JOURNAL OF TROPICAL LIFE SCIENCE
2024, Vol. 14, No. 1, 131 – 142
http://dx.doi.org/10.11594/jtls.14.01.14

Research Article

Molecular Docking and Pharmacokinetics Analysis of Phytochemicals from *Piper caninum* as Dengue NS2B-NS3 Protease Inhibitors

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Article history:
Submission June 2022
Revised September 2023
Accepted September 2023

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ABSTRACT

Dengue fever affects 390 million people each year. Currently, there is no specific medicine to treat this disease. Thus, the search for potential NS2B-NS3 protease inhibitors has attracted increasing research interest. The dengue protease NS2B-NS3 was used as a molecular target because of its vital function in viral replication. *Piper caninum* belongs to the *Piperaceae* family, found inhabited in Malaysia and Indonesia. This plant possesses various phytochemicals with various health benefits. However, the anti-dengue activity of this plant is yet to be discovered. Therefore, the objective of this research is to evaluate the inhibitory activity of phytochemicals from *P. caninum* against NS2B-NS3 using *in silico* experimentation. Molecular docking using AutoDock Vina was utilized to identify the binding interaction of phytochemicals on NS2B-NS3. SwissADME and ProTox-II web servers were used to analyse the ADMET (absorption, distribution, metabolism, excretion, and toxicity) of the phytochemicals. Results showed that cepharadione A, bornyl caffeate, and (+)-bornyl p-coumarate had comparable molecular interaction with the reference compound, curcumin. Analysis of *in silico* pharmacokinetics properties revealed that these phytochemicals have good pharmacokinetics profiles and excellent drug-ability, which obeyed Lipinski's Rule of Five. This study shows the potential inhibitory activity of the phytochemicals against NS2B-NS3 for a lead in the development of dengue inhibitors.

Keywords: Dengue, Molecular docking, NS2B-NS3, Pharmacokinetics, Phytochemicals

Introduction

Dengue is an endemic disease caused by the Flaviviridae family's dengue virus, which uses *Aedes aegypti* as a vector [1]. The infection can result in dengue fever, haemorrhagic fever, or shock syndrome, the latter of which is more severe and potentially fatal [2]. Each year, 390 million infections are reported worldwide [3]. Unfortunately, there is currently no medication available to treat these diseases. This enveloped virus contains a positive-sense single-stranded RNA encoding a polypeptide. The polypeptide undergoes post-translational processing by infected host cell proteases and dengue NS2B-NS3 protease enzyme [4]. This enzyme is a chymotrypsin-like serine protease with cofactor NS2B and the N-terminal domain consisting of NS3. The cofactor is crucial for the proper function of the enzyme [5]. Inhibiting this enzyme can prevent the cleavage of the polypeptides into individual proteins, which is crucial for viral replication. Thus, NS2B-NS3 is currently used as a main inhibitory target in the discovery of anti-dengue drugs.

Since ancient times, plants have been essential to human welfare due to their therapeutic properties. According to the WHO, approximately 80% of the world's population relies on medicinal

How to cite:
Mustafa NF, Cheng KK, Razali AS, Nadri MH (2024) Molecular Docking and Pharmacokinetics Analysis of Phytochemicals from *Piper caninum* as Dengue NS2B-NS3 Protease Inhibitors. Journal of Tropical Life Science 14 (1): 131 – 142. doi: 10.11594/jtls.14.01.14.

plants or herbs to meet their medical needs [6]. Plant-based inhibitors act as the potential lead for anti-dengue drugs due to less toxicity and a large structural diversity of phytochemicals [7]. *Piper caninum*, also known as *cabai hutan* comes from Piperaceae family. This species can be found in the rainforests of Malaysia and Indonesia. *P. caninum* was traditionally used to treat hoarseness and as a tonic after childbirth [8]. The plant exhibits several biological activities, including antioxidant, anti-bacterial and tyrosinase inhibitory activities [9–11, 8]. It contains several phytochemicals such as stilbene, flavonoids, and alkaloids [9]. Hence, it is beneficial to investigate the bioactivity of the phytochemicals as dengue inhibitors.

In silico methodologies refer to computer simulation-based experimentation. This computational technology is relevant in the drug discovery field due to its potential to speed up the rate of drug discovery and cost-effectiveness [12]. Molecular docking is a powerful tool in modern drug discovery. In fact, the molecular docking technique has been implemented in the development of several commercialized drugs [13]. It is employed in this research to get more insight into anti-dengue inhibitory compounds against the dengue NS2B-NS3 protease enzyme. Docking is a molecular modelling technique used to predict binding pose and binding affinity between ligand and protein targets [14].

Other than that, the advancement of machine learning has resulted in the development of an *in silico* ADMET (absorption, distribution, metabolism, excretion, and toxicity) platform to aid in discovering new drugs [15]. This computational method can reduce the development cost of drugs. Notably, *in silico* ADMET enables researchers to direct their focus towards the most promising their apeutic candidates.

Therefore, this study reported for the first time the NS2B-NS3 inhibition activity of selected phytochemicals from *P. caninum* using computational approaches. A molecular docking approach was used to predict the binding of these phytochemicals against the NS2B-NS3 protease enzyme. Besides, SwissADME and ProTox-II web servers were used to study the drug-ability and pharmacokinetics profile of the compounds.

Material and Methods

Selection of phytochemicals

Five compounds from *P. caninum* were selected, as shown in Table 1. These compounds have been reported to exhibit several biological activities. It is important to note that the selected compounds are predicted to have antiviral activity against a few viral strains, as demonstrated by the screening carried out via the Way2Drug platform (http://way2drug.com/passonline/) (Supplementary 1) [16, 17]. However, studies have yet to be reported on dengue inhibitory activity, which becomes the objective of this research.

Therefore, the compounds were further explored to identify potential anti-NS2B-NS3 dengue protease. All the phytochemical structures (*.sdf file) were downloaded from the PubChem database [21]. The files were then converted to *.pdb using OpenBabel version 2.4.0 [22].

Preparation of protein and ligand structure

The protein structure was downloaded from the Protein Data Bank website (PDB ID: 3U1I) [23]. The heteroatoms and water molecules were deleted from the structure using AutoDock Tools version 1.5.7 [24]. Then, polar hydrogen atoms and Kollman partial atomic charges were included

Table 1. The phytochemical substances reported to be present in *P. caninum* and its biological activities.

No.	Phytochemical	Class	Plant organs	Biological activity	PubChem ID
1.	(+)-Bornyl p-coumarate	Bornyl hydroxycinnamic esters	Bark	Antibacterial [18]	129317111
2.	Bornyl caffeate	Bornyl hydroxycinnamic esters	Bark	Antibacterial [18]	5477248
3.	Cepharadione A	Alkaloids	Leaves; Stems	Tyrosinase inhibitory activity [9]; DNA damaging yeast assay [19]	94577
4.	N-cis-feruloyl tyramine	Hydroxycinnamic esters	Twigs	Most potent DNA strand scission [20]	6440659
5.	Safrole	Phenylpropanoid	Leaves; Stems	Antimicrobial [8]	5144

to the receptor. The file format was created as *.pdbqt. Meanwhile, the structure of the ligands were added with Gasteiger charges by using AutoDock Tools and saved in *.pdbqt.

Molecular Docking

Molecular docking was conducted through AutoDock Vina version 1.2.0 [25] under rigid protein while the ligand was flexible. The grid map for receptor was 60×60×60 grid points. The spacing of 0.375 Å with dimensions of the box (x=36.086; y=-11.177; z=11.822) was set for all docking procedures. The binding site of this crystal structure was targeted at the allosteric pocket (Supplementary 2) [23]. The exhaustiveness was set as 16 with 100 modes. The docking simulations were carried out utilizing the iterated local search global optimization technique with the Broyden-Fletcher-Goldfarb-Shanno (BFGS) approach. This method is highly effective and precise for conducting virtual screening and molecular docking investigations in the fields of drug discovery and computational biology [25]. The successful docking simulation generated possible poses of the docked ligands in the protein target, which was classified by the binding energy values. The lowest binding energy indicates the best binding pose between the protein target and the ligand [26]. The molecular interactions between the molecules were visualized using PyMOL version 2.5 (https://pymol.org/2/) for 3D structures [27] and BIOVIA Discovery Studio 4.5 [28] for 2D interaction. Curcumin was used in this study as a positive control. The lowest binding energy values generate the best binding conformation, which signifies a strong interaction between molecules [20].

Table 2. The binding energy of ligand against dengue NS2B-NS3 protease enzyme.

No.	Ligand	Binding energy (kcal/mol)
1.	Curcumin (Positive control)	-7.3
2.	Cepharadione A	-8.4
3.	Bornyl caffeate	-7.2
4.	(+)-Bornyl p-coumarate	-7.0
5.	Safrole	-5.7
6.	N-cis-feruloyl tyramine	-5.5

Pharmacokinetics Analysis

The pharmacokinetics of the selected phytochemicals were analysed using the SwissADME (http://www.swissadme.ch.) [29]. The canonical SMILES of the molecules were uploaded to the server. The Lipinski's Rule of Five (LRF) was utilized for the determination of pharmacokinetics characteristics and drug-likeness. The rule comprised of four parameters that measured molecular weight of the phytochemicals, hydrogen donors, hydrogen acceptors, and lipophilicity. The server was also used to analyse bioavailability of the phytochemicals. Next, the toxicity of the phytochemicals was examined using ProTox-II webserver (https://tox-new.charite.de/protox_II/). The online server predicts compounds' toxicity by ranking the toxicity from 1 (toxic) to 6 (non-toxic).

Results and Discussion

Molecular Docking Analysis

Molecular docking is a computer simulation technique which predicts interaction between small molecules and macromolecular targets [30]. The NS2B-NS3 protease is essential for dengue virus infection, and inhibiting its activity can effectively hinder viral replication and prevent the progression of the disease [31]. However, an orally bioavailable drug targeting the active site of NS2B-NS3 protease is challenging to develop due to its shallow and charged binding site. A more promising target is an allosteric pocket behind the active site, which has been identified on the closed conformation of the protease [23, 2]. Thus, molecular docking using AutoDock Vina was used to dock the phytochemicals aimed at the allosteric pocket of protease.

Curcumin, the reference phytochemical was found to non-competitively inhibit the enzyme with IC_{50} value, 7.18 ± 0.62 µM and inhibitory constant (K_i), 4.35 ± 0.20 µM [32]. Molecular docking of curcumin against the protease produces a predicted binding energy of -7.3 kcal/mol (Table 2). Hence, the phytochemicals with binding energy lower or comparable to the curcumin underwent further interaction analysis.

It was observed that three phytochemicals exhibited comparable binding energy towards the enzyme except for safrole and N-cis-feruloyl tyramine. The phytochemicals belong to the alkaloid group which is the largest investigated class for antiviral activity [33]. Furthermore, the molecular contact of the top three phytochemicals including

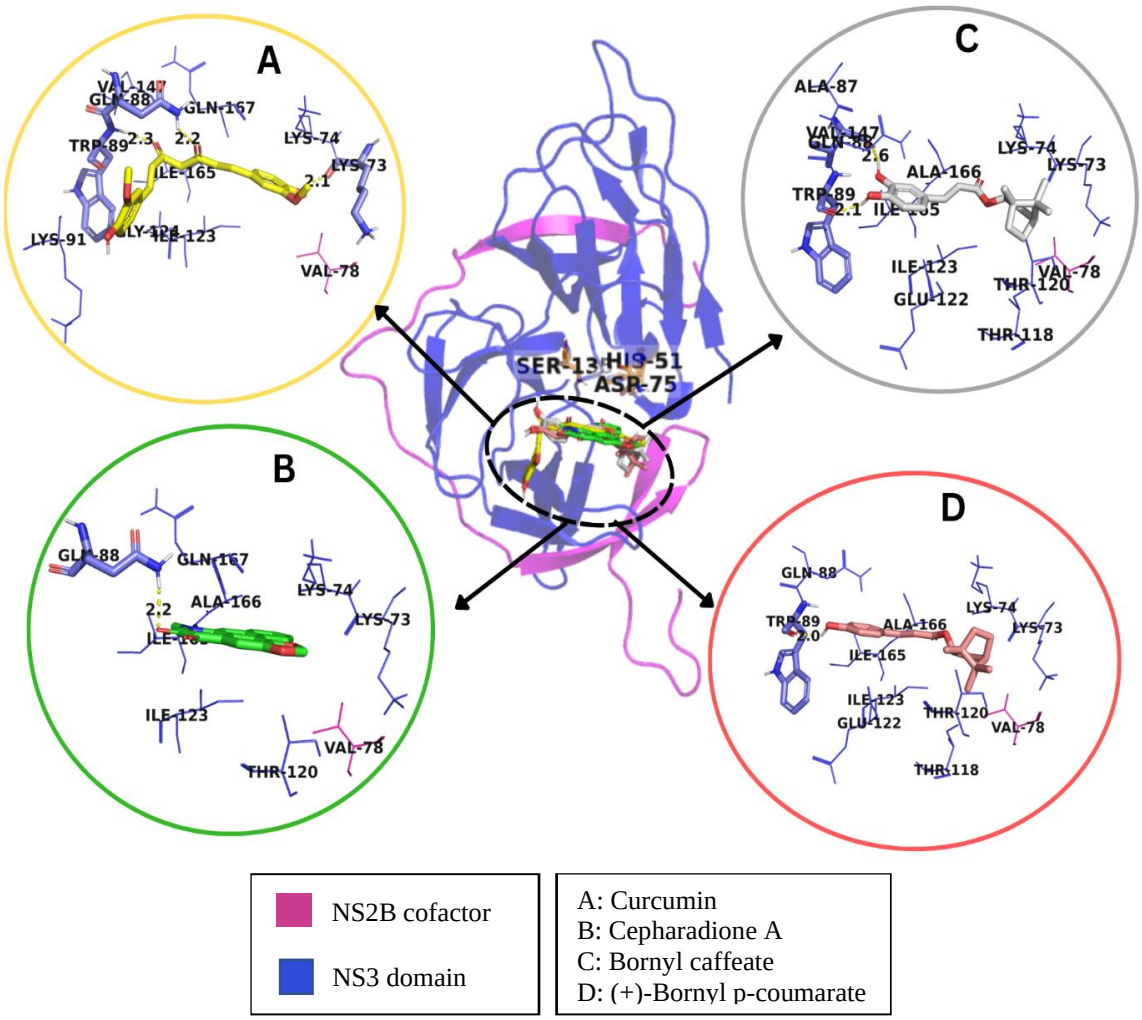

Figure 1. The molecular interaction of selected phytochemicals against NS2B-NS3.

reference compound was evaluated to understand their molecular interaction in the protein structure. The binding conformation in 3D structure was visualized in Figure 1. All phytochemicals were docked onto the allosteric site having similar binding interaction as the reference, which did not overlap with the catalytic triad (His51, Asp75, and Ser135).

Figure 2 displays the two-dimensional structure of the docked phytochemicals on the allosteric site. The NS2B-NS3-cepharadione A complex showed a hydrogen bond formation with Gln88 at 2.2 Å. The hydrophobic interaction was logged into the binding site at Lys73, Lys74, Val78, Thr120, Ile123, Ile165, Ala166, and Gln167. The predicted binding energy of the cepharadione A-NS2B-NS3 complex was -8.4 kcal/mol, which was the lowest among other phytochemicals. Aside from that, bornyl caffeate formed two hydrogen bond interactions with Trp89 (2.1 Å) and Val147 (2.6 Å). The phytochemical interacted hydrophobically with Lys73, Lys74, Val78, Ala87, Gln88, Thr118, Thr120, Glu122, Ile123, Ile165, and Ala166. Meanwhile, (+)-bornyl p-coumarate produced single hydrogen bond with Trp89 (2.0 Å) and hydrophobic interaction with Lys73, Lys74, Val78, Gln88, Thr118, Thr120, Glu122, Ile123, Ile165 and Ala166. In comparison with curcumin, the complex formed three hydrogen bond interactions in the allosteric region with Lys73 (2.1 Å), Gln88 (2.2 Å) and Trp89 (2.3 Å). Hydrophobic interactions were also discovered within the complex at residues Lys74, Val78, Lys91, Ile123, Gly124, Val147, Ile165, Ala166, and Gln167. Based on these results, it can be summarized that all the phytochemicals formed hydrogen bond interactions either with Gln88 or Trp89 similar as the reference phytochemical.

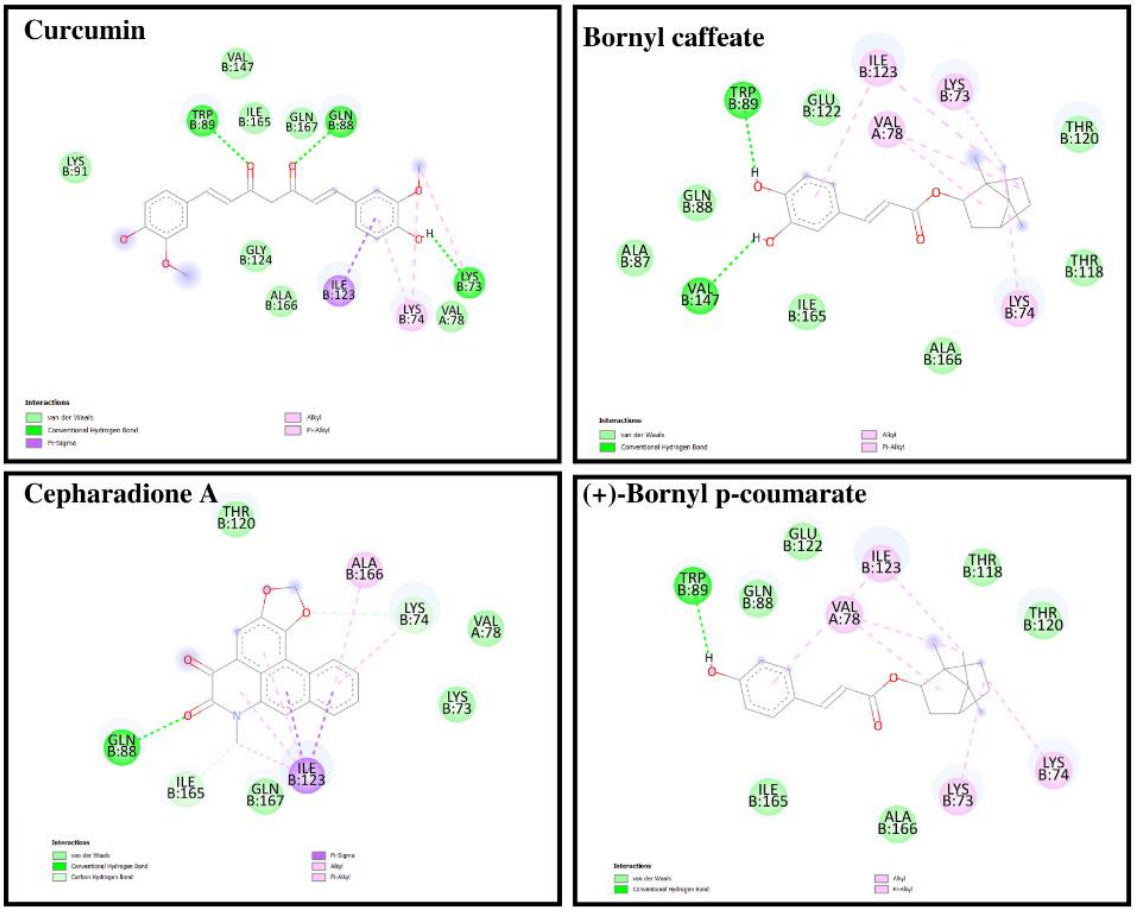

Figure 2. The interaction of compounds against NS2B-NS3.

Besides, all the phytochemicals formed alkyl interactions with Lys74 except cepharadione A, where this compound interacted by a conventional hydrogen bond with this residue. The interaction with Lys74 could potentially interrupt the activity of the enzyme as this residue is connected to Asp75, one of the catalytic triads of NS2B-NS3 protease. Fascinatingly, all the phytochemicals formed hydrophobic interaction with Ile165 which is an important residue for the catalytic activity of NS3. Past studies reported that mutation of this residue significantly reduced the activity of NS2B-NS3. This residue is conserved across flavivirus such as West Nile virus (WNV) whereby the phytochemicals may also have inhibitory activity against WNV [34]. Based on the docking results, it can be anticipated that the binding of phytochemicals to these residues could stabilize the closed conformation of NS2B-NS3 and inhibit conformational changes required for catalysis. In summary, the study predicted that three phytochemicals found in *P. caninum* have the potential to inhibit NS2B-NS3 at allosteric site, showing similar binding interactions to the reference phytochemical. Nonetheless, additional investigations are required to confirm their inhibition activity.

Pharmacokinetics Analysis

Molecular docking results showed that cepharadione A, bornyl caffeate and (+)-bornyl p-coumarate have a high possibility of inhibiting the dengue NS2B-NS3 protease enzyme. These phytochemicals were further analyzed for their physicochemical and pharmacokinetics properties, which both contribute to drug effectiveness when administered in the human body.

Pharmacokinetics predict the movement of a drug inside the human body and are often referred to as ADME properties [35]. The absorption of drugs is associated with gastrointestinal absorption (GI), whereby a high value of GI indicates better absorption of the drug. Table 3 shows that all phytochemicals exhibit high absorption before they reach the bloodstream. Another important factor that influences absorption and distribution is drug permeability which was highly relatable to

Table 3. Pharmacokinetics predictions of the analyzed compounds.

Pharmacokinetics Properties	Curcumin	Cepharadione A	Bornyl caffeate	(+)-Bornyl p-coumarate
Molecular weight (g/mol) ≤ 500	368.38	305.28	316.39	300.39
WLOGP ≤ 5	3.15	2.37	3.76	4.05
Hydrogen Bond Donors ≤ 5	2	0	2	1
Hydrogen Bond Acceptors ≤ 10	6	4	4	3
GI	High	High	High	High
BBB	No	Yes	Yes	Yes
P-gp substrate (p-glycoprotein)	No	No	No	No
CYP1A2 (Cytochrome P450 1A2) inhibitor	No	Yes	No	Yes
CYP2C19 (Cytochrome P450 2C19) inhibitor	No	No	No	Yes
CYP2C9 (Cytochrome P450 2C9) inhibitor	Yes	Yes	Yes	Yes
CYP2D6 (Cytochrome P450 2D6) inhibitor	No	No	No	No
CYP3A4 (Cytochrome P450 3A4) inhibitor	Yes	No	No	No
TPSA (Å^2)	93.06	57.53	66.76	46.53
Lipinski's Rule of Violations	No	No	No	No

molecular size and lipophilicity. The result depicted that the molecular weight of the top three phytochemicals was below 500 g/mol and had lipophilicity lower than 5. For a drug to arrive at blood circulation, it is required to travel across semipermeable cell membranes. This prediction suggested that the phytochemicals were permeable to cross the cell membrane based on their smaller size and acceptable lipophilicity value, according to LRF. Besides, the absorption of drugs is incorporated by transporters, which facilitates the process. P-glycoprotein, however, can hinder the process of absorption by discarding the substrate from cells [36]. The three phytochemicals do not serve as substrates for p-glycoprotein, which can be effectively absorbed through the cells.

Next, the distribution of the phytochemicals crossing various tissues was estimated by the permeability of the blood-brain barrier (BBB). Analysis of BBB (Table 3) suggested that the compounds were well distributed and able to cross the blood-brain barrier, unlike curcumin as this compound illustrated the opposite result for BBB permeability. A similar outcome can be observed at Brain or Intestinal Estimated permeation (BOILED-Egg) of Figure 3 displayed the lipophilicity (WLOGP) and polarity of small molecules (TPSA). The BOILED-Egg estimates the permeability of molecules either up to the intestines or across the brain barrier [37]. According to the data, curcumin falls into the white region while the investigated phytochemicals were around yellow (egg yolk). It can be deduced that the phytochemicals in the yellow region have higher lipophilicity than curcumin. Hence, this observation showed that the studied phytochemicals were able to pass the BBB when administered in the human body. Meanwhile, curcumin was found in a white region, which indicates a high probability of passive absorption through the gastrointestinal tract.

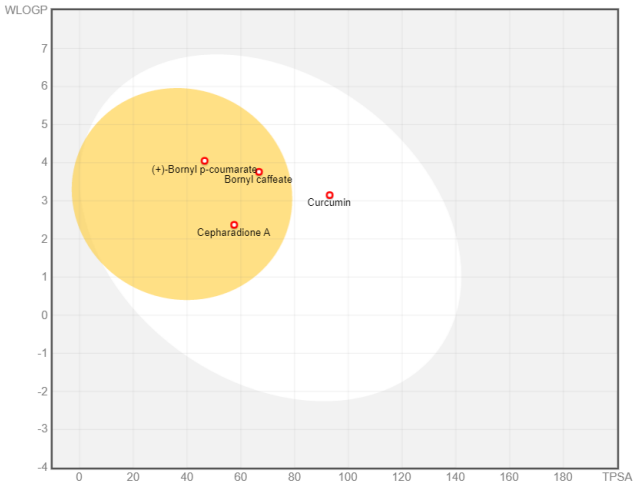

Figure 3. BOILED-Egg results of three compounds in comparison with curcumin, generated by SwissADME server

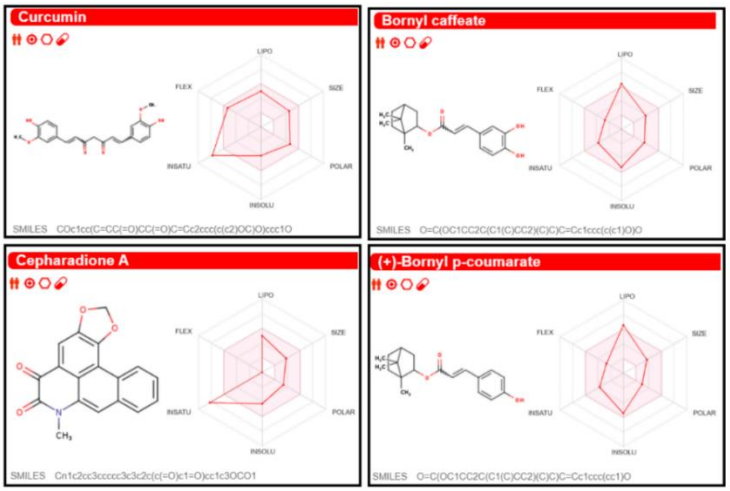

Figure 4. Bioavailability radar of tested compounds using the SwissADME server.

However, crossing BBB can have undesired effects on the central nervous system (CNS), which requires further investigation [38].

Furthermore, the metabolism of the drug is mainly participated by cytochrome P450 (CYP). The most crucial cytochrome involved in metabolizing drugs are CYP1, CYP2 and CYP3 families, which contribute to 80% of drug metabolism [39]. From Table 3, (+)-bornyl p-coumarate inhibits three CYP, which were CYP1A2, CYP2C19 and CYP2C9. Followed by cephradine A, which inhibits two CYP (CYP1A2 and CYP2C9) but acts as a non-inhibitor to CYP2C19, CYP2D6 and CYP3A4. Meanwhile, bornyl caffeate acts as an inhibitor for CYP2C9 only. Among the phytochemicals, bornyl caffeate was less likely to encounter drug-drug interaction because it only inhibits one CYP compared to other compounds, including positive control. The drawbacks of drug-drug interaction are related to a reduction of therapeutic activity and an increase in drug accumulation, which is highly toxic to the human body [29]. Nevertheless, all the phytochemicals have good bioavailability and are considered drug-like compounds.

The drug-likeness of phytochemicals was analyzed according to LRF. The rule defines the bioavailability of a molecule when administered in the human body [35]. Bioavailability refers to the absorption rate of drugs entering the systemic circulation in a complete form. Based on Figure 4, all the investigated phytochemicals were within the pink area of the bioavailability radar, indicating their promising bioavailability properties. Additionally, analysis showed that all the compounds comply with the LRF which predicts these

Table 4. Toxicity profiles of tested phytochemicals.

Toxicity	Curcumin	Cepharadione A	Bornyl caffeate	(+)-Bornyl p-coumarate
Predicted LD_{50} (mg/kg)	2000	2000	9600	5000
Predicted Toxicity Class (Class 1-3: toxic, lethal if consumed; Class 4-5: moderate toxicity, harmful if consumed; Class 6: not toxic)	4	4	6	5
Hepatotoxicity	No	No	No	No
Carcinogenicity	No	Yes	No	No
Immunotoxicity	Yes	Yes	Yes	Yes
Mutagenicity	No	Yes	No	No
Cytotoxicity	No	No	No	No

compounds as orally available drug. The molecular weight for all the compounds were below 500 which indicates suitability of compounds to be absorbed by human body in a proper speed and quantity.

In addition, the toxicity of the phytochemicals was illustrated in Table 4 using ProTox-II, an online webserver which predicts the toxic levels of compounds using 33 models from *in-vitro* and *in-vivo* data [40]. This study examines hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. Besides, the server also estimates the toxic doses of compounds whereby it calculates a lethal dose of 50% compound exposure, which can lead to fatality of test subjects (LD_{50}). Among the bioactive compounds, bornyl caffeate had the lowest toxicity compared to the other phytochemicals, which were under Class 6 (non-toxic). The LD_{50} of this phytochemical was the highest, 9600 mg/kg, which implies the compound was only toxic when the concentration was higher. The compound can cause toxicity to the immune system if taken in a higher dose.

Furthermore, (+)-bornyl p-coumarate exhibits a safe orally bioavailable drug similar to bornyl caffeate, where this phytochemical had LD_{50} more than 2000 mg/kg and falls into Class 5, indicating less toxic drug and might have the potential to cause harm when swallowed. However, cepharadione A is toxic to the liver, increases the risk of cancer and affects the immune system if consumed more than the predicted LD_{50} (2000 mg/kg). Despite that, the compound was categorized in Class 4, which is still in an acceptable range of safe drug administration. In comparison to the recommended dose of paracetamol, the dose taken for an adult was 1000 mg, with a weight of more than 50 kg [41]. Besides, the skeletal structure can be optimised in future research for improved inhibition activity with reduced toxicity [42]. Overall, the toxicity results suggested the safety of the molecules to be used as an orally bioavailable drug and followed all LRF whereby no violations occurred [43].

Collectively, cepharadione A, bornyl caffeate and (+)-bornyl p-coumarate displayed safe and good bioavailability properties, which estimate the potentiality of compounds reaching their biological destination in unchanged structure. The use of a prediction system in the development of drugs such as SwissADME and ProTox-II server can produce a better screening process of lead compounds for experimentation, reduce cost as well as save time. Aside from that, the prediction of pharmacokinetic properties and bioavailability is crucial in developing new drugs. Hence, all these selected phytochemicals can be investigated further for molecular dynamics to gain a better understanding of their binding interaction and conformational behaviours. The *in vitro* assessments are warranted to discover their potential as NS2B-NS3 inhibitors.

Conclusion

The *in-silico* prediction was utilized in this study to identify the potential of phytochemicals from *P. caninum* as dengue NS2B-NS3 protease. From the result, cepharadione A, bornyl caffeate, and (+)-bornyl p-coumarate were found to have binding confirmation comparable to curcumin, the positive control. Besides, the phytochemicals were safe and had good pharmacokinetic properties, which might be good candidates for the development of anti-dengue drugs. This study presents new findings on the potential anti-dengue activity of these phytochemicals through binding interaction with the NS2B-NS3. The investigation led to the finding of anti-dengue activity which warrants further validation.

Acknowledgement

This work was funded by the Ministry of Higher Education under the Fundamental Research Grant Scheme (FRGS/1/2021/STG01/UTM/02/-8). The authors also acknowledge Universiti Teknologi Malaysia (UTM) for research support.

References

1. Das B, Ghosal S, Mohanty S (2018) Aedes: What Do We Know about Them and What Can They Transmit? Vectors and Vector-Borne Zoonotic Diseases. doi: 10.5772/intechopen.81363.
2. Chen J, Jiang H, Li F, et al. (2018) Computational insight into dengue virus NS2B-NS3 protease inhibition: A combined ligand- and structure-based approach. Computational Biology and Chemistry 77: 261–271. doi: 10.1016/j.compbiolchem.2018.09.010.
3. Zacheo A, Hodek J, Witt D et al. (2020) Multi-sulfonated ligands on gold nanoparticles as virucidal antiviral for Dengue virus. Scientific Reports 10 (1): 1-9. doi: 10.1038/s41598-020-65892-3.
4. Zhang X, Zhang Y, Jia R et al. (2021) Structure and function of capsid protein in flavivirus infection and its applications in the development of vaccines and therapeutics. Veterinary research 52 (1): 98. doi: 10.1186/s13567-021-00966-2.
5. Wahaab A, Mustafa BE, Hameed M et al. (2021) Potential Role of Flavivirus NS2B-NS3 Proteases in Viral Pathogenesis and Anti-flavivirus Drug Discovery Employing Animal Cells and Models: A Review. Viruses 14 (1): 44. doi: 10.3390/v14010044.
6. World Health Organization (2019) WHO global report on traditional and complementary medicine 2019.
7. Ghildiyal R, Prakash V, Chaudhary VK et al. (2020) Phytochemicals as antiviral agents: Recent updates. In: Plant-derived Bioactive: Production, Properties and Therapeutic Applications. Springer Singapore pp 279–295.
8. Salleh WMNHW, Ahmad F, Yen KH, Sirat HM (2011) Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Piper caninum* blume. International Journal of Molecular Sciences 12 (11): 7720–7731. doi: 10.3390/ijms12117720.
9. Hashim NA, Ahmad F, Salleh WMNHW, Khamis S (2019) Phytochemicals and tyrosinase inhibitory activity from *Piper caninum* and *Piper magnibaccum*. Pharmaceutical Sciences 25 (4): 358–363. doi: 10.15171/PS.2019.47.
10. Nadri MH, Naser MA, Zukifli RM et al. (2015) Antioxidant activity of *Piper caninum* and cyclooxygenase-2 inhibition by methoxylated flavones. African Journal of Traditional, Complementary and Alternative Medicines 12 (2): 120–125. doi: 10.4314/ajtcam.v12i2.18.
11. Salleh WMNHW, Ahmad F, Yen KH (2015) Chemical constituents from *Piper caninum* and antibacterial activity. Journal of Applied Pharmaceutical Science 5 (6): 020–025. doi: 10.7324/JAPS.2015.50604.
12. Brogi S, Ramalho TC, Kuca K, Valko MM (2020) Editorial: In silico Methods for Drug Design and Discovery. Frontiers in Chemistry 8: 612. doi: 10.3389/fchem.2020.00612.
13. Abrigach F, Rokni Y, Takfaoui A et al. (2018) In vitro screening, homology modeling and molecular docking studies of some pyrazole and imidazole derivatives. Biomedicine and Pharmacotherapy 103: 653–661. doi: 10.1016/j.biopha.2018.04.061.
14. Boittier ED, Tang YY, Buckley ME, Schuurs ZP, Richard DJ, Gandhi NS (2020) Assessing molecular docking tools to guide targeted drug discovery of cd38 inhibitors. International Journal of Molecular Sciences 21 (15): 1–19. doi: 10.3390/ijms21155183.
15. Göller AH, Kuhnke L, Montanari F et al. (2020) Bayer's in silico ADMET platform: a journey of machine learning over the past two decades. Drug Discovery Today 25 (9): 1702–1709. doi: 10.1016/j.drudis.2020.07.001.
16. Setzer WN, Setzer MC, Bates RB et al. (1999) Antibacterial hydroxycinnamic esters from *Piper caninum* from Paluma, north Queensland, Australia. The crystal and molecular structure of (+)-bornyl coumarate. Planta medica, 65 (08): 747-749. doi: 10.1055/s-2006-960855
17. Filimonov DA, Lagunin AA, Gloriozova TA et al. (2014) Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds 50: 444-457. doi: 10.1007/s10593-014-1496-1
18. Ma J, Jones SH, Marshall R et al. (2004) A DNA-damaging oxoaporphine alkaloid from *Piper caninum*. Journal of Natural Products 67 (7): 1162–1164. doi: 10.1021/np040056x.
19. Wicaksono A, Raihandhany R, Zen TV et al. (2022). Screening Rafflesia and sapria metabolites using a bioinformatics approach to assess their potential as drugs. Philippine Journal of Science 151 (5): 1771 – 1791. doi: 10.56899/151.05.20
20. Ma J, Jones SH, Hecht SM (2004) Phenolic acid amides: A new type of DNA strand scission agent from *Piper caninum*. Bioorganic and Medicinal Chemistry 12 (14): 3885–3889. doi: 10.1016/j.bmc.2004.05.007.
21. Kim S, Chen J, Cheng T et al. (2021) PubChem in 2021: New data content and improved web interfaces. Nucleic Acids Research 49 (D1): D1388–D1395. doi: 10.1093/nar/gkaa971.
22. O'Boyle NM, Banck M, James CA et al. (2011) Open Babel: An Open chemical toolbox. Journal of Cheminformatics 3 (1): 1-14. doi: 10.1186/1758-2946-3-33.

23. Noble CG, Seh CC, Chao AT, Shi PY (2012) Ligand-Bound Structures of the Dengue Virus Protease Reveal the Active Conformation. Journal of Virology 86 (1): 438–446. doi: 10.1128/jvi.06225-11.
24. Morris GM, Ruth H, Lindstrom W et al. (2009) Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry 30 (16): 2785–2791. doi: 10.1002/jcc.21256.
25. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry: 31(2): pp.455-461. doi: 10.1002/jcc.21334
26. Rahman MM, Biswas S, Islam KJ et al. (2021) Antiviral phytochemicals as potent inhibitors against NS3 protease of dengue virus. Computers in Biology and Medicine 134: 104492. doi: 10.1016/j.compbiomed.2021.104492.
27. Schrodinger LLC (2015) The PyMOL Molecular Graphics System, Version 1.8.
28. Systèmes D (2020) BIOVIA, discovery studio visualizer, release 2019. San Diego: Dassault Systèmes
29. Daina A, Michielin O, Zoete V (2017) SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports 7 (1): 42717. doi: 10.1038/srep42717.
30. Sethi A, Joshi K, Sasikala K, Alvala M (2020) Molecular Docking in Modern Drug Discovery: Principles and Recent Applications. Drug Discovery and Development - New Advances. doi: 10.5772/intechopen.85991.
31. Sarwar MW, Riaz A, Dilshad SMR et al. (2018) Structure activity relationship (SAR) and quantitative structure activity relationship (QSAR) studies showed plant flavonoids as potential inhibitors of dengue NS2B-NS3 protease. BMC structural biology 18 (1): 1-10. doi: 10.1186/s12900-018-0084-5.
32. Lim L, Dang M, Roy A et al. (2020) Curcumin Allosterically Inhibits the Dengue NS2B-NS3 Protease by Disrupting Its Active Conformation. ACS Omega 5 (40): 25677–25686. doi: 10.1021/acsomega.0c00039.
33. Abookleesh FL, Al-Anzi BS, Ullah A (2022) Potential Antiviral Action of Alkaloids. Molecules 27: 903. doi: 10.3390/molecules27030903.
34. Salaemae W, Junaid M, Angsuthanasombat C, Katzenmeier G (2010) Structure-guided mutagenesis of active site residues in the dengue virus two-component protease NS2B-NS3. Journal of Biomedical Science 17: 68. doi:10.1186/1423-0127-17-68.
35. Isyaku Y, Uzairu A, Uba S (2020) Computational studies of a series of 2-substituted phenyl-2-oxo-, 2-hydroxyl- and 2-acylloxyethylsulfonamides as potent anti-fungal agents. Heliyon 6 (2020): e03724. doi: 10.1016/j.heliyon.2020.e03724.
36. Nguyen TTL, Duong VA, Maeng HJ (2021) Pharmaceutical formulations with p-glycoprotein inhibitory effect as promising approaches for enhancing oral drug absorption and bioavailability. Pharmaceutics 13: 1103. doi: 10.3390/pharmaceutics13071103.
37. Bojarska J, Remko M, Breza M et al. (2020) A supramolecular approach to structure-based design with a focus on synthons hierarchy in ornithine-derived ligands: Review, synthesis, experimental and in silico studies. Molecules 22: 1135. doi: 10.3390/molecules25051135.
38. Han Y, Zhang J, Hu CQ et al. (2019) In silico ADME and toxicity prediction of ceftazidime and its impurities. Front Pharmacol 10: 434. doi: 10.3389/fphar.2019.00434.
39. Zhao M, Ma J, Li M et al. (2021) Cytochrome p450 enzymes and drug metabolism in humans. International Journal of Molecular Sciences 22: 12808. doi: 10.3390/ijms222312808.
40. Banerjee P, Eckert AO, Schrey AK, Preissner R (2018) ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Research 46 (W1): W257–W263. doi: 10.1093/nar/gky318.
41. Saragiotto BT, Abdel Shaheed C, Maher CG (2019) Paracetamol for pain in adults. The BMJ 367: I6693. doi: 10.1136/bmj.I6693.
42. Hsu KH, Su BH, Tu YS et al. (2016). Mutagenicity in a molecule: identification of core structural features of mutagenicity using a scaffold analysis. PloS One 11 (2): p.e0148900. doi: 10.1371/journal.pone.0148900
43. Guan L, Yang H, Cai Y et al. (2019) ADMET-score-a comprehensive scoring function for evaluation of chemical drug-likeness. MedChemComm 10 (1): 148–157. doi: 10.1039/C8MD00472B.

Supplementary 1

Table 5. Antiviral activity of the selected compounds was screened using Way2Drug

Phytochemicals	Activity	Pa	Pi
Cepharadione A Binding energy: -8.4 kcal/mol	Antiviral (Adenovirus)	0,484	0,008
	Antiviral (Hepatitis B)	0,255	0,049
	Antiviral (Picornavirus)	0,315	0,203
(+)-Bornyl p-coumarate Binding energy: -7.0 kcal/mol	Antiviral (Influenza)	0,739	0,004
	Antiviral (Herpes)	0,490	0,011
	Antiviral (Hepatitis B)	0,237	0,058
	Antiviral	0,227	0,075
	Antiviral (Influenza A)	0,240	0,116
	Antiviral (Picornavirus)	0,277	0,267
Bornyl caffeate Binding energy: -7.2 kcal/mol	Antiviral (Influenza)	0,748	0,004
	Antiviral (Herpes)	0,491	0,011
	Antiviral (Rhinovirus)	0,400	0,090
	Antiviral (Hepatitis B)	0,247	0,052
	Antiviral	0,249	0,060
	Antiviral (Influenza A)	0,228	0,143
N-cis-feruloyl tyramine Binding energy: -5.5 kcal/mol	Antiviral (Influenza)	0,353	0,063
	Antiviral (Herpes)	0,286	0,101
	Antiviral (Hepatitis B)	0,207	0,081
	Antiviral (HIV)	0,146	0,071
Safrole Binding energy: -5.7 kcal/mol	Antiviral (Adenovirus)	0,356	0,050
	Antiviral (CMV)	0,319	0,020
	Antiviral (Influenza)	0,202	0,194

*The compound is very likely to exhibit activity in experiment when Pa>0.7; very likely to exhibit activity in experiment when 0.5<Pa<0.7; compounds unlikely to exhibit activity in experiment when Pa<0.5, however the compound might be a new chemical entity if the activity is confirmed in experiment [17].

Supplementary 2

Table 6. Preliminary assessment of binding site using blind docking (CB-Dock2) of the selected compounds and positive control.

Phytochemicals	Binding energy (kcal/mol)	Contact residues
Curcumin	-7.6	NS2B: VAL78, GLY82 MET84 NS3: LYS73, LYS74 ASP75, LEU76, GLN88, TRP89, THR118, THR119, THR120, GLU122, ILE123, GLY124, ASN152, ILE165, ALA166, GLN167
Cepharadione A	-8.2	NS3: LYS74, GLN88, TRP89, GLU122, ILE123, GLY124, VAL147, ILE165, ALA166, GLN167, ASN169
(+)-Bornyl p-coumarate	-6.7	NS2B: VAL78, GLY82, MET84 NS3: VAL72, LYS73, LYS74, ASP75, GLN88, THR120, GLY121, GLU122, ILE123, ASN152, ILE165, ALA166, GLN167, THR168, ASN169
Bornyl caffeate	-7.6	NS2B: VAL78 NS3: LYS73 LYS74 ALA87 GLN88 TRP89 GLN90 THR118 THR120 GLU122 ILE123 VAL147 ILE165 ALA166 GLN167