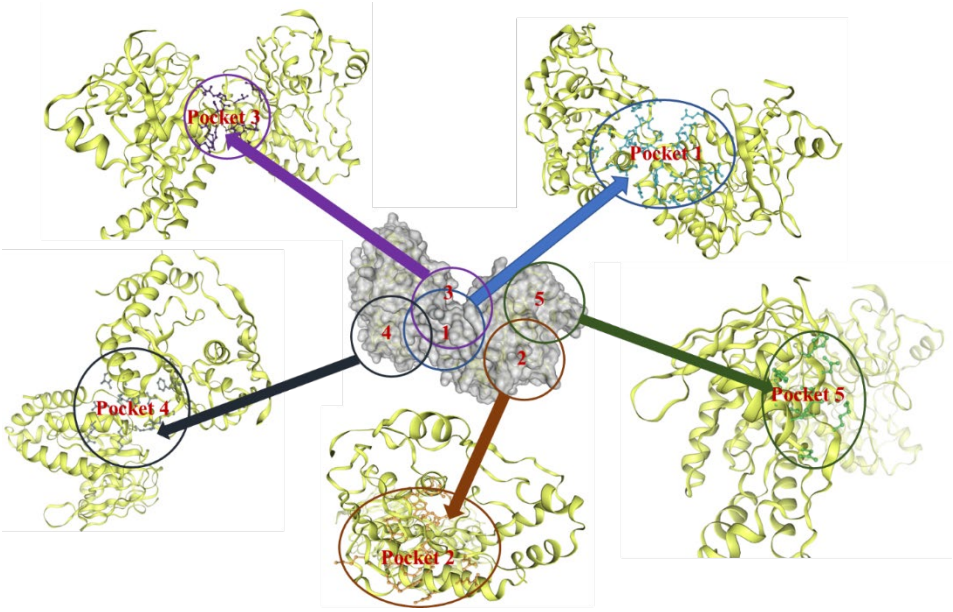

Figure 2. Ligand binding pocket in the DENV-3 methyltransferase protein (4R8S)

Table 1. Active binding pocket of DENV-3 methyltransferase protein (4R8S)

Pocket Number	Volume	Center (X,Y,Z)	Size (X,Y,Z)	Chain	Ligand binding pocket residues
1	1277	-16.8,5.6,21.3	18,18,22	A, B	A: Cys140, Glu138, Trp171, Pro170, Leu135, Asn175, Tyr103, Lys173, Pro137, Pro136, Lys139, Lys130 and Cys140, Hsd200, GLu74, Glu138, Trp171, Asp141, Pro170, Asn174, Asn175, Lys139, Lys173, Pro137, Gln176, Glu169, Leu172, Lys199
2	632	-13.3,-15.3,5.2	18,19,19	B	Asp146, Trp87, Val164, Gly86, Cys82, Lys130, Gly83, Arg84, Gly81, Lys105, Ser56, Glu111, Hsd110, Lys61, Ile147, Phe133, Asp131, Thr104, Gly148, Gly106, Gu149, Gly85, Arg160, Arg163, Val132, Gly58, Asp79
3	549	-9.4,-8.6,29.3	17,14,15	A, B	A: Phe133, Arg163, Pro136, Leu135, Tyr134, Asp131, Lys130 and B: Met203, Arg68, Val205, Asn222, Gly223, Ile72, Met70, Gln176, Asn69, Ile220, Thr224
4	358	-2.3,11.8,20.8	16,18,16	A, B	A: Gly202, Asn174, Hsd200, Ser221, Arg195, Phe177, Leu196, Asn175, Lys199, Glu169, Leu172, Lys173 and B: Arg195, Arg198, Lys199
5	229	-21.9,-33.0,18.9	14,12,11	B	Ala54, Ala41, Asp37, Lys253, Val255, Asp256, Hsd53, Hsd52, Asp254, Glu40

3.2 Molecular Docking

The compatibility of UA with the DENV-3 methyltransferase was investigated using the current in-silico method. Because it had improved docking metrics for the bound dengue protein, such as docking score, hydrogen, and hydrophobic bonding, this studied ligand eventually became a potential medication. The docking scores showed that it contains potential therapeutic benefits to fight this infection. The affinities of this protein to the tested chemical, including hydrogen bonds, hydrophobic interactions, ionic, and π-cation, served as supplementary evidence.

The DENV-3 methyltransferase protein and UA have the best docking score of -9.3 kcal/mol (Table 2). Asp131, Glu149, Gly148, Asp146, His110, Thr104, Tyr103, Cys82, and Gly83 were discovered to form hydrogen bond connections with UA, whereas Phe133, Arg160, Ile147, Val132, Lys105, and Tyr103 were shown to establish

hydrophobic interactions. Additionally, UA displayed ionic connections with the residues Arg160 and Lys180 as well as π-cation contacts with Lys105, His110, and Arg163 residues (Figure 3A).

Table 2. Molecular docking results analysis of (A) UA and (B) Reference compound (Quercetin) with DENV-3 methyltransferase (PDB ID: 4R8S)

Compound Name	Vina Score	Number of H-B	Bound amino acids
UA	-9.3	9	Asp131, Glu149, Gly148, Asp146, His110, Thr104, Tyr103, Cys82, Gly83 (H-B), Phe133, Arg160, Ile147, Val132, Lys105, Tyr103 (C-H), Arg160, Lys180 (ionic), Lys105, His110, Arg163 (pi-cation)
Quercetin	-8.8	10	Asp131, Lys130, His110, Gly148, Asp146, Glu111, Thr104, Gly83, Cys82, Tyr103 (H-B), Lys105, Val132, Tyr103, Ile147 (C-H), Arg84, Lys180 (ionic), Lys105 (pi-cation)

The DENV-3 methyltransferase protein and the reference substance (quercetin) have a docking score of -8.8 kcal/mol (Table 2). Lys105, Val132, Tyr103, and Ile147 were discovered to interact hydrophobically with quercetin, whereas Asp131, Lys130, His110, Gly148, Asp146, Glu111, Thr104, Gly83, Cys82, and Tyr103 were shown to form hydrogen bond connections with quercetin. As seen in Figure 3B, quercetin also demonstrated ionic interactions with the residues Arg84 and Lys180 as well as a π-cation with Lys105.

In this study, we used CB-Dock software to run the blind docking of the reference compound (Quercetin) and the selected compound (UA) with the target protein. UA or its derivatives are novel compounds to the DENV. In our previous work, we studied for the first time the anti-DENV activity of UA through the in-silico approaches [25]. In this study, we used blind docking to predict the UA binding site to the MTase protein to discover UA as a competitive or non-competitive compound as anti-DENV. Both compounds have been found to have interaction in the binding pocket 2 (Table 1; Figure 2) which indicated that these compounds bound in the active site of the target protein. The docking data reveal that the UA has good docking values and remarkable hydrogen bonding interactions. UA will be a therapeutic chemical to build anti-viral drugs against DENV based on the interaction in the active site of the target protein, bound amino acids, and binding energy as well as compared to the reference compound (Quercetin).

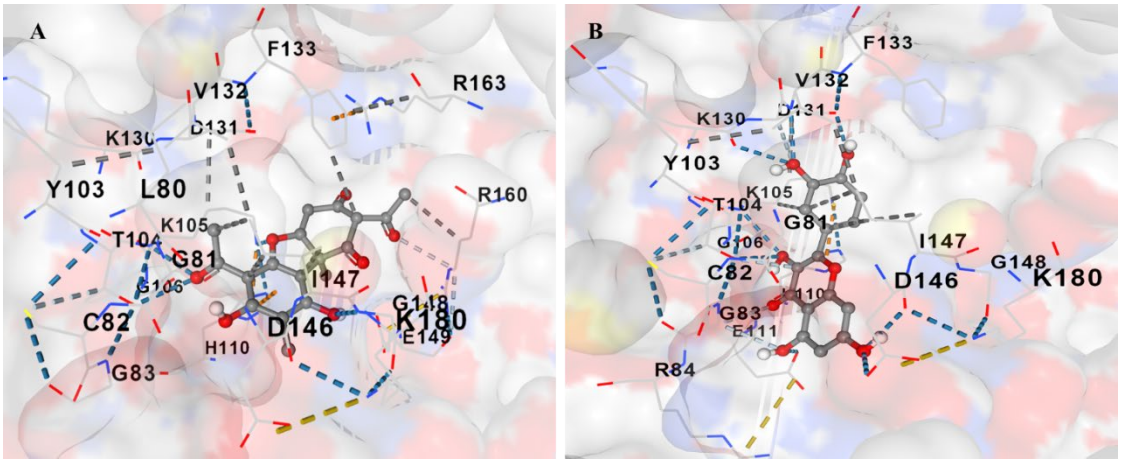

Figure 3. Molecular docking results of (A) UA and (B) Reference compound (Quercetin) with DENV-3 methyltransferase (PDB ID: 4R8S)

3.3 Pharmacokinetics Analysis

Based on their physicochemical characteristics, which are displayed in Table 3, the drug-likeness of UA and the reference molecule were investigated. The MiLogP value was used to assess the lipophilicity of the drug candidate. GB/SA (Generalized-Born and Solvent Accessible) surface area model is used by MiLopP to determine the free solvation energies in n-octanol and water [26]. For both compounds, the MiLopP value was acceptable. According to Palm et al., the topological polar surface area (TPSA) is made up of all polar atoms, namely oxygen and nitrogen as well as associated hydrogen [27]. With TPSA values of 117.97 $Å^2$ and 131.35 $Å^2$, respectively, which are not greater than 140 $Å^2$, the UA and Quercetin compounds were well within the permissible range [25]. A substance having a low molecular weight is simple to absorb in the human gut. With a few rare exceptions, as molecular weight rises, so does the bulkiness of the molecules [28]. In the permissible range (MWT ≤500), the molecular weights of UA and quercetin were 344.32 and 302.24, respectively. Additionally, the number of HB-A and HB-D was within an acceptable range and did not violate Lipinski's Rule of Five (Ro5) [29].

Table 3. Drug-likeness properties of UA and Reference compound (Quercetin)

Compound Name	MiLogP	TPSA	MW	nON	nOHNH	No. of Violation	No. of Rotat	Volume
Reference Value	---	140 Å^2	≤500	≤10	≤5	0	≤10	---
UA	1.01	117.97	344.32	7	2	0	2	290.31
Quercetin	1.68	131.35	302.24	7	5	0	1	240.08

Table 4 clearly displayed the pharmacological characteristics of UA and the reference substance. High gastrointestinal absorption and a strong blood-brain barrier were demonstrated by the reference substance and the UA. In order to build complex molecules, various bodily organs used the absorbed drugs that were provided by blood vessels [30]. Different xenobiotics must be biotransformed in the human body using CYP450 enzymes. More than fifty isoforms of this family of enzymes exist, although CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 are usually regarded as crucial CYP450 enzymes since they metabolise 90% of medications [31]. The predictions of pkCSM program showed that the UA could not interact with CYP450 as a substrate or an inhibitor, whereas the reference substance may interact with CYP1A2 inhibitor. According to predictions, neither substance is a probable substrate of renal organic cation transporter 2 (Renal OCT2), which means Renal OCT2 is unlikely to be involved in their renal clearance and disposal [32]. The human ether-á-go-go related gene (hERG) creates a potassium ion receptor that takes part in electrical heart activity by repolarizing the cardiac action potential [33]. Drugs that block this channel may cause an arrhythmia, which can result in potentially fatal symptoms [34]. None of the substances examined by the hERG inhibitor predictor showed the ability to block this channel, which suggests their potential as a therapeutic alternative. The chosen molecule had a hepatotoxic profile, which suggested that it may be able to harm the liver [20].

Table 4. Physicochemical properties of UA and Reference compound (Quercetin)

	Absorption		Distri-bution	Metabolism						Ex-cretion	Toxicity		
C/Name	AS	HIA	BBB	CYP2D	CYP 3A4	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4	Renal OCT2	hERG I	hERG II	HT
Reference Value	---	>70	---	No	No	No	No	No	No	No	No	No	No	No
UA	-2.80	84.18	-0.53	No	No	No	No	No	No	No	No	No	No	Yes
Quercetin	-2.92	77.20	-1.09	No	No	Yes	No	No	No	No	No	No	No	No

3.4 E-Pharmacophore Analysis

By preserving the activity criterion in the range of 6.5 to 7.9, the data set was split into regions that were actively, moderately, and inactively occupied. Due to the UA binding domain's strong survival value, the generic pharmacophore hypotheses were added among its four properties, as illustrated in Figure 4. The e-pharmacophore also reveals that the UA is composed of seven acquired acceptors (A1 to A7), two obtained donors (D8 and D9), two obtained hydrophobics (H10 and H11), and one obtained aromatic ring (R12).

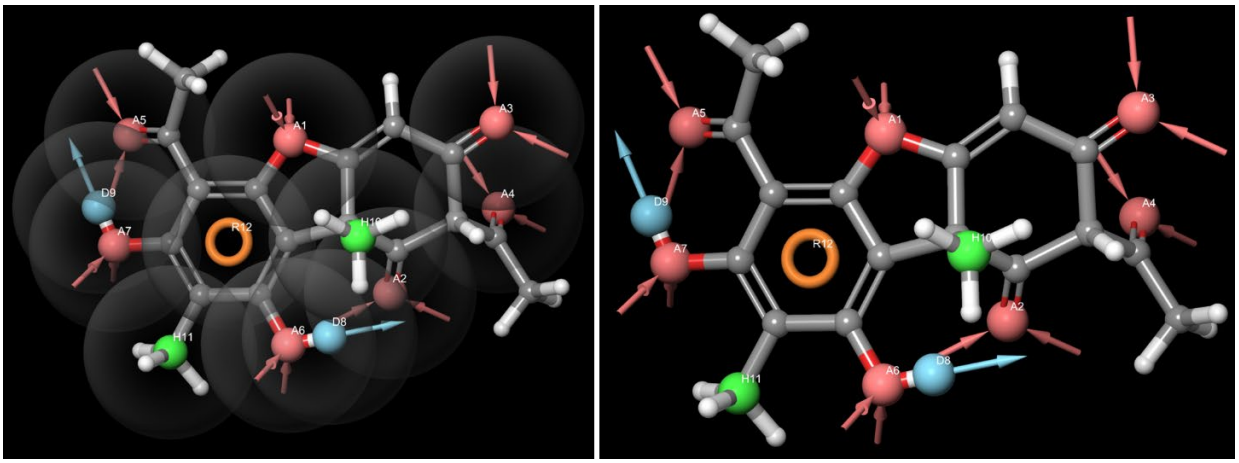

Figure 4. Pharmacophore hypothesis of Usnic acid. A denotes hydrogen bond acceptor in pink color, D denotes hydrogen bond donor in blue, H denotes hydrophobic in green color and R denotes aromatic rings in brown color from docked phytochemicals

4.0 CONCLUSION

There is currently no FDA-approved antiviral medication to treat dengue virus. On the other hand, individuals all over the world frequently employ plant extracts to lessen the problems of viral infections in the human body. As a result, the current in-silico investigation using nicotinic acid was chosen to examine the anti-DENV potential. The docked usnic acid showed exceptional docking values and bound amino acids compared to the reference chemical, we discovered in the end. This docking finding led to an additional investigation into the pharmacological potential of usnic acid utilizing pharmacokinetics and pharmacophore, where usnic acid demonstrated promising medicinal potential.

5.0 ACKNOWLEDGEMENTS

The authors would like to thank to Universiti Malaysia Pahang for providing financial support under the grant RDU1803148 and for providing lab facilities.

6.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

7.0 AUTHORS CONTRIBUTION

M. Roney (Searched the data; performed the experiments (docking and pharmacokinetics); drafted the manuscript)

A. Dubey (Experimented (E-Pharmacophore); reviewed the manuscript)

A. M. Huq (Manuscript-review and editing)

M. F. F. Mohd Aluwi (Conceptualized; supervised the work)

8.0 REFERENCES

[1] A. Madushanka, N. Verma, M. Freindorf, E. Kraka, "Papaya leaf extracts as potential dengue treatment: an in-silico study," *International Journal of Molecular Sciences*, vol. 23, no. 20, pp. 12310, 2022.

[2] V. Jarerattanachat, C. Boonarkart, S. Hannongbua, P. Auewarakul, R. Ardkhean, "In silico and in vitro studies of potential inhibitors against Dengue viral protein NS5 Methyl Transferase from Ginseng and Notoginseng," *Journal of Traditional and Complementary Medicine*, vol. 13, no. 1, pp. 1-10, 2023.

[3] S. N. Adawara, G.A. Shallangwa, P.A. Mamza, A. Ibrahim, "In-silico approaches towards the profiling of some anti-dengue virus as potent inhibitors against dengue NS-5 receptor," *Scientific African*, vol. 13, pp. e00907, 2021.

[4] C. M. Alam, A. Iqbal, B. Thadari, S. Ali, "Imex based analysis of repeat sequences in flavivirus genomes, including dengue virus," *Journal of Data Mining Genomics Proteomics*, vol. 7, no. 187, pp. 2153-0602, 2016.

[5] M.T. Qamar, U.A. Ashfaq, K. Tusleem, A. Mumtaz, Q. Ul Ain, A. Goheer et al., "In-silico identification and evaluation of plant flavonoids as dengue NS2B/NS3 protease inhibitors using molecular docking and simulation approach," *Pakistan Journal of Pharmaceutical Sciences,* vol. 30 no. 6, pp. 2119-2137, 2017.

[6] G. Lu, P. Gong, "Crystal structure of the full-length Japanese encephalitis virus NS5 reveals a conserved methyltransferase-polymerase interface," *PLoS pathogens*, vol. 9, no. 8, pp. e1003549, 2013.

[7] P. O. Fernandes, M.A. Chagas, W.R. Rocha, A.H. Moraes, "Non-structural protein 5 (NS5) as a target for antiviral development against established and emergent flaviviruses," *Current Opinion in Virology*, vol. 50, pp. 30-39, 2021.

[8] D. N. Sokolov, V.V. Zarubaev, A.A. Shtro, M.P. Polovinka, O.A. Luzina, N.I. Komarova, et al., "Anti-viral activity of (−)-and (+)-usnic acids and their derivatives against influenza virus A (H1N1) 2009," *Bioorganic & Medicinal Chemistry Letters*, vol. 22, no. 23, pp. 7060-7064, 2012.

[9] A. A. Shtro, V.V. Zarubaev, O.A. Luzina, D.N. Sokolov, O.I. Kiselev, N.F. Salakhutdinov, "Novel derivatives of usnic acid effectively inhibiting reproduction of influenza A virus," *Bioorganic & Medicinal Chemistry*, vol. 22, no. 24, pp. 6826-6836, 2014.

[10] C. S. Vijayakumar, S. Viswanathan, M.K. Reddy, S. Parvathavarthini, A.B. Kundu, E. Sukumar, "Anti-inflammatory activity of (+)-usnic acid," *Fitoterapia*, vol. 71, no. 5, pp. 564-566, 2000.

[11] A. Zugic, V. Tadic, S. Savic, "Nano-and microcarriers as drug delivery systems for usnic acid: review of literature," *Pharmaceutics*, vol. 12, no. 2, pp. 156, 2020.

[12] M. Cardarelli, G. Serino, L. Campanella, F.D. Nardone, O. Alesiani, et al., "Antimitotic effects of usnic acid on different biological systems," *Cellular and Molecular Life Sciences*, vol. 53, pp. 667-672, 1997.

[13] H. Cetin, O. Tufan-Cetin, A.O. Turk, et al., "Insecticidal activity of major lichen compounds, (−)-and (+)-usnic acid, against the larvae of house mosquito, Culex pipiens L," *Parasitology Research*, vol. 102, pp. 1277-1279, 2008.

[14] M. Takai, Y. Uehara, J.A. Beisler, "Usnic acid derivatives as potential antineoplastic agents," *Journal of medicinal chemistry*, vol. 22, no. 11, pp. 1380-1384, 1979.

[15] M. Maciąg-Dorszyńska, G. Węgrzyn, B. Guzow-Krzemińska, "Antibacterial activity of lichen secondary metabolite usnic acid is primarily caused by inhibition of RNA and DNA synthesis," *FEMS Microbiology Letters*, vol. 353, no. 1, pp. 57-62, 2014.

[16] X. Yu, Q. Guo, G. Su, A. Yang, Z. Hu, C. Qu, et al., "Usnic acid derivatives with cytotoxic and antifungal activities from the lichen Usnea longissima," *Journal of Natural Products*, vol. 79, no. 5, pp. 1373-1380, 2016.

[17] R. R. Bomfim, A.A. Araujo, S.C.-Orellana, M.G.D. Melo, L. J. Quintans-Júnior, S. Cavalcanti, "Larvicidal activity of Cladonia substellata extract and usnic acid against Aedes aegypti and Artemia salina," *Latin American Journal of Pharmacy*, vol. 28, no. 4, pp. 580-584, 2009.

[18] C. G. Noble, S.H. Li, H. Dong, S.H. Chew, P.Y. Shi, "Crystal structure of dengue virus methyltransferase without S-adenosyl-L-methionine," *Antiviral Research*, vol. 111, pp. 78-81, 2014.

[19] J. H. Gan, J. X. Liu, Y. Liu, S.W. Chen, W.T. Dai, Z.X. Xiao et al., "DrugRep: an automatic virtual screening server for drug repurposing," *Acta Pharmacologica Sinica*, vol. 44, no. 4, pp. 888-896, 2023.

[20] M. Roney, G. Singh, A.M. Huq, M.S. Forid, W.M.W. Ishak, K. Rullah et al., "Identification of Pyrazole derivatives of usnic acid as novel inhibitor of SARS-CoV-2 main protease through virtual screening approaches," *Molecular Biotechnology*, vol. 66, no. 4, pp. 1-11, 2023.

[21] D. Kashyap, S. Jakhmola, D. Tiwari, R. Kumar, N.S.H.N. Moorthy, M. Elangovan et al., "Plant derived active compounds as potential anti SARS-CoV-2 agents: an in-silico study," *Journal of Biomolecular Structure and Dynamics*, vol. 40, no. 21, pp. 10629-10650, 2022.

[22] M. Abdul-Hammed, I.O. Adedotun, V.A. Falade, A.J. Adepoju, S.B. Olasupo, M.W. Akinboade, "Target-based drug discovery, ADMET profiling and bioactivity studies of antibiotics as potential inhibitors of SARS-CoV-2 main protease (Mpro)," *VirusDisease*, vol. 32, no. 4, pp. 642-656, 2021.

[23] A.C. Rani, K. Kalaimathi, S. Jayasree, S. Prabhu, S. Vijayakumar, R. Ramasubbu et al., "Exploring the drug potential of phytochemicals as a novel therapeutic drug candidate for herpesvirus: An in-silico evaluation," *Chemistry Africa*, vol. 6, pp. 1-14, 2022.

[24] C.R.A. Samy, K. Karunanithi, J. Sheshadhri, M. Rengarajan, P. Srinivasan, P. Cherian, "(R)-(+)-Rosmarinic acid as an inhibitor of herpes and dengue virus replication: An in-silico assessment," *Revista Brasileira de Farmacognosia*, pp. 1-8, 2023.

[25] M. Roney, A.M. Huq, K. Rullah, H.A. Hamid, S. Imran, M.A. Islam, et al., "Virtual screening-based identification of potent DENV-3 RdRp protease inhibitors via in-house usnic acid derivative database," *Journal of Computational Biophysics and Chemistry*, vol. 20, no. 08, pp. 797-814, 2021.

[26] A. Nag, A. Dasgupta, S. Sengupta, T.K. Lai, K. Acharya, "An in-silico pharmacophore-based molecular docking study to evaluate the inhibitory potentials of novel fungal triterpenoid Astrakurkurone analogues against a hypothetical mutated main protease of SARS-CoV-2 virus," *Computers in Biology and Medicine*, vol. 152, pp. 106433, 2023.

[27] K. Palm, P. Stenberg, K. Luthman, P. Artursson, "Polar molecular surface properties predict the intestinal absorption of drugs in humans," *Pharmaceutical Research*, vol. 14, pp. 568-571, 1997.

[28] S.S. Mishra, C.S. Sharma, H.P. Singh, H. Pandiya, N. Kumar, "In silico ADME, bioactivity and toxicity parameters calculation of some selected anti-tubercular drugs," *International Journal of Pharmaceutical and Phytopharmacological Research*, vol. 6, no. 6, pp. 77-79, 2016.

[29] C.A. Lipinski, "Lead-and drug-like compounds: the rule-of-five revolution," *Drug discovery today: Technologies*, vol. 1, no. 4, pp. 337-341, 2004.

[30] A.L.M. de Sousa, R.R. Pinheiro, J.F. Araujo, R.M. Peixoto, D.A.A. de Azevedo, A.M.C. Lima et al., "In vitro antiviral effect of ethanolic extracts from Azadirachta indica and Melia azedarach against goat lentivirus in colostrum and milk," *Scientific Reports*, vol. 13, no. 1, pp. 4677, 2023.

[31] F. Lin, X. Lin, X. Wang, G. Mei, B. Chen, H. Yao et al., "Inhibitory effect of Selaginella doederleinii hieron on human cytochrome P450," *Frontiers in Pharmacology*, vol. 14, 2023.

[32] J.X. Shen, W.W. Du, Y.L. Xia, Z.B. Zhang, Z.F. Yu, Y.X. Fu et al., "Identification of and mechanistic insights into sars-cov-2 main protease non-covalent inhibitors: An In-Silico study," *International Journal of Molecular Sciences*, vol. 24, no. 4, pp. 4237, 2023.

[33] K. Mika, M. Szafarz, M. Bednarski, A. Siwek, K. Szczepańska, K. Kieć-Kononowicz et al., "Evaluation of some safety parameters of dual histamine h3 and sigma-2 receptor ligands with anti-obesity potential," *International Journal of Molecular Sciences*, vol. 24, no. 8, pp. 7499, 2023.

[34] H. Feng, and G.W. Wei, "Virtual screening of DrugBank database for hERG blockers using topological Laplacian-assisted AI models: *Computers in Biology and Medicine*, vol. 153, pp. 106491, 2023.