STM JOURNALS

International Journal of
Genetic Modifications and Recombinations

Volume 1, Issue 1, 2023
DOI (Journal): 10.37591/IJGMR

https://journals.stmjournals.com/ijgmr/

Research IJGMR

Molecular Docking of *Cocculus hirsutus* Phytocompounds with the Selective DENV Virus Stereotypes

Prabhuram K[1,*], Shubham Wanarase[2]

Abstract

Dengue is a tropical disease with a high infection rate worldwide. Though treatments are available for the symptoms, anti-dengue drugs are unavailable. To tackle this issue, the study aims to shortlist the potential phytocompounds from Cocculus hirsutus medicinal plant, prevalent in South and South East Asia. Using molecular docking and ADME analysis, two potential compounds possessing high drug-likeness are identified namely, Sinococuline and Morphinan.

Keywords: DENV, NS1, anti-dengue, molecular docking.

INTRODUCTION

Arthropod-borne human diseases are prevalent worldwide in tropical, sub-tropical, and equatorial areas. Dengue is one of the rapidly spreading seasonal diseases in tropical areas due to the female mosquito, *Aedes aegypti*, which acts as the transfer agent to spread Dengue. Dengue is caused by the dengue virus, popularly known as DENV virus which belongs to the *Flaviviridae* family. Some notable human disease-causing viruses belonging to this family are the Zika virus, Yellow fever virus, and Japanese encephalitis virus [1].

Four DENV virus stereotypes are recognized–DENV 1–4, with a positive sense viral genome. The viral genome is structured with 3 structural proteins–Capsid (C protein), Envelop (E protein), and pre-Membrane/Membrane (prM/M protein) and 7 Non-Structural proteins (NS)–NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The interesting aspect of the viral genome replication within the host is that the structural proteins prM and E protein, and non-structural proteins NS1 and NS4B are synthesized in the endoplasmic reticulum (ER) of the host cell [2].

Each non-structural proteins perform a certain function that greatly increases the viral replication rate. NS2A assists in virion assembly and more importantly, it deals in inhibiting the host immune system response. NS5 is a highly conserved region in all the DENV stereotypes and serves an important function for the DENV virus i.e to suppress the interferon response and also evade the Toll-like receptors from the host immune system [3]. NS3 exists as NS3helicase which involves in initiating RNA synthesis and NS3 protease (works jointly with NS2B) plays a key role in cleaving crucial sites of the DENV polyprotein [4]. Recently, the role of NS3 in triggering fatty liver conditions in the host due to dengue was uncovered ([5]. NS4A and NS4B contribute to viral genome replication subtly by inducing autophagy in the infected host cell and assisting in interaction with the NS3 helicase which is essential for viral replication [2].

***Author for Correspondence**
Prabhuram K
E-mail: prabhuramkpr@gmail.com

[1,2]Student, Department of Bioinformatics, Bionome, Bengaluru, Karnataka, India

Received Date: April 13, 2023
Accepted Date: May 06, 2023
Published Date: May 20, 2023

Citation: Prabhuram K, Shubham Wanarase. Molecular Docking of *Cocculus hirsutus* Phytocompounds with the Selective DENV Virus Stereotypes. International Journal of Genetic Modifications and Recombinations. 2023;1(1): 17–29p.

Among the DENV non-structural proteins, NS1 stands out as a unique one because it is the only protein to be continuously secreted from the infected host cells to the external environment [1]. Since the NS1 proteins are also among the priority components synthesized in the host's ER, it is an attractive target for drug development. It is crucial to note that there is no standard drug or vaccine for dengue, the current treatments only tackle the symptoms. However, the approaches to developing antibodies and/or vaccines against DENV using NS1 are being tried and tested but were not fruitful in terms of efficiency [6, 1].

It is reported that the NS1 proteins are secreted in the vector cells (mosquito) and also during the infection of human cells in a significant quantity, however, the mechanism of secretion differs in mosquito cells and human cells [7]. They validated the claim using the Fli-06, a novel ER protein exit sites inhibitor that failed to prevent the NS1 secretions from the mosquito cells [8]. This might be a reason the medicine targeting early secretions of NS1 fails to act interfering with NS1 secretions. These findings also suggest the lack of complete understanding or information availability about different DENV stereotypes to proceed in developing targeted vaccines at higher efficiency. Another aspect of complications in developing drugs against the DENV virus is the mutation noted in the patients [9].

Cocculus hirsutus (C. hirsutus) is widely known for its medicinal properties–anti-microbial properties, anti-viral properties, anti-cancer properties, insecticidal properties, anti-diabetic, and anti-oxidant properties, and was in use as an Ayurveda medicine [10]. In modern medicine, the phytocompounds from *C. hirsutus* are being tested in a wide variety of applications, particularly for the medicinal features, ABB demonstrated the anti-bacterial, anti-oxidant, and anti-diabetic potential of the plant can be extrapolated to be used at nanoscales. Results of different cell line studies increase the reliability of the potential of *C. hirsutus*, the extracts of *C. hirsutus* suppressed LPS, PAM3CSk-induced secretions on RAW264.7 cell lines and reduced stomach lesions inflammation (gastric injuries) (phytomedicine). Mouse model studies also showed the significant potential of *C. hirsutus* extracts as pharmaceutical drugs for different ailments including dengue [11, 12]. This study aims to shortlist the potential ligands for drug development against dengue from the list of phytocompounds of *C. hirsutus* using molecular docking and ADME analysis techniques.

MATERIALS AND METHODS

Protein Preparation

Crystal structure of two proteins NS1 of DENV type 1 (PDB ID: 4IOG) and NS1 of DENV type 2 (PDB ID: 4O6B) were retrieved from the Protein Database (PDB https://www.rcsb.org/) in the PDB file format. The retrieved raw protein structures in the PDB file format were purified using the Biovia Discovery Studio software. Purification of the proteins were executed by removing the water molecules, heteroatoms and the secondary chain structures. Finally, polar hydrogens are added to the protein molecules and the purified proteins are saved in PDB format for docking.

Ligand Selection

The three Dimensional (3D) structure of the phytocompounds of *C. hirsutus* was retrieved from the NCBI PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in SDF (Structure Data File) format. From the 22 available compounds, a total of 11 compounds were retrieved based on the molecular weight and 3D structure availability.

Molecular Docking

The molecular docking technique provides a simulation environment to comprehend the interaction of a ligand and a protein at the molecular level, thus enhancing the preciseness of drugs. Using the software BIOVIA Discovery Studio and PyRx the targeted DENV NS proteins are docked with the selected phytocompounds (ligands) of *C. hirsutus* to evaluate the binding affinity of the ligands. The protein structures were purified in the BIOVIA Discovery Studio and the files were submitted to PyRx software for ligand-protein docking. The resulting output was sorted according to the root mean square deviation (rmsd) and the binding affinity efficiency. The compound with the greatest binding affinity

for each protein was subjected to model simulation in the PyRx software and was further analyzed for the ligand–protein interactions in the BIOVIA Discovery Studio.

RESULTS

Molecular docking

The results showed that all 11 compounds from the plant showed binding affinity listed in Table 1 and Table 2. Among the compounds, 9H-Xanthene (PubChem Compound ID: 7107) showed higher affinity against type 1 DENV 4IOG and Sinococuline (PubChem Compound ID: 5489400) showed a significant affinity against type 2 DENV 4O6B. The compounds with the highest binding affinity are listed in Table 3 and Table 4.

Table 1. List of compounds binding affinity with DENV 1 (PDB ID: 4IOG)

S.N.	PubChem Compound ID	Compound name	Binding Affinity
1.	7107	9H-Xanthene	-6.4
2.	11077	Resazurin	-6.4
3.	3440	Furosemide	-6.1
4.	5489400	Sinococuline	-6
5.	6857497	Morphinan	-6
6.	588	Creatinine	-5
7.	241	Benzene	-4.1
8.	8058	Hexane	-3.9
9.	8857	Ethyl acetate	-3.9
10.	180	Acetone	-3
11.	6212	Chloroform	-2.9

Table 2. List of compounds binding affinity with DENV 2 (PDB ID: 4O6B)

S.N.	PubChem Compound ID	Compound name	Binding Affinity
1.	5489400	Sinococuline	-6.9
2.	6857497	Morphinan	-6.7
3.	11077	Resazurin	-6.6
4.	3440	Furosemide	-6.1
5.	7107	9H-Xanthene	-5.8
6.	588	Creatinine	-4.3
7.	241	Benzene	-4.1
8.	8857	Ethyl acetate	-3.5
9.	180	Acetone	-3.3
10.	6212	Chloroform	-3.1
11.	8058	Hexane	-3

Table 3. List of selected compounds with highest binding affinity for DENV 1 (PDB ID: 4OIG)

S.N.	PubChem Compound ID	Compound name	Binding Affinity
1.	7107	9H-Xanthene	-6.4
2.	11077	Resazurin	-6.4
3.	3440	Furosemide	-6.1

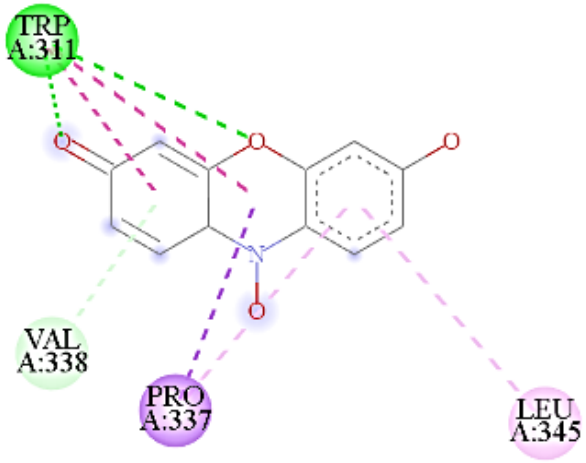
Table 4. List of selected compounds with highest binding affinity for DENV 2 (PDB ID: 4O6B)

S.No	PubChem Compound ID	Compound name	Binding Affinity
1.	5489400	Sinococuline	-6.9
2.	6857497	Morphinan	-6.7
3.	11077	Resazurin	-6.6

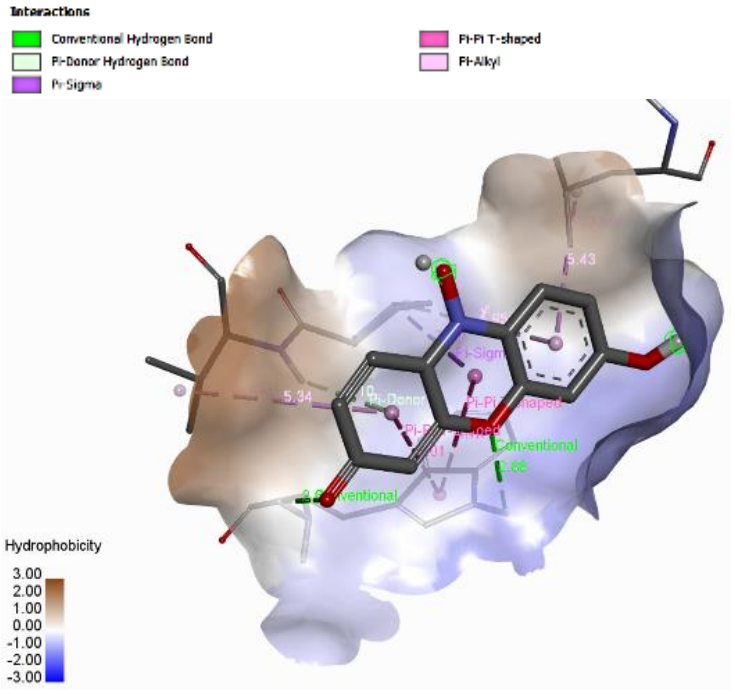
Molecular Visualization

The binding analysis between the selected phytocompounds and both the proteins resulted in two final ligands, the ligands proceeded with model simulation. The docking results of DENV1 NS1 protein showed two strong hydrogen bond formations with the ligand 9H-Xanthene. In addition to the hydrogen bonds, two pi-alkyl bonds, one pi-donor hydrogen bond, and one pi-sigma bonds are observed between the interaction of the protein and the ligand. Figure 1 (a) shows the 2D structure of the 9-H Xanthene, (b)-(g) displays the interaction on the receptor surfaces.

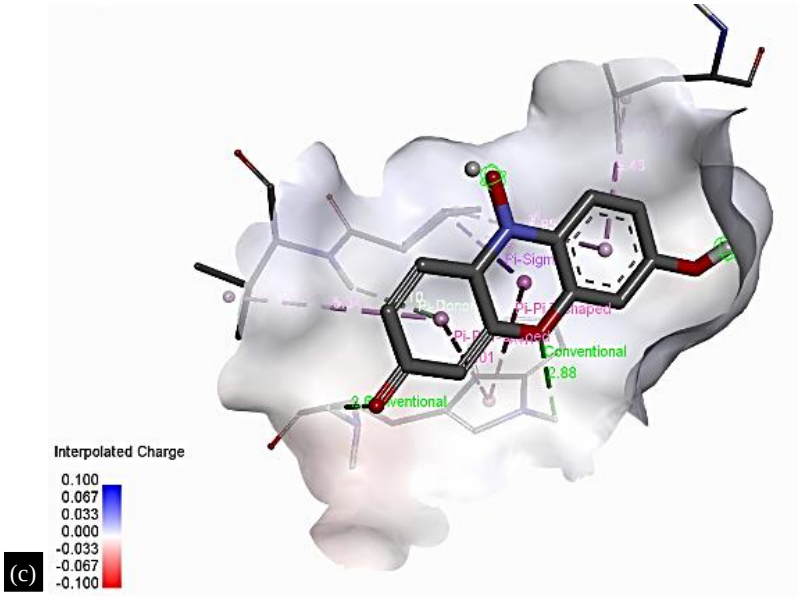
Docking of the ligand Sinococuline with the DENV2 NS1 proteins resulted with three hydrogen bonds of which two forming with a nitrogen base and an oxygen base respectively. The remaining hydrogen bonds with a carbon base, making it a little weaker than the other hydrogen bonds. Two alkyl and pi-alkyl bonds are noted as well supposedly indicating a strong hydrophobic interactions between the ligand and the protein. Figure 2 (a) shows the 2D structure of Sinococuline, (b)-(g) displays the interaction on the receptor surfaces.



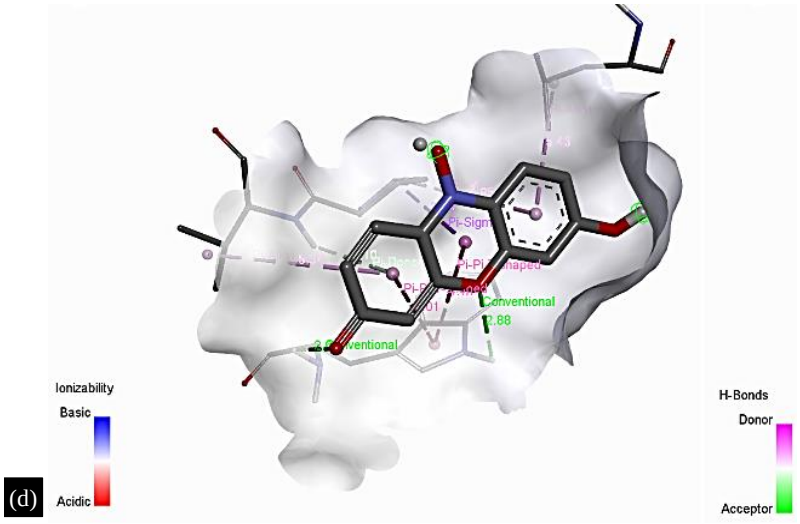
(a)



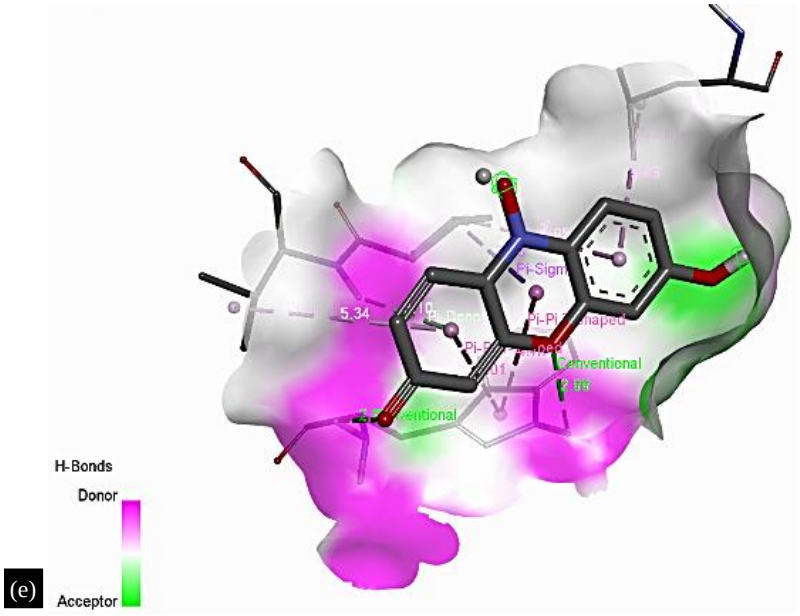
(b)



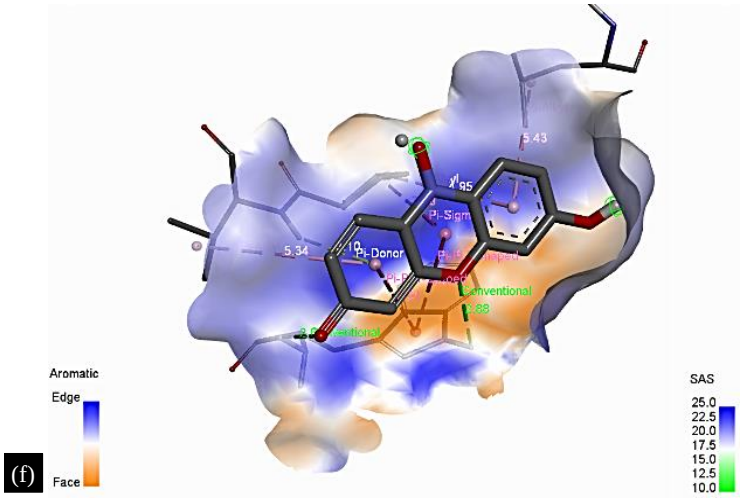
(c)



(d)



(e)



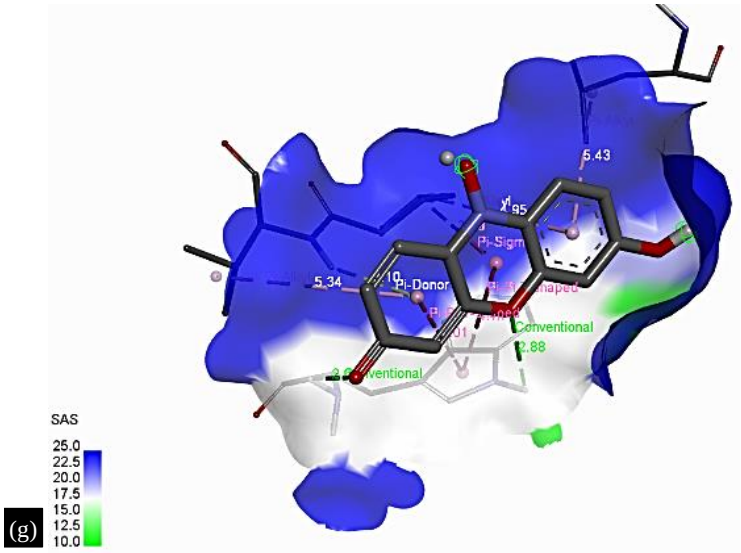
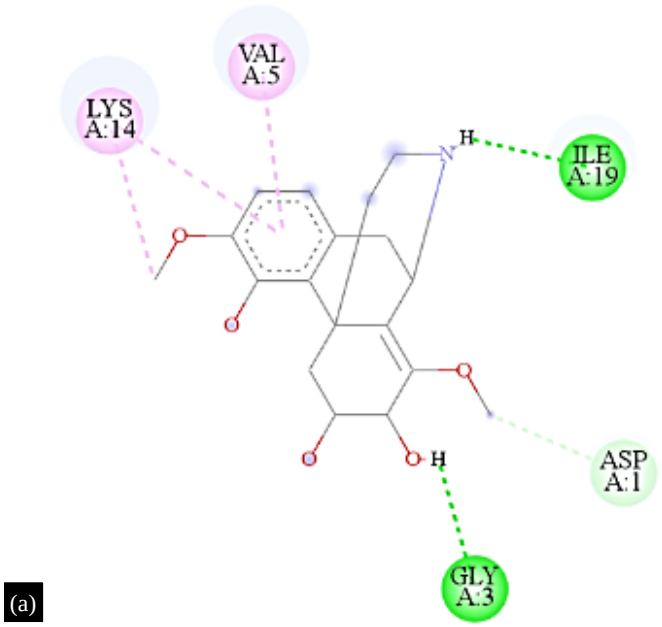
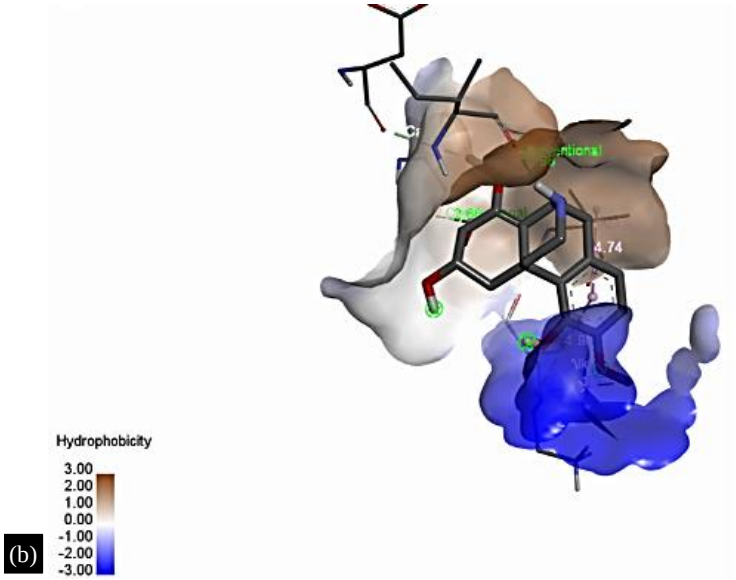
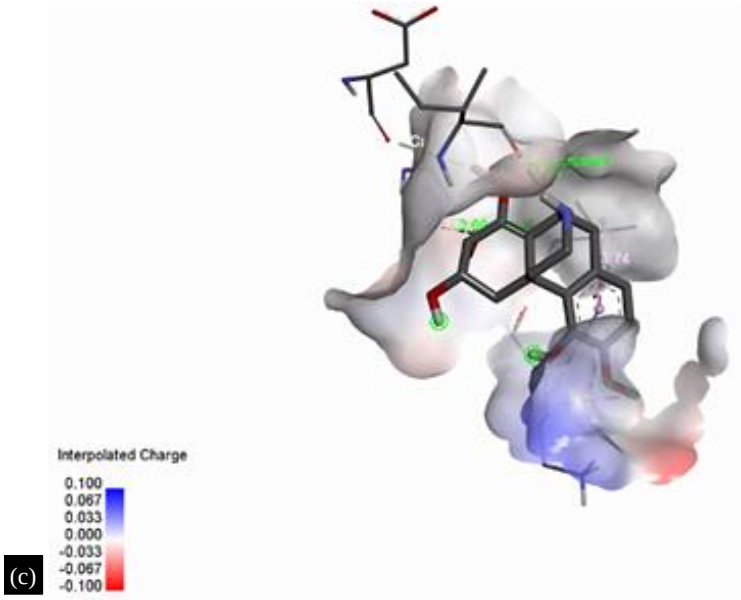


Figure 1. Molecular visualization of 9H-Xanthene (a) 2D structure (b) hydrophobicity (c) Interpolated Charge (d) Ionizability (e) H-bonds (f) Aromatic (g) SAS.

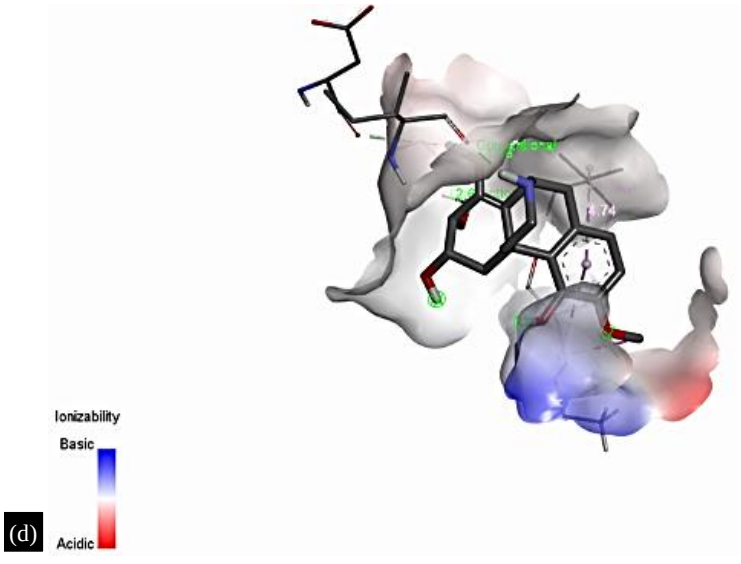




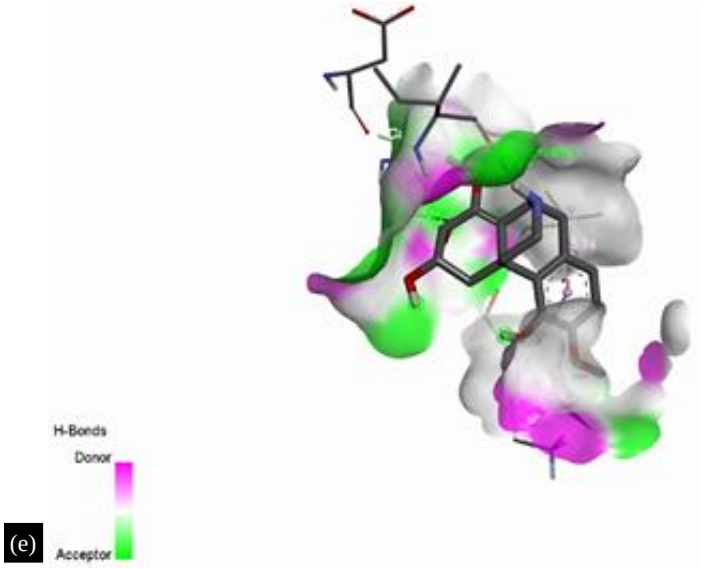
(b)



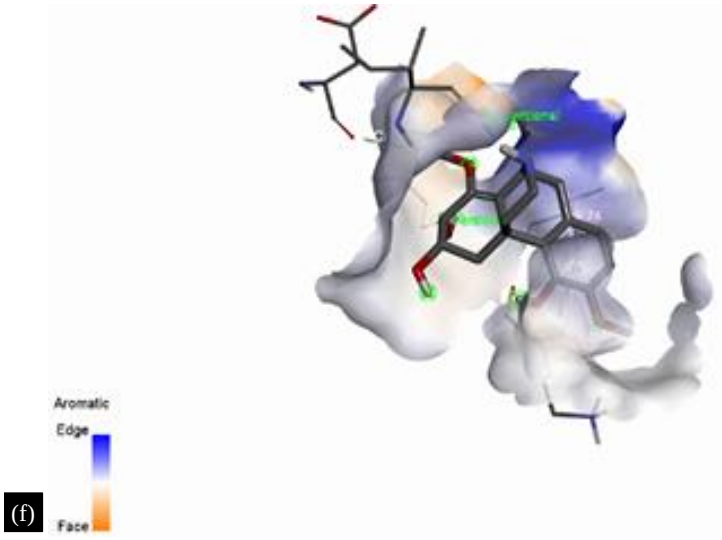
(c)



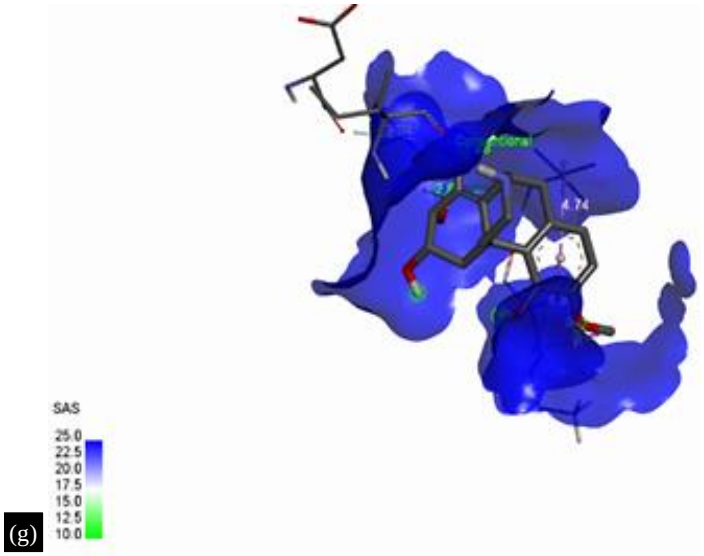
(d)



(e)



(f)



(g)

Figure 2. Molecular visualization of Sinococuline (a) 2D structure (b) hydrophobicity (c) Interpolated Charge (d) Ionizability (e) H-bonds (f) Aromatic g) SAS.

ADME Analysis

The 3 highest affinity compounds for each protein were subjected to ADME analysis and visualized as a BOILE-egg plot [13] using the SwissADME [14] application to evaluate the drug-likeness and the chemistry of the small molecules. The ADME analysis results are listed in the Table 5 and Table 6. The ADME analysis of DENV1 molecules shows there were no Lipinski violations and provides the hydrogen acceptor and donor status of each molecule. Though 9H-Xanthene showed a strong binding affinity with DENV1 NS1 the molar refractivity was observed to be low.

Table 5. ADME analysis of DENV 1 docking using ADMET application

S.N.	Molecule	Molecular Weight	H-bond donors	H-bond acceptors	Molar Refractivity	Lipinski violations
1.	9H-Xanthene	182.2	0	1	55.97	0
2.	Resazurin	229.2	1	4	62.29	0
3.	Furosemide	330.7	3	6	75.46	0

Table 6. ADME analysis of DENV 2 docking using ADMET application

S.N	Molecule	Molecular Weight	H-bond donors	H-bond acceptors	Molar Refractivity	Lipinski violations
1.	Sinococuline	333.38	4	6	91.34	0
2.	Morphinan	227.34	1	1	75.09	0
3.	Resazurin	229.19	1	4	62.29	0

The drug-likeness visualized as the BIOLED-egg plot shows that the molecules 2 and 3 are more likely to be absorbed by the gastrointestinal tract, whereas molecule 1 (9H-Xanthene) with the properties more likely to permeate the brain (Figure 3).

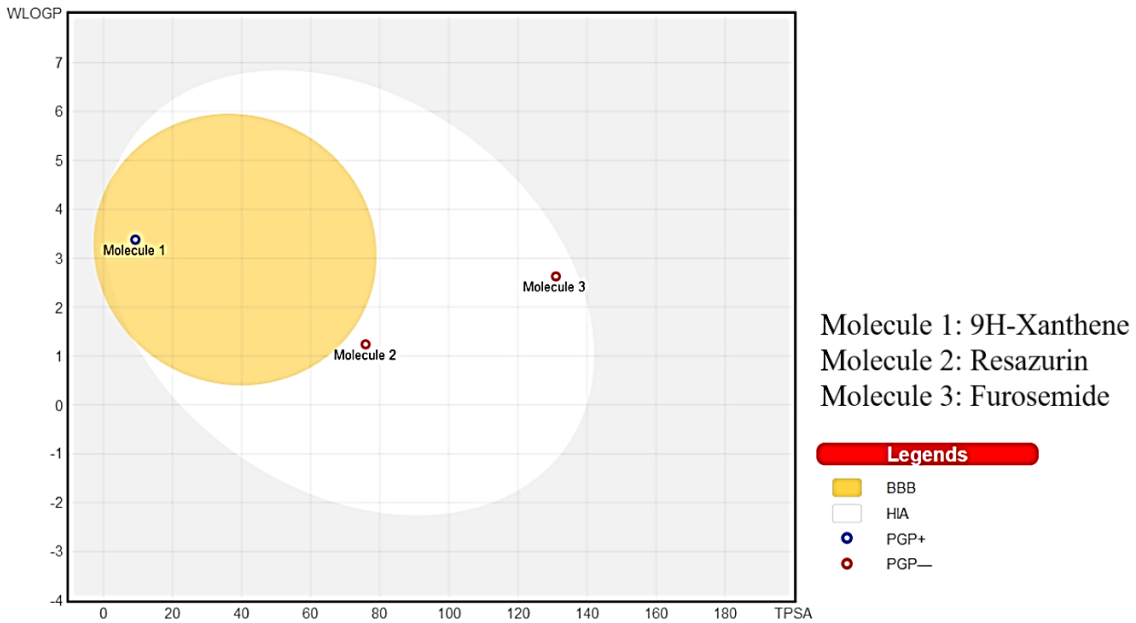


Figure 3. BOILED-Egg analysis: 9H-Xanthene, Resazurin, Furosemide.

The ADME analysis of DENV2 molecules shows there were no Lipinski violations and supports the compound Sinococuline being a potent drug with the high molar refractivity and strong hydrogen bonding. The remaining molecules were relatively stronger with higher refractivity but the BIOLED-egg plot suggests that the molecule 2 is likely to permeate the brain. The molecules 1 and 3 are more likely absorbed by the gastrointestinal tract (Figure 3 & 4). Figure 5 shows the bioavailability radar for the selected compounds.

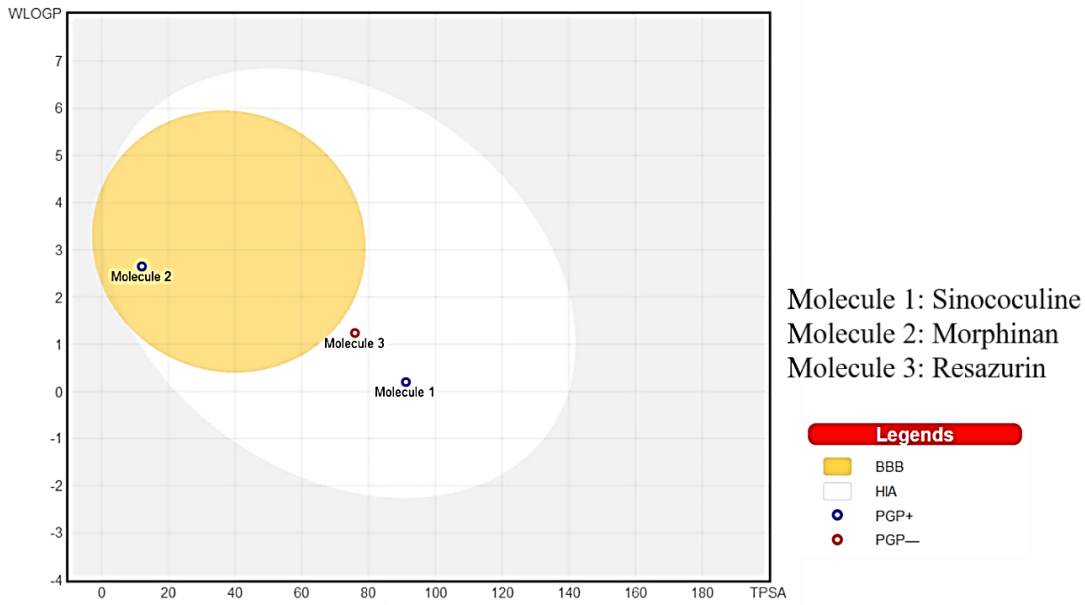


Figure 4. BOILED-Egg analysis: Sinococuline, Morphinan, Resazurin.

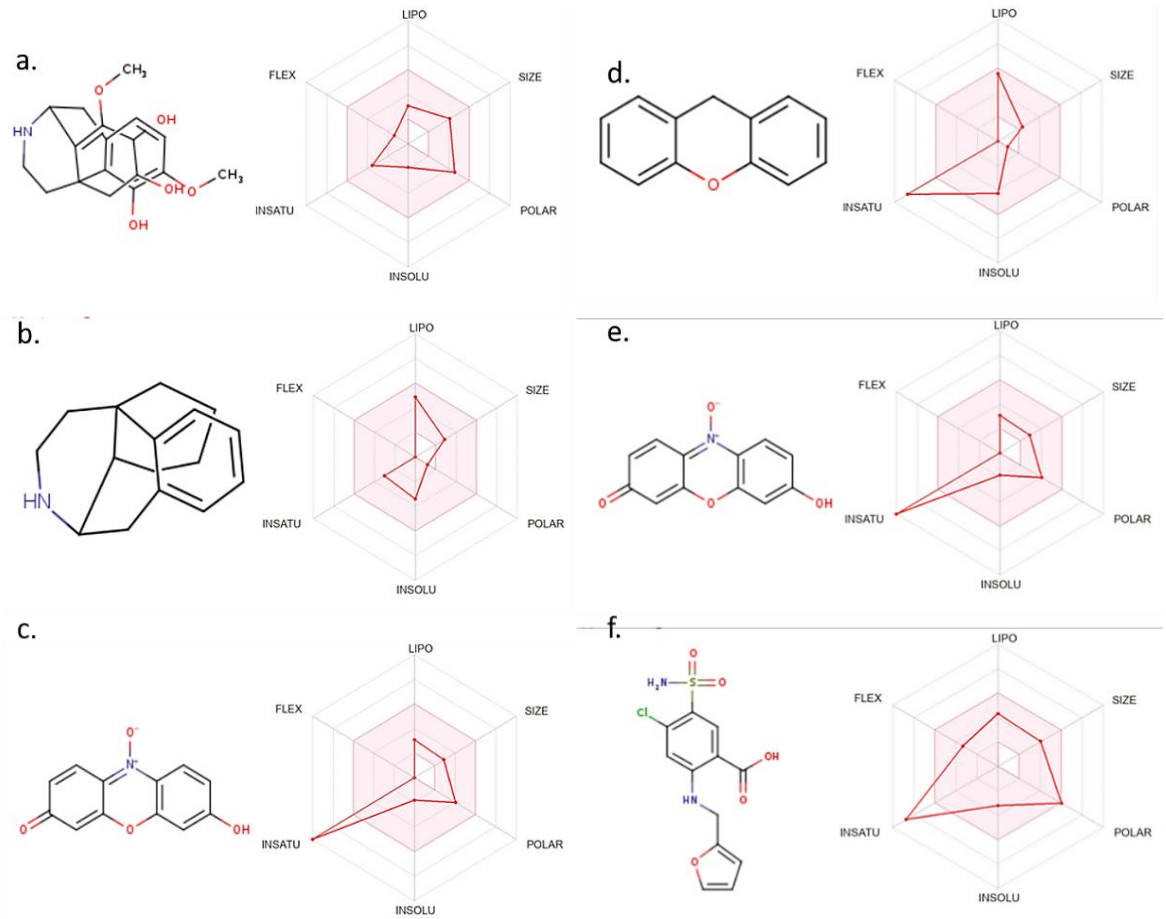


Figure 5. Compound structure and Bioavailability radar (a) Sinococuline (b) Morphinan (c) Resazurin (d) 9H-Xanthene € Resazurin (f) Furosemide.

DISCUSSION

India reported 110000 cases and 86 cases of death in the year 2022 due to dengue as per the National Center for Vector Borne Disease Control, India (link). Though the number of deaths has reduced from

325 deaths in 2017, the number of infection cases has remained significantly on the same scale at 180000 reported cases in 2017 (Figure 6). The cohort study from [15] revealed the presence of different DENV stereotypes within India and the prevalence of different patterns among the stereotypes infection in different states of India. The study identified the first-time infection report of DENV 1 in Himachal Pradesh, India and overall DENV 2 has the highest infection rate in different states. From this study, it was evident that DENV 2 and DENV 1 stereotypes are the highest prevalent viral types causing dengue infections in India. With this information and the influence of NS1 proteins in pathogenesis, the NS1 proteins of DENV 2 and DENV 1 were shortlisted for in-silico analysis with the medicinal plant.

Results of molecular docking of the ligands revealed an interesting outcome indicating that all the 11 compounds showed less root mean square deviation (rmsd). However, the fiercest binding affinity was observed with 9H-Xanthene, Resazurin, and Furosemide for the DENV Type 1 NS1 protein. In DENV Type 2 NS1 protein-ligand interaction, the compounds Sinococuline, Morphinan, and Resazurin showed strong potential with the highest binding affinity. When looking at ADME analysis, the 5 selected compounds showed no Lipinski violations (Table 5 and 6).

But the bioavailability radar accessed the drug-likeness and illustrated the prime 6 physicochemical properties where Sinococuline and Morphinan stayed within the radar zone (area highlighted in Pink) in all the parameters. All the compounds in DENV 1 protein interaction showed out-of-zone spike for solubility making it ineligible to be an effective drug (Figure 5).

DENGUE/DHF SITUATION IN INDIA

Back

Dengue Cases and Deaths in the Country since 2017

Sl. No.	Affected States/UTs	2017		2018		2019		2020		2021		2022 (Prov.till 31st Oct)	
		C	D	C	D	C	D	C	D	C	D	C	D
1	Andhra Pradesh	4925	0	4011	0	5286	0	925	0	4760	0	4754	0
2	Arunachal Pradesh	18	0	1	0	123	0	1	0	7	0	163	0
3	Assam	5024	1	166	0	196	0	33	0	103	0	191	0
4	Bihar	1854	0	2142	0	6712	0	493	2	633	2	9374	7
5	Chattisgarh	444	0	2674	10	722	0	57	0	1086	0	2436	0
6	Goa	235	0	335	1	992	0	376	0	649	0	429	0
7	Gujarat	4753	6	7579	5	18219	17	1564	2	10983	14	4811	2
8	Haryana	4550	0	1898	0	1207	0	1377	0	11835	13	4551	12
9	Himachal Pradesh	452	0	4672	7	344	2	21	0	349	0	2563	0
10	J & K	488	0	214	0	439	0	53	0	1709	4	4927	10
11	Jharkhand	710	5	463	1	825	0	79	0	220	1	123	0
12	Karnataka	17844	10	4427	4	16986	13	3823	0	7393	7	7317	4
13	Kerala	19994	37	4083	32	4652	16	4399	5	3251	27	3446	24
14	Lakshadweep	0	0	0	0	0	0	0	0	1	0	56	0
15	Madhya Pradesh	2666	6	4506	5	4189	2	806	0	15592	11	1669	0
16	Meghalaya	52	0	44	0	82	0	4	0	129	0	15	0
17	Maharashtra	7829	65	11011	55	14907	29	3356	10	12720	42	6330	5
18	Manipur	193	1	14	0	359	0	37	0	203	0	313	2
19	Mizoram	136	0	68	0	42	0	67	0	83	0	437	0
20	Nagaland	357	0	369	0	8	0	1	0	24	0	68	0
21	Odisha	4158	6	5198	5	3758	4	496	0	7548	0	5728	0
22	Punjab	15398	18	14980	9	10289	14	8435	22	23389	55	5139	5
23	Rajasthan	8427	14	9587	10	13706	17	2023	7	20749	96	9283	6
24	Sikkim	312	0	320	0	444	0	11	0	243	1	209	0
25	Tamil Nadu	23294	65	4486	13	8527	5	2410	0	6039	8	4771	4
26	Tripura	127	0	100	0	114	0	24	0	349	0	44	0
27	Telangana	5369	0	4592	2	13331	7	2173	0	7135	0	13091	0
28	Uttar Pradesh	3092	28	3829	4	10557	26	3715	6	29750	29	6758	1
29	Uttrakhand	849	0	689	3	10622	8	76	1	738	2	1878	0
30	West Bengal	37746	46			NR	NR	5166	0	8264	7	1753	3
31	A& N Island	18	0	49	0	168	0	98	0	175	0	982	0
32	Chandigarh	1125	0	301	0	286	0	265	0	1596	3	623	0
33	Delhi	9271	10	7136	4	5077	0	1269	0	13089	23	4609	0
34	D&N Haveli	2064	0	493	0	1491	2	248	0	547	0	511	0
35	Daman & Diu	59	0	163	0	625	2	71	0	279	0	156	0
36	Puduchery	4568	7	592	2	2030	2	633	1	1625	1	827	1
	Total	188401	325	101192	172	157315	166	44585	56	193245	346	110473	86

C=Cases | D=Deaths | NR=Not Reported

Figure 6. Official reported Dengue cases and deaths in India from 2017.

Searching for compounds with medicinal properties and turning it into viable drugs have been common throughout medicinal history, there are a number of drugs for dengue which has been reported to function effectively at in-vitro level but fail in mouse models. Niclosamide, an effective inhibitor of DENV and similar virus showed a strong reduction in DENV 2 replication but failed to defend the DENV 2 infected mouse [16]. It is important to note that there are no previous reports of these compounds – Morphinan, Resazurin, Furosemide, and 9H-Xanthene focusing in anti-dengue activities making it evident a potential area of phytocompounds to explore. Based on the molecular docking and ADME analysis report, the compound Morphinan is an eligible candidate but further studies on mouse models, drug optimization cohort studies, and drug feasibility are yet to be analyzed. Though Morphine, a painkiller agent, is a derivative of Morphinan, the modelling of ligand-protein interaction showed a significant attraction. However, ADME analysis eliminated the compound indicating the likeliness of permeating the brain upon intake. Unlike Morphinan, the compound Sinococuline relatively studied and is reported for inhibiting NS1 secretions from mammalian cells and their effectiveness against antibody-mediated secondary dengue infection where [12] infected mice models with DENV Type 2. Furthermore, cohort studies of Sinococuline in humans for pharmacokinetic evaluation presented a healthy toleration of the medicine against dengue [17]. These work supports the claims of Sinococuline being a latent anti-dengue drug.

CONCLUSION

Dengue is one of the major seasonal disease without a permanent vaccine or cure that affects a significant population of the world. Observing the prevalence of dengue infection and spread pattern from the recent cohort studies in India two types of DENV virus NS1 crystal structures are subjected to molecular docking. The study aims to identify potential phytocompounds from the Indian native medicinal plant *Cocculus hirsutus* for anti-dengue drug development.

From the reported 22 compounds extracted from the medicinal plant 11 has been shortlisted based on the 3D structure availability and the molecular weight. Conclusively, the study reports five compounds based on strong binding affinity namely, Sinococuline, Morphinan, Resazurin, 9H-Xanthene, and Furosemide, to be developed to a potential drug. Further examining with ADME analysis, it is evident that Sinococuline is a strong candidate for anti-dengue drug. However, recent studies of Sinococuline on DENV Type 2 infected mouse models validate the current findings of Sinococuline being a potent drug. But no studies or reports are present on compounds – Morphinan, Resazurin, 9H-Xanthene, and Furosemide. This study is the first report indicating the potential test compounds from an Indian native medicinal plant especially for anti-dengue drug development. With these latent property compounds it is clear that the phytocompounds are relatively less studied for development of drugs.

REFERENCES

1. D.R. Glasner, H. Puerta-Guardo, P.R. Beatty, E. Harris, The good, the bad, and the shocking: The multiple roles of dengue virus nonstructural protein 1 in protection and pathogenesis, Annu. Rev. Virol. 5 (2018) 227–253. https://doi.org/10.1146/annurev-virology-101416-041848.
2. H. Norazharuddin, N.S. Lai, Roles and prospects of dengue virus nonstructural proteins as antiviral targets: An easy digest, Malaysian J. Med. Sci. 25 (2018) 6–15. https://doi.org/10.21315/mjms2018.25.5.2.
3. A. El Sahili, J. Lescar, Dengue virus non-structural protein 5, Viruses. 9 (2017) 1–20. https://doi.org/10.3390/v9040091.
4. K.-H. Lin, A. Ali, L. Rusere, D.I. Soumana, N. Kurt Yilmaz, C.A. Schiffer, Dengue Virus NS2B/NS3 Protease Inhibitors Exploiting the Prime Side, J. Virol. 91 (2017) 1–10. https://doi.org/10.1128/jvi.00045-17.
5. E.M. Silva, J.N. Conde, D. Allonso, G.T. Ventura, D.R. Coelho, P.H. Carneiro, M.L. Silva, M. V. Paes, K. Rabelo, G. Weissmuller, P.M. Bisch, R. Mohana-Borges, Dengue virus nonstructural 3 protein interacts directly with human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and reduces its glycolytic activity, Sci. Rep. 9 (2019) 1–19. https://doi.org/10.1038/s41598-019-39157-7.

6. H.R. Chen, Y.C. Lai, T.M. Yeh, Dengue virus non-structural protein 1: A pathogenic factor, therapeutic target, and vaccine candidate, J. Biomed. Sci. 25 (2018) 1–11. https://doi.org/10.1186/s12929-018-0462-0.
7. R.R. Ramirez, E. Ludert, crossm from Mosquito Cells in Association with the Intracellular Cholesterol Transporter Chaperone Caveolin Complex, 93 (2019) 1–25.
8. A.C. Alcalá, F. Medina, A. González-Robles, L. Salazar-Villatoro, R.J. Fragoso-Soriano, C. Vásquez, M. Cervantes-Salazar, R.M. del Angel, J.E. Ludert, The dengue virus non-structural protein 1 (NS1) is secreted efficiently from infected mosquito cells, Virology. 488 (2016) 278–287. https://doi.org/10.1016/j.virol.2015.11.020.
9. M.C. Torres, A.L. Martins Karl, M. Müller Pereira da Silva, L.E. Dardenne, A.M. Bispo de Filippis, In Silico Analysis of Dengue Virus Serotype 2 Mutations Detected at the Intrahost Level in Patients with Different Clinical Outcomes, Microbiol. Spectr. 9 (2021) 1–29. https://doi.org/10.1128/spectrum.00256-21.
10. P. Activities, R. Logesh, N. Das, A. Adhikari-devkota, H.P. Devkota, A Review on Traditional Uses, Phytochemistry and, 8 (2019) 4–6.
11. R. Shukla, R.K. Rajpoot, A. Poddar, R. Ahuja, H. Beesetti, R.K. Shanmugam, S. Chaturvedi, K. Nayyar, D. Singh, V. Singamaneni, P. Gupta, A.P. Gupta, S. Gairola, P. Kumar, Y.S. Bedi, T. Jain, B. Vashishta, R. Patil, H. Madan, S. Madan, R. Kalra, R. Sood, R.A. Vishwakarma, D.S. Reddy, A.A. Lal, U. Arora, N. Khanna, Cocculus hirsutus-Derived Phytopharmaceutical Drug Has Potent Anti-dengue Activity, Front. Microbiol. 12 (2021) 1–16. https://doi.org/10.3389/fmicb.2021.746110.
12. R. Shukla, R. Ahuja, H. Beesetti, A. Garg, C. Aggarwal, S. Chaturvedi, K. Nayyar, U. Arora, A.A. Lal, N. Khanna, Sinococuline, a bioactive compound of Cocculus hirsutus has potent anti-dengue activity, Sci. Rep. 13 (2023) 1–13. https://doi.org/10.1038/s41598-023-27927-3.
13. A. Daina, V. Zoete, A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules, ChemMedChem. (2016) 1117–1121. https://doi.org/10.1002/cmdc.201600182.
14. A. Daina, O. Michielin, V. Zoete, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 1–13. https://doi.org/10.1038/srep42717.
15. K. Alagarasu, J.A. Patil, M.B. Kakade, A.M. More, B. Yogesh, P. Newase, S.M. Jadhav, D. Parashar, H. Kaur, N. Gupta, N. Vijay, J. Narayan, P.S. Shah, D.A. Sharma, D.A. Jain, D.A. Bhargava, D.B. Anukumar, D.A. M, D.B. Malhotra, D.B.J. Borkakoty, D.D. Biswas, D.S. Jaryal, D.K.S. Dhingra, D.K. Kaveri, D.P.K. Khatri, D.M. Kumar, D.K. Nagamani, D.R. Dhodapkar, D.R. Bakshi, D.P.S. Gill, D.S. Pati, D.S. Dutta, D.S. G, D.U. Kalawat, D.D.A. Patel, D.P. Vijayachari, Serotype and genotype diversity of dengue viruses circulating in India: a multi-centre retrospective study involving the Virus Research Diagnostic Laboratory Network in 2018, Int. J. Infect. Dis. 111 (2021) 242–252. https://doi.org/10.1016/j.ijid.2021.08.045.
16. J.C. Kao, W.C. HuangFu, T.T. Tsai, M.R. Ho, M.K. Jhan, T.J. Shen, P.C. Tseng, Y.T. Wang, C.F. Lin, The antiparasitic drug niclosamide inhibits dengue virus infection by interfering with endosomal acidification independent of mTOR, PLoS Negl. Trop. Dis. 12 (2018) 1–16. https://doi.org/10.1371/journal.pntd.0006715.
17. S.K. Dar, S. Kumar, S. Maiti, S. Dhawan, S. Joglekar, U. Arora, R. Kalra, S. Madan, A.A. Lal, V. Singamaneni, P. Gupta, U. Nandi, D. Singh, A.H. Khuroo, Clinical safety and pharmacokinetic evaluation of aqueous extract of Cocculus hirsutus, an anti-viral phytopharmacetical drug as a potential for the treatment of dengue and COVID-19, Heliyon. 8 (2022) e09416. https://doi.org/10.1016/j.heliyon.2022.e09416.

