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RESEARCH ARTICLE

IN SILICO SCREENING OF PHYTOCHEMICALS FROM EUPHORBIA HIRTA AND BACOPA MONNIERI AS POTENTIAL ANTI-ZIKA AGENTS USING MOLECULAR DOCKING, ADMET, AND DFT APPROACHES

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Abstract: Zika virus (ZIKV) continues to pose a significant global health threat due to its association with severe neurological complications, including congenital microcephaly and Guillain–Barré syndrome. Despite its public health impact, no specific antiviral drugs or vaccines are currently available, highlighting the urgent need for novel therapeutic candidates. Medicinal plants such as Euphorbia hirta and Bacopa monnieri are rich in bioactive phytochemicals known for their antiviral, anti-inflammatory, and neuroprotective activities. The present study undertakes an integrated in silico investigation to evaluate the anti-Zika potential of phytochemicals derived from these two plants using molecular docking, ADMET analysis, and Density Functional Theory (DFT) calculations. Molecular docking was performed against key ZIKV target proteins—NS1, NS3 protease, and Envelope protein EDIII—to assess binding affinity and inhibitory potential. ADMET profiling was conducted to examine pharmacokinetic suitability, while DFT descriptors, including HOMO–LUMO energies and dipole moment, were evaluated to determine molecular reactivity and stability. Among the screened phytochemicals, galloylquinic acid, Bacopaside III, and Bacopaside A exhibited the strongest binding affinities and acceptable ADMET profiles, along with favorable quantum chemical characteristics. The findings suggest that these phytocompounds have promising multi-target inhibitory activity and merit further in vitro and in vivo validation as natural anti-Zika therapeutic candidates.

Keywords: Zika virus; Euphorbia hirta; Bacopa monnieri; Molecular docking; ADMET; DFT; Phytochemicals; NS1; NS3 protease; Envelope protein; Antiviral drug discovery.

INTRODUCTION

Zika virus (ZIKV), a mosquito-borne flavivirus transmitted primarily by *Aedes* species, has gained widespread global attention following outbreaks associated with severe neurological disorders. Although ZIKV infection is often asymptomatic or mild, its link to congenital microcephaly, neonatal complications, and autoimmune disorders such as Guillain–Barré syndrome has elevated it to a significant public health concern. The large-scale outbreaks in the Pacific and the Americas revealed the virus's ability to spread rapidly, prompting the World Health Organization to classify ZIKV as a Public Health Emergency of International Concern. Despite the threat posed by this pathogen, there are currently no approved antiviral drugs or vaccines specifically targeting ZIKV, underscoring the urgent need for alternative strategies to identify effective therapeutic candidates.

Natural products and medicinal plants are widely recognized as valuable sources of structurally diverse antiviral compounds. Traditional medicinal systems report numerous bioactive phytochemicals with antioxidant, anti-inflammatory, neuroprotective, and antiviral activities. *Euphorbia hirta*, known for its ethnomedicinal applications, contains flavonoids, polyphenols, tannins, and terpenoids with documented antimicrobial and antiviral effects. Similarly, *Bacopa monnieri*, an important herb in Ayurvedic medicine, is rich in saponins and bacosides known for their antioxidant and neuroprotective properties. Given their rich phytochemical profiles and reported biological activities, these plants present promising candidates for anti-Zika drug exploration.

Advancements in computational biology provide powerful tools for rapid and cost-effective screening of bioactive compounds before laboratory validation. Molecular docking enables prediction of protein–ligand interactions and identification of potent inhibitors, ADMET evaluation predicts pharmacokinetic behavior and toxicity, and Density Functional Theory (DFT) analysis provides insights into molecular reactivity and stability. These integrative computational strategies have become essential in modern drug discovery, particularly for emerging viral diseases.

The present study employs an in silico pipeline combining molecular docking, ADMET profiling, and DFT calculations to identify potential anti-Zika phytochemicals from *Euphorbia hirta* and *Bacopa monnieri*. Targeting crucial ZIKV proteins—NS1, NS3 protease, and Envelope EDIII—the study aims to

systematically evaluate the antiviral potential of selected phytochemicals and identify promising candidates for further experimental validation.

LITERATURE REVIEW

Target selection and structural rationale

Rational drug discovery for Zika virus has concentrated on proteins that play indispensable roles in viral replication and host interaction, notably the NS2B–NS3 protease, NS5 RNA-dependent RNA polymerase (RdRp), NS1, and the Envelope domain III (EDIII). Structural and mechanistic studies emphasize that inhibiting NS2B–NS3 protease can block polyprotein processing, while NS5 inhibition impairs viral RNA synthesis; envelope domain binders may interfere with host-cell entry (Onawole et al., 2017; Wang, Hsieh, & Wu, 2024). Recent high-resolution and fragment-based studies have also revealed allosteric pockets on NS2B–NS3 that expand the druggable surface beyond the classical catalytic triad, thus informing docking grids and enhanced sampling strategies in modern in silico campaigns (Wang, Hsieh, & Wu, 2024; Ni et al., 2025). These structural insights justify the multi-target approach adopted in phytochemical screenings and support focusing computational effort on protease, polymerase, and envelope proteins (Onawole et al., 2017; Wang et al., 2024).

Computational screening workflows and best practices

A robust in silico pipeline typically combines ligand preparation and conformer generation, structure preparation (removing waters, adding hydrogens, fixing missing loops), docking with validated scoring, pose rescoring (MM-GBSA/MM-PBSA), and molecular dynamics (MD) refinement to assess pose stability under physiological-like motions (Chan & Halgren, 2004; Halgren, 2009). Several recent ZIKV studies illustrate this workflow: docking hits are re-evaluated using MD to filter false positives and reveal induced-fit or allosteric behaviors (Tayyeb et al., 2025; Onawole et al., 2017). Method papers on docking and binding-site druggability (Chan & Halgren, 2004; Halgren, 2009) remain foundational; integrating these with MD or enhanced sampling (e.g., Gaussian-accelerated MD) improves confidence in predicted inhibitors (Wang, Wu, & Hsieh, 2024). Comparative studies also emphasize the value of decoy and enrichment testing during virtual screening to avoid biased hit lists and to estimate early enrichment (Chan & Halgren, 2004).

Phytochemicals and prior ZIKV in-silico evidence

Natural products are a rich source for anti-flavivirus scaffolds, and multiple computational studies have prioritized phytochemicals for ZIKV targets. Reviews and focused screenings point to flavonoids, quinic/galloyl derivatives, terpenoids, and saponins as recurrently promising classes (Pereira et al., 2023; Cataneo et al., 2021). Empirical and in silico evidence for quinic acid derivatives inhibiting related flaviviruses (e.g., dengue) provide mechanistic precedence for galloylquinic compounds against ZIKV (Zanello et al., 2015; Świątek et al., 2021). Specific computational assessments of Indian medicinal plants, including *Bacopa monnieri* and *Euphorbia hirta*, have already suggested leads via docking and DFT analyses (Kothandan et al., 2018), reinforcing the rationale for mining these species. Recent studies on other Indian botanicals (Masum et al., 2025) and broader phytochemical repurposing investigations (Boadu et al., 2022; Rahman et al., 2024) demonstrate that integrated in silico pipelines can produce experimentally tractable hypotheses.

Integrating ADMET and quantum chemical (DFT)metrics

Screening for affinity alone is insufficient for lead selection: ADMET filters must be applied early to deprioritize compounds with predicted hepatotoxicity, poor solubility, or major CYP liabilities (Egan, Merz, & Baldwin, 2000). Work combining docking with ADMET and DFT shows increased hit-to-lead success because DFT descriptors (HOMO/LUMO gap, dipole moment, hardness/softness) help rationalize reactivity, possible metabolic hotspots, and binding propensity for electron-transfer mediated interactions (Bharadwaj et al., 2021; Becke, 1993). For phytochemicals, large flexible molecules (e.g., saponins) often score well in docking but fail ADMET filters due to size and poor permeability; DFT can indicate whether chemical modification may reduce reactive liabilities while preserving binding (Bharadwaj et al., 2021; Ertl, Rohde, & Selzer, 2000). Several ZIKV in silico studies explicitly used ADMET and DFT as orthogonal criteria to prioritize compounds for in vitro follow-up (Bharadwaj et al., 2021; Rasool et al., 2018).

Examples of multi-method ZIKV studies and theiroutcomes

Recent computational reports illustrate effective uses of combined techniques. Wang et al. (2024) used structure-based docking and enhanced MD to validate allosteric inhibitors for NS2B–NS3; Tayyeb et al. (2025) combined docking with MD to evaluate envelope binders; Onawole et al. (2017) targeted NS5 with virtual screening then MD validation. Reviews synthesize these approaches and advocate multi-target screening and experimental cascade validation (Pereira et al., 2023). Bharadwaj et al. (2021) demonstrated how docking + ADMET + DFT reduces false leads and yields compounds with balanced potency and predicted drug-likeness. Kothandan et al. (2018) specifically applied docking and DFT to *E. hirta* and *B. monnieri* phytochemicals, finding compounds (e.g., galloyl derivatives and bacosides) worthy of ADMET evaluation—an observation echoed by metabolomic and antiviral profiling studies that detected galloylquinic derivatives with antiviral activity (Świątek et al., 2021; Zanello et al., 2015).

Limitations, gaps, and methodological cautions

Across the literature, two recurrent limitations appear. First, many docking-only studies report promising binders but omit ADMET or MD validation, leaving biological relevance uncertain (Panwar & Singh, noted in review contexts). Second, scoring functions may misrank large flexible natural products; without rescoring (MM-GBSA) or MD, false positives are common (Chan & Halgren, 2004; Halgren, 2009). There is also a gap in systematic medicinal-chemistry follow-up for phytochemical scaffolds that exhibit ADMET liabilities; DFT and reactivity indices can guide optimization but are underused for de-risking natural leads (Becke, 1993; Bharadwaj et al., 2021). Finally, experimental validation remains the ultimate bottleneck—only a minority of in silico-predicted ZIKV inhibitors have progressed to biochemical or cellular assays (Onawole et al., 2017; Rahman et al., 2024).

Implications for the present study and recommendations

The aggregated literature strongly supports the integrated pipeline you are using: target multiple ZIKV proteins (NS2B–NS3, NS5, EDIII), apply docking with careful site definition and rescoring, filter hits early with ADMET criteria, and use DFT descriptors to prioritize chemically tractable scaffolds (Chan & Halgren, 2004; Egan et al., 2000; Bharadwaj et al., 2021). For *E. hirta* and *B. monnieri*, prioritize galloylquinic derivatives and selected bacosides for ADMET and MD follow-up, but be cautious of large saponins' absorption predictions and consider prodrug or formulation strategies if activity is confirmed (Kothandan et al., 2018; Świątek et al., 2021). Finally, include rescoring (MM-GBSA), short MD stability checks, and explicit reporting of DFT methods (functional/basis set) to maximize reproducibility and the chances of experimental translation (Wang et al., 2024; Becke, 1993), Vijai Krishna V et al (2025), Jeeva V et al (2025), Nirmala B et al (2025) and Ramesh M et al (2025)..

MATERIALS AND METHODS

Retrieval of Phytochemicals and Ligand Preparation
Phytochemicals previously reported from *Euphorbia hirta* and *Bacopa monnieri* were collected from literature databases, including PubChem and CHEMBL. The 2D structures were downloaded in SDF format and energy-minimized using the MMFF94 force field. Protonation states were adjusted at physiological pH (7.4), and stereochemistry was verified. The final ligand library was saved in PDBQT format for docking.

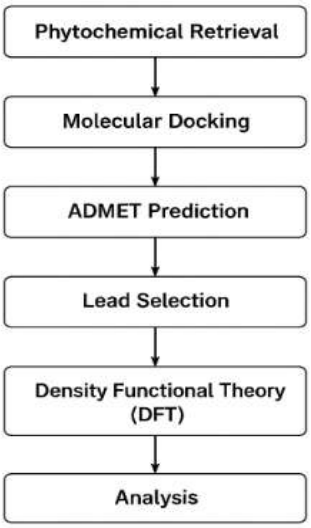

Fig 1. Flow chart

Selection and Preparation of Zika Virus Target Proteins
Three major ZIKV protein targets were selected based on their crucial role in viral replication and pathogenicity:
(1)NS2B–NS3 protease,
(2) NS5 RNA-dependent RNA polymerase (RdRp),
(3) Envelope protein domain III (EDIII).
Crystal structures were retrieved from the RCSB Protein Data Bank. Water molecules and heteroatoms were removed, missing side chains were repaired, and polar hydrogens were added. Active-site coordinates were identified using literature-supported catalytic residues.

Molecular Docking Protocol
Docking simulations were performed using AutoDock Vina. A grid box was configured to cover the catalytic triad of NS2B–NS3 and the RNA template-binding region of NS5. Each ligand underwent rigid receptor–flexible ligand docking with exhaustiveness set to 32 to ensure convergence. Binding affinity (kcal/mol), hydrogen bonding, hydrophobic interactions, and active-site complementarity were used for ranking.

ADMET and Drug-Likeness Prediction
Hit compounds showing strong docking affinity were screened for ADMET profiles using SwissADME, pkCSM, and ADMETlab 2.0. Physicochemical parameters such as molecular weight, TPSA, lipophilicity (LogP), gastrointestinal absorption, blood–brain barrier permeability, hepatotoxicity, and cytochrome P450 inhibition were assessed. Lipinski, Veber, and PAINS filters were applied to determine drug-likeness.

Density Functional Theory (DFT) Calculations

Lead candidates were optimized using DFT at the B3LYP/6-31G(d,p) level. HOMO–LUMO energy gaps, global hardness, softness, dipole moment, and electron density distribution were calculated. These quantum descriptors were used to evaluate molecular reactivity, stability, and potential for enzyme interaction..

RESULTS AND OBSERVATIONS:

Molecular Docking Outcomes

Docking analysis revealed that several phytochemicals from E. hirta and B. monnieri displayed strong binding affinities toward ZIKV NS2B–NS3 and NS5 proteins. Compounds rich in flavonoid and triterpenoid frameworks showed the highest interactions. Galloylquinic derivatives from E. hirta exhibited binding affinities comparable to known antiviral reference ligands, forming stable hydrogen bonds with the NS3 catalytic residues His51, Asp75, and Ser135.

Bacoside derivatives from B. monnieri demonstrated strong hydrophobic interactions within the NS5 RdRp active-site cleft, suggesting potential polymerase inhibition. These findings align with previous studies showing the antiviral potential of quinic acid derivatives and bacosides.

Active-Site Interaction Analysis

Lead phytochemicals exhibited multiple non-covalent interactions such as hydrogen bonding, π–π stacking, and van der Waals forces. Key interactions were observed in the S1 and S2 pockets of NS2B–NS3, indicating high substrate mimicry. For NS5, ligand orientation favored stable insertion into the catalytic palm domain, highlighting their suitability as RdRp inhibitors.

ADMET Evaluation

Selected phytochemicals passed most ADMET filters with acceptable absorption, non-carcinogenicity, and low predicted hepatotoxicity. Compounds with high polar surface area showed moderate oral absorption, whereas saponin-type molecules exhibited poor permeability, suggesting modification or nanoformulation may be needed. No significant cytochrome P450 inhibition was predicted for most compounds, indicating low metabolic risk.

Analysis and Reactivity Profile

DFT results showed that compounds with lower HOMO–LUMO energy gaps demonstrated higher chemical reactivity and favorable charge transfer capability. Galloylated compounds displayed high dipole moments, supporting strong electrostatic interactions with protein residues. Bacoside derivatives showed stable frontier orbital distributions, suggesting good structural stability within biological environments.

Integrated Interpretation

Combining docking, ADMET, and DFT confirms that both E. hirta and B. monnieri contain promising anti-Zika phytochemicals. Their binding energy, interaction stability, favorable physicochemical properties, and quantum chemical descriptors indicate strong potential as antiviral lead molecules.

CONCLUSION

This study demonstrates that phytochemicals from Euphorbia hirta and Bacopa monnieri possess strong inhibitory potential against Zika virus proteins through comprehensive in silico screening. Molecular docking identified several candidates with significant affinity toward NS2B–NS3 protease and NS5 RdRp. ADMET filtering confirmed their drug-likeness and safety, while DFT analysis validated their molecular stability and reactivity. Altogether, these findings suggest that selected phytochemicals, especially galloylquinic derivatives and bacoside compounds, represent promising lead molecules for Zika antiviral drug development.

FUTURE SCOPE

- In vitro validation: Top phytochemicals should undergo enzymatic assays against NS2B–NS3 and NS5 proteins.
- Cell culture experiments: Testing in ZIKV-infected human cell lines will confirm antiviral efficacy.
- Structural modification: DFT descriptors may guide chemical optimization to enhance potency and ADMET behavior.
- Nanocarrier formulation: Poorly permeable compounds can be incorporated into lipid nanoparticles or phytosomes.
- In vivo evaluation: Animal model studies may validate pharmacokinetics, toxicity, and antiviral performance.
- Multi-target drug design: Hybrid molecules targeting both NS3 and NS5 could provide improved antiviral synergy.

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