IN SILICO ANALYSIS OF THE INHIBITION OF DENGUE VIRUS NS-1 BY THE PHYTOCHEMICALS SOURCE FROM SRI LANKAN MEDICINAL PLANTS

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The incidence of dengue has grown intensely across the globe in recent decades. Dengue-related deaths have been increasing due to the lack of an effective vaccine and antiviral therapy. However, antiviral drug development is a complex problem due to the lack of animal models and the development of drug resistance for chemically synthesized compounds. Hence, it is essential to investigate alternative sources such as phytochemicals as antivirals against dengue using *In silico* analysis. This study investigated the potential of inhibition of DENV NS-1 protein by the phytochemicals source from Sri Lankan medicinal plants using *in silico* analysis. A phytochemical database source from the Sri Lankan Plant Database was tested in silico for the potential of inhibiting the DENV NS-1. Initial bioavailability screening via the SwissAdme server which identified 34 compounds as bioavailable compounds and the toxicity analysis using ProTox-2 produced 8 compounds as less toxic bioavailable compounds. Molecular docking studies revealed 10 phytochemicals with the lowest binding energies ranging from -8.5 to -7.5 Kcal/mol to the DENV NS1. Detailed molecular docking analysis using Discovery Studio 2024 Client evaluated the interactions between the selected compounds and the NS-1 protein and highlighted that ɤ-Taraxasterol, despite having the lowest binding affinity, demonstrated significant non-bonding interactions with critical active site residues. Other notable compounds, such as Calozeyloxanthone, Alkaloid II, Alkaloid IV, and Myoinosiol also exhibited a significant binding activity to the DENV NS1. Molecular dynamics simulations using ChimeraX and ISOLDE software provided insights into the stability and conformational changes of the protein-ligand complexes over time. Ramachandran Plots further confirmed the steric clashes and deviations from ideal structural conformations, ensuring the reliability of the docking results. Notably, Alkaloid II engaged in hydrogen bond interactions with key active site residues, highlighted its potential as a bioactive compound. The present study has identified Alkaloid II sources from Sri Lankan medicinal plants as the most effective phytochemical that could bind to the NS1 protein and inhibit the dengue virus. However, in *vitro* and in vivo investigations are warranted to confirm the efficacy and safety of these phytochemicals as antiviral agents against the dengue virus, potentially leading to new treatments for dengue fever.

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INTRODUCTION

Dengue fever, a mosquito-borne viral infection, poses a significant public health threat globally, particularly in tropical and subtropical regions. The disease is caused by the dengue virus (DENV), which has four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Among the viral proteins, the non-structural protein 1 (NS-1) plays a crucial role in the pathogenesis and immune evasion of the virus, making it a promising target for antiviral drug development. Current treatment options for dengue are primarily supportive, with no specific antiviral therapies available, underscoring the urgent need for effective therapeutic agents. With its rich biodiversity and traditional medicinal heritage, Sri Lanka offers several medicinal plants that have been used for centuries to treat various diseases, including viral infections. Phytochemicals, the bioactive compounds found in these plants, have shown potential in combating numerous viral diseases. However, the antiviral properties of many of these phytochemicals against dengue virus, particularly the NS-1 protein, remain largely unexplored (Perera & Kuhn, 2008).

OBJECTIVES

The primary objective of this research is to investigate the anti-viral potential of phytochemicals isolated from Sri Lankan medicinal plants against the dengue NS-1 protein used *in silico* analysis. Further to investigate the bioavailability and toxicity of phytochemicals to ensure the potential of leading to a drug lead.

METHODOLOGY

Selection of phytochemicals:

This study involved a comprehensive computational and network pharmacology approach to investigate the antiviral potential of phytochemicals from Sri Lankan medicinal plants against the dengue virus NS-1 protein. Initially, 120 phytochemicals were selected from the Sri Lankan plant database based on their documented medicinal properties and potential antiviral activities (BERMAN, 2000).

Bioavailability and toxicity screening

These compounds underwent bioavailability screening using the SwissAdme server (Sravika et al., 2021). Further toxicity analysis was conducted using the ProTox-2 server narrowed this down to 8 compounds with acceptable safety profiles.

Molecular Docking Studies:

Molecular docking studies using Autodock Vina assessed the binding affinities of these phytochemicals with the dengue NS-1 protein (PDB ID: 7BSC) of the DEN-2 strain, revealing 10 compounds with binding energies ranging from -8.5 to -7.5 kcal/mol (Dallakyan & Olson, 2015). Detailed molecular docking analysis with Discovery Studio 2024 client elucidated interactions between the selected compounds and critical active site residues of the NS-1 protein (Pettersen et al., 2021).

Molecular Dynamics Simulations:
To understand the dynamic behaviour of these interactions, molecular dynamics simulations using Chimerax and ISOLDE software provided insights into the stability and conformational changes of the protein-ligand complexes over time (Croll, 2018).

Structural Validation:
Ramachandran plots were used to identify any steric clashes and deviations from ideal structural conformations, ensuring the reliability of the docking results. This methodology led to the identification of several phytochemicals with potential antiviral activity against the dengue NS-1 protein, contributing to natural product-based drug discovery and paving the way for future experimental validation and the development of new treatments for dengue fever.

RESULTS AND DISCUSSION

Bioavailability screening and toxicity analysis:
Out of the initial 120 phytochemicals selected from the Sri Lankan plant database, 34 compounds passed the bioavailability screening conducted via the SWISSADME Server, demonstrating favourable pharmacokinetic properties. These 34 compounds were subsequently subjected to toxicity analysis using ProTox-2, which narrowed the list to 8 compounds with acceptable safety profiles for further study.

Molecular docking studies:
Molecular docking studies were performed to evaluate the binding affinities of the 8 shortlisted phytochemicals with the dengue NS-1 protein (PDB ID: 7BSC) of the DEN-2 strain. The docking results revealed that 10 phytochemicals exhibited the lowest binding energies, ranging from -8.5 to -7.5 kcal/mol, indicating strong potential for interaction with the NS-1 protein.

A more detailed analysis using Discovery Studio 2024 client elucidated the interactions between the selected phytochemicals and the NS-1 protein. Among the top compounds, ɤ-taraxasterol, despite having the lowest binding affinity, demonstrated significant non-bonding interactions with critical active site residues. Calozeyloxanthone, alkaloid II, alkaloid IV, and myoinositol were also notable for their promising interactions. These compounds exhibited key interactions with amino acids essential for the function of the NS-1 protein, suggesting their potential as effective inhibitors.

Molecular dynamics simulations:
Molecular dynamics simulations were conducted using Chimerax and Isolde software to assess the stability and conformational changes of the protein-ligand complexes over time. The simulations confirmed the stability of the interactions for the top phytochemicals. The trajectories of these simulations showed consistent binding conformations and minimal deviations, indicating robust and stable complexes.

Structural validation:
Ramachandran plots were generated to evaluate the structural integrity of the protein-ligand complexes. These plots confirmed the absence of steric clashes and deviations from ideal structural conformations, further validating the reliability of the docking results.

In this investigation, alkaloid II and alkaloid IV obtained from *sarcococca zeylanica* plant were identified as bioavailable and non-toxic phytochemicals, making them promising candidates for antiviral therapy against the dengue virus.

Despite their favourable profiles, these alkaloids exhibited relatively high binding energies compared to other phytochemicals. Strategies to enhance their efficacy include structural modifications to improve binding affinities, combination therapies to leverage synergistic effects, and dose optimization to balance efficacy with safety. Additionally, addressing steric clashes observed in Ramachandran plots through structural optimization can further enhance their therapeutic potential (Reiter et al., 2010). Notably, Alkaloid II (Figure 1) engaged in hydrogen bond interactions with key active site residues, highlighted its potential as a bioactive compound. The present study has identified Alkaloid II sources from Sri Lankan medicinal plants as the most effective phytochemical that could bind to the NS1 protein and inhibit the dengue virus.

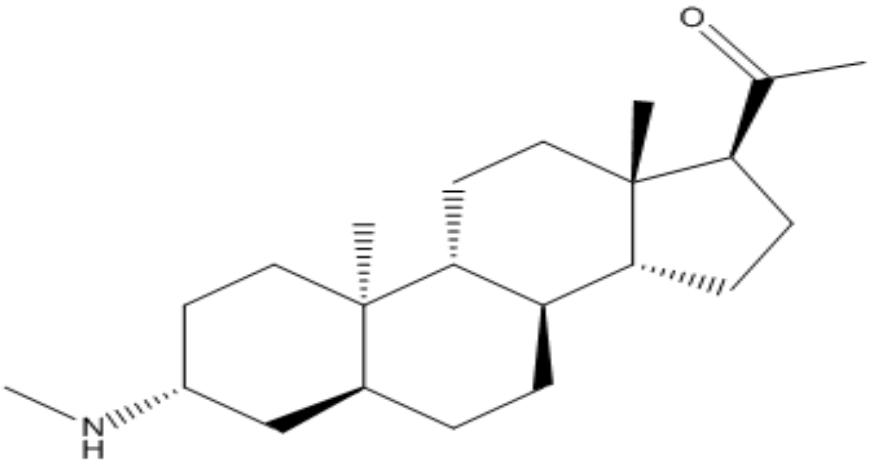

Figure 1: Structure of Alkaloid II

CONCLUSIONS/RECOMMENDATIONS

In conclusion, alkaloid ii and alkaloid iv from *sarcococca zeylanica* demonstrate promising potential as bioavailable and non-toxic antiviral agents against the dengue virus, though their relatively high binding energies necessitate further optimization. Recommendations for enhancing their efficacy include structural modifications to improve target interactions, such as altering functional groups or adjusting bond angles to increase binding affinity and reduce steric clashes. Combination therapies can be explored to leverage synergistic effects, potentially allowing for lower doses and reducing the risk of adverse effects. Dose optimization is crucial to balance therapeutic benefits with safety, ensuring effective antiviral activity while minimising toxicity. Additionally, considering alternative administration routes, such as intravenous or intramuscular injections, can bypass gastrointestinal issues and first-pass metabolism, improving systemic availability. Enteric-coated formulations could also protect these compounds from degradation in the stomach, enhancing absorption in the small intestine. Continued research is essential to refine these strategies, ensuring the safe and effective development of alkaloid II as antiviral agents, ultimately contributing to the fight against the dengue virus.

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