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Short Communications

Exploring *Centella asiatica*-derived Compounds as Inhibitors of Chikungunya Virus nsP2 Protease: Insights into Pharmacokinetics Prediction

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ABSTRACT

Background: Chikungunya virus (CHIKV) infection poses a significant global health threat, with limited treatment options. **Objective:** This study explores the potential of flavonoids, terpenoid, and triterpenoid compounds derived from *Centella asiatica* to inhibit the CHIKV nsP2 protease, a crucial enzyme in viral replication. **Method:** Through computational modelling, we demonstrate the inhibitory activity of these compounds against the nsP2 protease, highlighting their potential as therapeutic agents against CHIKV. **Results:** The results indicate that flavonoid derivatives from *Centella asiatica*, including castilliferol and castillicetin, exhibit lower binding energy values (-10 and -9.9, respectively) compared to the FDA drug emetine. Additionally, these flavonoid derivatives demonstrate specific inhibition of Lys1045 and Lys1239, active sites of the CHIKV nsP2 protease. Pharmacokinetic predictions provide valuable insights into the compounds' absorption, distribution, metabolism, and excretion. This indicated that castilliferol and castillicetin fulfil Lipinski's rule parameters in terms of physicochemical and ADMET properties.

INTRODUCTION

Indonesia's tropical location near the equator results in a hot, humid climate and abundant natural resources, but it also makes it vulnerable to tropical diseases like Chikungunya. This mosquito-borne disease, caused by the Chikungunya virus (CHIKV), is classified as a neglected tropical disease (NTD), receiving insufficient attention in global health initiatives. Despite recording 5042 cases in 2019, Chikungunya remains relatively ignored in Indonesia. Globally, Chikungunya cases in Asia surged to 1.9 million, underscoring the urgent need for greater attention [1].

Global epidemics of the alphavirus CHIKV present significant threats to public health. Chikungunya fever is distinguished by intense joint pain, a high body temperature, and a skin rash. Due to the absence of licensed antiviral medications or immunisations, it is imperative to develop effective treatments [2]. The CHIKV non-structural protein 2 (nsP2) protease is essential for viral replication and a prospective antiviral therapeutic target. This protease breaks down the viral polyprotein precursor into functional proteins and disrupts host antiviral responses to facilitate immune evasion [3].

Natural products are a rich source of bioactive compounds. *Centella asiatica*, known as Gotu Kola, is a medicinal herb with reported antiviral [4], anti-inflammatory [5] and neuroprotective properties [6], making it a candidate for antiviral agent exploration. This study identifies compounds derived from *Centella asiatica* as potential inhibitors of the CHIKV nsP2 protease. Using *in silico* screening and molecular docking studies, the study investigates the inhibitory potential and molecular interactions of these compounds with nsP2. Additionally, *in silico* pharmacokinetics prediction models evaluate the drug-likeness and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the identified inhibitors [7]. The research findings support the ongoing efforts in the development of effective treatments against CHIKV infections and highlight the potential of natural product-derived compounds in antiviral drug discovery.

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METHODS

Materials

The Protein Data Bank database (www.rcsb.org) provided the nsP2 protease (PDB ID: 3TRK), which served as the macromolecule in this study. *Centella asiatica* has compounds that could be used as ligands. These are C1 (apigenin), C2 (luteolin), C3 (naringin), C4 (patuletin), C5 (castillicetin), C6 (castilliferol), C7 (centelloside D), C8 (centellasaponin B), C9 (astragalin), C10 (centellasaponin C), C11 (galanal A), and C12 (galanal B) (Yan, Shi, and Jiang 2023).

Preparation and Optimisation of the Protein, Ligands, and Control

The X-ray crystal structure of nsP2 protease (PDB ID: 3TRK) was obtained from the Protein Data Bank and subsequently optimised. The target proteins were prepared by removing cofactors and water molecules from their structures using the Molegro Virtual Docker software. The three-dimensional (3D) structures were created using Avogadro software and optimised using the DFT B3LYP 3-21G method. The control used in the study was the FDA-approved drug emetine.

Molecular Docking and Statistical Analysis

The prepared proteins and ligands were converted into the PDBQT format using AutoDock Tools. The grid box was set to encompass the active site of the nsP2 protease, ensuring that the binding pocket was thoroughly covered. Docking simulations were conducted using AutoDock Vina. To validate the docking protocol, the FDA-approved drug emetine was used as a control ligand, and its binding affinity was compared against the potential ligands from Centella asiatica. Post-docking analysis was performed to evaluate the binding affinities and interaction modes. The results were visualised and interpreted using molecular visualisation tools to understand the interaction patterns and potential efficacy of the ligands.

The physicochemical and ADMET analyses

All compounds were converted into SMILES format and subsequently uploaded individually to the pkCSM server using Discovery Studio. The physicochemical and ADMET properties were determined by selecting the ADMET menu. The pkCSM server then provided data for each compound, including Lipinski's rule of five as well as their absorption, distribution, metabolism, excretion, and toxicity profiles.

RESULTS AND DISCUSSION

Molecular Docking Study of Centella asiatica towards nsP2 Protease

The objective of this study was to identify derivatives of *Centella asiatica* compounds [8] capable of inhibiting the non-

Table I. Demographic characteristics by age group

Compounds	Binding Affinity	Amino Acid Interaction	Secondary metabolites
C10 (Centellasaponin C)	-10,1	**Leu1203, Val1077, Gly1089, Glu1050, Ser1048, Gln1241**, Ala1046, Gly1090	Triterpenoid
C5 (Castillicetin)	-10	**Tyr1079, Asn1082, Gly1206, Lys1239, Lys1045, Gln1241**, Leu1205, Ala1046, Val1051, Tyr1047	Flavonoid
C6 (Castilliferol)	-9,9	**Asn1082, Gly1206, Lys1045**, Leu1205, Lys1239,	Flavonoid
C3 (Naringin)	-9,6	Val1051, Tyr1047, Ala1046, Tyr1079	Flavonoid
C7 (Centelloside D)	-8,9	**Cys1013, Asp1246, Gly1206, Lys1239**, Tyr1079, Ala1046, Leu1203, Ser1048 **Leu1243, Tyr1079, Tyr1047,** Glu1204, Leu1203, Asn1082	Triterpenoid
C9 (Astragalin)	-8,2	**Leu1243, Ser1048**, Lys1239, Gln1241	Flavonoid
C8 (Centellasaponin B)	-8,2	**Tyr1079, Gln1241, Leu1205, Glu1050**, Asp1246,	Triterpenoid
C11 (Galanal A)	-8,1	**Lys1045, Lys1239, Leu1243, Leu1203**	Terpenoid
C12 (Galanal B)	-8	**Lys1045, Lys1239, Leu1243**, Glu1204	Terpenoid
Emetine	-7,8	**Lys1239, Ser 1048**, Val1051, Tyr1047, Trp1084, Tyr1079	Control
C2 (Luteolin)	-7,8	**Lys1239**, Ala123, Ala1046, Tyr1079, Tyr1047, Val1051	Flavonoid
C4 (Patuletin)	-7,8	**Lys1239, Tyr1079, Leu1243, Asp1246**, Gly1206, Leu1205, Asn1082	Triterpenoid
C1 (Apigenin)	-7,6	**Leu1243, Ser1048**, Lys1239, Gln1241	Flavonoid

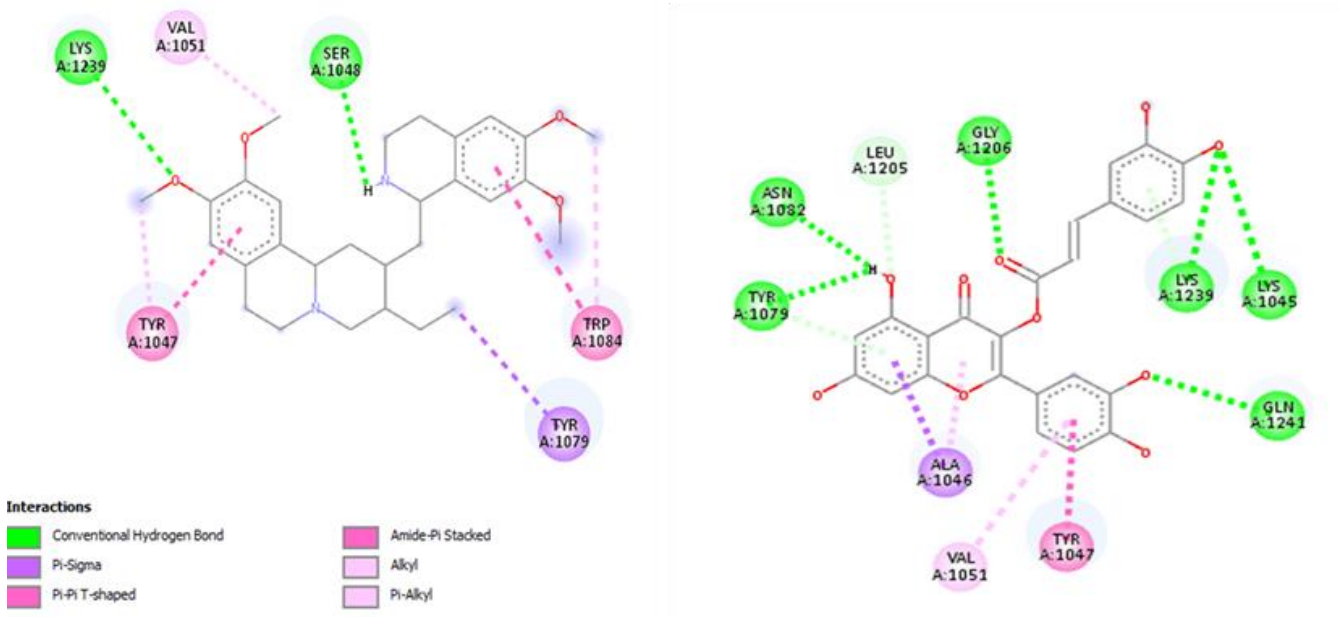

Figure I. Visualisation of C5 (castillicetin) and emetine interactions towards nsP2 protease

structural nsP2 protease protein using molecular docking. The nsP2 protease (PDB: 3TRK) was prepared by removing the Na+ ligand and water molecules present in the protein. The docking results of *Centella asiatica* derivatives are shown in Table I. The data reveals that C10 (centellasaponin C) has the highest binding affinity, whereas C5 (castillicetin) demonstrates binding values of -10.1 kcal/mol and -10 kcal/mol, respectively.

Binding affinity is a quantitative measure used in molecular docking analysis to assess the strength of the interaction between a ligand and a protein. A higher binding affinity value corresponds to a larger effect on the nsP2 protease. According to Table I, flavonoid compounds (C5, C6, and C3) exhibit superior binding affinity values compared to other compounds. Furthermore, this indicates that flavonoid molecules possess advantageous inhibitory properties. The nsP2 protease cleaves the viral polyprotein into functional components needed for viral assembly and maturation, enabling Chikungunya virus replication. Interfering with this protease can stop viral replication and reduce disease severity. This protease is a viral enzyme, therefore inhibitors targeting it are less likely to disrupt host cellular processes, minimising off-target effects and toxicity from antiviral medicines. Lys1045, Gly1176, His1222, and Lys1239 are the essential residues for interaction at binding site 1 (pocket 1), the possible inhibitor target [9].

The binding modes obtained from docking at potential binding sites were analysed to detail the interactions and identify key residues by examining hydrogen bonds (H-bonds) and hydrophobic contacts. Based on Table I, showed that compounds C5 (castillicetin) and C6 (castilliferol) have the best binding affinity values and form hydrogen bonds at the active site of nsP2 protease (Fig. 1), specifically with Lys1045 and Lys1239. Additionally, compound C3 forms a hydrogen bond with Cys1013, an amino acid that plays a role as a conserved catalytic dyad together with histidine. Except for C7 and C8, all compounds interact with the nsP2 active site through either hydrogen bonds or hydrophobic interactions. Emetine, an FDA-approved medication, was utilised as a control. Prior investigations using computational and laboratory experiments have demonstrated that emetine exhibits inhibitory properties against the Chikungunya virus nsP2 at doses of 200 µM and 100 µM [10]. According to the docking data, the chemical emetine exhibited a binding affinity of -7.8 kcal/mol, forming hydrogen bonds with the active site Lys1239. These findings suggest that *Centella asiatica* derivatives bind to the same active site as emetine and exhibit stronger binding affinities, particularly for C5 (castillicetin) and C6 (castilliferol). Based on the statistical approach of this inquiry, it can be concluded that there is no significant difference in the binding affinities of flavonoid compounds to other compounds for the target protein. This conclusion is supported by the fact that the p-value (0.508) is higher than the significance level (0.05).

Physicochemical and ADMET Properties of Compounds

The pkCSM website was used to evaluate the physicochemical and ADMET parameters. In order to adhere to the "rules of five," a drug must possess a molecular weight that is less than 500 Da, a log P (logarithm of the octanol-water partition coefficient) value that is lower than 5, less than 10 rotatable bonds (ROTB), less than 10 hydrogen bond acceptors (HBA),

less than 5 hydrogen bond donors (HBD), and a polar surface area (PSA) that is below 140 Å. None of the substances, including the control medication emetine, fit the "rules of five" requirements based on their physicochemical features. These findings indicate that compounds C1–C12 are unsuitable for oral administration as pharmaceutical medicines.

The ADMET study assesses the pharmacokinetics of a drug, specifically examining the toxicity, metabolism, distribution, absorption, and excretion of small-molecule medicines. Absorption is influenced by water solubility, CaCO-2 permeability, intestinal absorption, and skin permeability. Most medications on the market have a water solubility of approximately 4. However, galangal A and galangal B have the highest water solubility among them. Furthermore, castilliferol has superior water solubility in comparison to the control substance, emetine. The Caco-2 cell line is frequently employed as an in vitro model to mimic the human intestinal mucosa to forecast oral medication absorption. Medications are classified as having high Caco-2 permeability if their permeability coefficient (Papp) values are greater than 0.90. The most favourable compounds in this regard are galangal A (1.363), galangal B (1.363), and apigenin (1.076), which belong to the category of flavonoid compounds. All chemicals satisfy the criterion for skin permeability, which is greater than -2.7.

When discussing distribution, the relevant factor is VDss (human), which refers to the overall amount of medicine that can be evenly spread throughout the body. Compounds with VDS values greater than 0.45 are considered to be of high quality. Therefore, the best compounds in this case are galanal A, galanal B, and emetine. Measuring the permeability of the blood-brain barrier offers valuable information on the capacity of drugs to pass across the barrier. Molecules with a logBB value higher than 0.3 are easily taken up by the brain, whereas those with a logBB value lower than -1 do not spread uniformly throughout it. This suggests that the chemicals C1-C12 and emetine have limited distribution in the brain. Concerning metabolism parameters, castilliferol can be broken down by substances that inhibit CYP2C9. Additionally, results on excretion indicate that none of the compounds can be eliminated by the renal OCT2 substrate.

CONCLUSIONS

Based on molecular docking analysis, it is shown that the compounds centellasaponin C and Castillicetin have the lowest binding affinity values of -10.1 and -10 kcal/mol, respectively. Based on the interaction with the active site of nsP2, the compound castillicetin is capable of binding to the amino acids Lys1239 and Lys1045, which are the active sites of nsP2. Interestingly, these same sites are also bound by the drug emetine. Based on the physicochemical analysis and ADMET predictions, it appears that all compounds are likely unsuitable for oral drugs, while the compounds castillicetin and castilliferol meet the ADMET criteria.

CONFLICT OF INTEREST

None

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Not available.

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