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Original article

Flavonoids and Alkaloids from *Murraya paniculata* Exhibit Potential as DDI1 Inhibitors in *Plasmodium falciparum*: A Molecular Docking Analysis

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Abstract

The emergence of drug-resistant *Plasmodium falciparum* strains has created an urgent need for new malarial therapeutic targets. The DNA Damage-Inducible Protein 1 (DDI1), which plays a critical role in protein degradation and parasite survival, has been identified as a promising molecular target for antimalarial drug development. *Murraya paniculata*, a plant rich in alkaloids, flavonoids, and tannins, has shown antimicrobial and antiparasitic properties, but its interaction with DDI1 remains unexplored. This study aimed to evaluate the binding potential, pharmacokinetics, and toxicity of *M. paniculata* phytoconstituents as DDI1 inhibitors using an integrated in silico approach. Molecular docking was performed using Molegro Virtual Docker to assess binding affinity and interaction profiles. Furthermore, QSAR-based pharmacokinetics and toxicity predictions were conducted using SwissADME and ProTox-II, respectively. The results showed that alkaloid compunds exhibited the strongest binding affinity (-7.2 kcal/mol), followed by flavonoids (-6,9 kcal/mol) and tannins (-4,3 kcal/mol). Docking stability analysis showed that all DDI-1 complexes exhibited fluctuations, with tannin having the widest range (0.128–5.394 Å), followed by flavonoid (0.264–4.331 Å), and alkaloid showing relatively moderate fluctuations (0.161–4.03 Å). Key interactions included hydrogen bonding and hydrophobic interactions within the active site of DDI1. Pharmacokinetics analysis indicated favorable gastrointestinal absorption and compliance with Lipinski's rule of five, while toxicity predictions indicated low acute toxicity (Class IV, LD50: 1190 mg/kg) with potential hepatotoxicity. In conclusion, alkaloids and flavonoids from *M. paniculata* have promising potential as DDI1 (PDB ID: 2i1a) inhibitors, supporting their development as lead structures for novel antimalarial agents.

Keywords: ADMET, Alkaloid, Malaria, Molecular docking, *Murraya paniculata*

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Introduction

Malaria remains a significant global health threat, with an estimated 263 million cases and 597,000 deaths reported in 2023 (Venkatesan, 2025). Despite numerous preventive and treatment interventions, including vaccines and insecticidal bed nets, the malaria burden remains high in several countries, including Indonesia which accounts for the majority of cases and deaths (Lindsay et al. 2021). Resistance to key drugs such as chloroquines and artemisinins have increased, contributing to the stagnation or increase in the global malaria burden (Okello & Aucamp, 2026). In this context, the development of new antimalarial therapies with alternative molecular targets is urgent (Belete 2020).

One such target of interest is DNA Damage-Inducible Protein 1 (DDI1), an essential protein in *Plasmodium falciparum* (*P. falciparum*) that plays a key role in the ubiquitin-based protein degradation pathway and parasite survival. (Kumar et al. 2022). DDI1 possesses a unique retroviral protease-like (RVP) domain, making it a potential target for antimalarial drug discovery (Kumar and Suguna 2018). The crystal structure of DDI1 is available in the Protein Data Bank under PDB ID: 2i1a, enabling structure-based analysis for rational drug design. Inhibition of DDI1 is believed to disrupt the degradation process of proteins essential for the parasite's life cycle, ultimately inhibiting the growth and replication of *P. falciparum* in the host (Oduro-Kwateng et al. 2025). To validate whether DDI1 is a functionally relevant target for the identified lead compoundss, a chemical-protein interaction analysis was performed using the STITCH 5.0 database. This analysis integrates evidence from experimental data, curated databases, and molecular pathways to confirm the bioactivity potential of the leads against the target protein.

Murraya paniculata, also known as orange jasmine, is a traditional medicinal plant that has long been used to treat various health problems, including fever, inflammation, and infection (Joshi and Gohil 2023). Numerous phytochemical studies have shown that *M. paniculata* contains a variety of bioactive compoundss, such as alkaloids, flavonoids, and tannins, which possess antimicrobial, anti-inflammatory, and antiparasitic activities (Sonter et al. 2021). Several compounds from this plant have been reported to inhibit the growth of malaria-causing parasites through various mechanisms, however no comprehensive study has yet examined their specific interactions with the DDI1 protein (PDB ID: 2i1a) (Tanneru et al., 2023). The

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diverse chemical composition of this plant offers significant opportunities for discovering effective natural inhibitors for this target (Najmi et al. 2022).

In silico approaches, particularly molecular docking and dynamic analysis, are efficient and cost-effective methods for predicting the interaction of ligand molecules with protein targets (Babalola et al., 2025). This analysis can provide an initial overview of the binding affinity, type of interaction, and stability of the ligand-protein complex, thus screening compounds with high potential as drug candidates. Furthermore, the integration of Pharmacokinetics analysis and toxicity prediction can help evaluate the feasibility of compounds for further development (Han et al. 2024). By utilizing the crystal structure data of DDI1 (PDB ID: 2i1a) and bioactive compounds from *M. paniculata*, this study aims to identify promising inhibitor candidates for the development of new antimalarial drugs (Saini 2021).

Methods

Ligand Structure Preparation

Information on the IUPAC names of alkaloid (compound CID: 73073), flavonoid (compounds CID: 44258292), and tannin (compounds CID: 996) compounds reported in *M. paniculata* was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The 2D structures of the ligands were drawn using ChemBioDraw Ultra 16 and converted into 3D models with ChemBio3D Ultra 16. Energy minimization was then performed using the Merck Molecular Force Field 94 (MMFF94) method to obtain stable structural conformations prior to docking (Bora & Crisan, 2024).

Protein Structure Preparation and Target Validation Analysis

The target protein used in this study was *P. falciparum* DDI1 with the Protein Data Bank code PDB ID: 2i1a. The crystal structure of the protein was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org) in *.pdb* format (Figure 1).

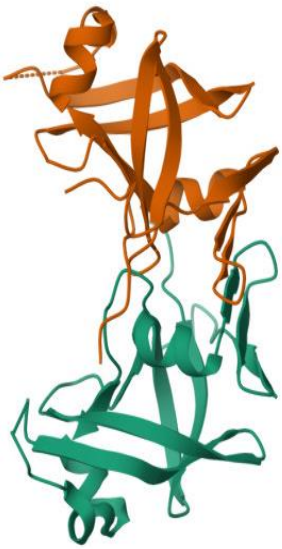


Fig. 1. Crystal structure of DDI1 protein

Before docking, water molecules around the protein structure were removed, and polar hydrogen atoms were added to optimize the protein conformation. To validate whether DDI1 is a functionally relevant target for the identified lead compounds, a chemical-protein interaction analysis was performed using the STITCH 5.0 database (http://stitch.embl.de/). The SMILES (Simplified Molecular Input Line Entry System) strings of the representative alkaloid, flavonoid, and tannin from *M. paniculata* were used as queries. The search was specifically conducted against the *P. falciparum* proteome to identify potential interaction networks. The confidence score (combined score) was used to assess the strength of the interaction, where a higher score indicates a stronger probability that the protein is a true biological target for the compounds (Szklarczyk et al., 2016).

Virtual Screening

The biological activity of the natural compounds, including anticarcinogenic and apoptosis agonist properties, was evaluated based on the probability of activity (Pa) and probability of inactivity (Pi) values obtained from the Prediction of Activity Spectra for Substances (PASS) Online web server (http://way2drug.com/passonline/index.php). This analysis relies on the relationship between the chemical structure of a compounds and its potential biological activity. The Pa value represents the likelihood that a compound exhibits a specific biological activity and belongs to the class of active compunds, whereas the Pi value indicates the probability that the compounds is inactive and does not demonstrate the predicted activity (Mahayasih et al., 2026).

Prediction of Compounds Toxicity

Toxicity prediction was performed using the ProTox-II web server (https://tox-new.charite.de/protox_II/). The canonical SMILES codes of each ligand were input into the system to obtain predicted LD50 values, toxicity classes, and potential mutagenic, carcinogenic, hepatotoxic, and cytotoxic effects (Arulanandam et al., 2022).

Pharmacokinetics Analysis

Pharmacokinetics properties were analyzed using the SwissADME platform (http://www.swissadme.ch/). The SMILES codes of the compounds were entered into the SwissADME input field, and the system was run to generate drug-likeness parameters according to Lipinski's rule of five, gastrointestinal absorption, solubility, and bioavailability scores (Alqaaf et al., 2024).

Molecular Docking

Molecular docking was performed by loading the prepared ligands and the DDI1 protein structure (PDB ID: 2i1a) into Molegro Virtual Docker (MVD) version 6.0. The process began with cavity detection to identify potential ligand-binding sites. Docking method validation was carried out by re-docking the native ligand to ensure an RMSD value below 2 Å. Subsequently, docking simulations between the test compounds and the target protein were conducted to determine binding affinities and the types of molecular interactions formed (Tsaqif et al., 2025).

To evaluate the stability and residue-level flexibility of the protein-ligand complexes, dynamic simulations were performed using the CABS-flex 3.0 server (https://lcbio.pl/cabsflex3/). CABS-flex 3.0 employs a well-established coarse-grained protein modeling tool for fast yet efficient simulations of protein structure flexibility. This approach provides a high-resolution consensus of protein dynamics that is comparable to all-atom molecular dynamics (MD) simulations.

The docked complexes of DDI1-ligands were used as input in PDB format. The simulation time was adjusted to represent a 10 ns MD trajectory, while other parameters, including the number of cycles and temperature, were kept at default settings. The primary output analyzed was the Root-Mean-Square Fluctuation (RMSF), which describes the local flexibility of each amino acid residue throughout the simulation. High RMSF values indicate flexible regions (loops), whereas lower values signify stable regions, such as the binding pocket, confirming the structural integrity of the complex upon ligand binding (Hossain et al., 2024).

Results

PASS Prediction

The PASS (Prediction of Activity Spectra for Substances) analysis reveals a synergistic multi-target antimalarial mechanism among the phytoconstituents of *M. paniculate* as shown in Table 1. Alkaloids primarily function as inhibitors of the parasite's genetic and structural machinery, as evidenced by their predicted activity against DNA polymerase I (Pa 0.279), RNA synthesis (Pa 0.222), and microtubule formation (Pa 0.255), which correlates with their high structural stability in binding to the DDI1 protein. Flavonoids exhibit a dual role, acting as potent cytoprotective agents and antioxidants; they show an exceptional probability as membrane integrity agonists (Pa 0.960) and free radical scavengers (Pa 0.889), while also contributing to mitochondrial disruption through ubiquinol-cytochrome-c reductase inhibition (Pa 0.856). Furthermore, Tannins emerge as the most potent metabolic disruptors, displaying high probability scores for inhibiting ubiquinol-cytochrome-c reductase (Pa 0.924), methylenetetrahydrofolate reductase (Pa 0.893), and the thioredoxin system (Pa 0.879). Collectively, these findings suggest that while alkaloids target protein degradation and replication, flavonoids protect host cells and tannins cripple the parasite's energy production, forming a comprehensive biochemical attack against Plasmodium falciparum (Chaniad et al., 2023).

Toxicity Prediction

The toxicity profiles of the selected *M. paniculata* phytochemicals were predicted using the ProTox-II server (Agrawal et al. 2024), and the results are summarized in Table 2. All three compounds—alkaloid, flavonoid, and tannin—were categorized under Toxicity Class IV, with an estimated LD50 value of 1190 mg/kg, which indicates low acute toxicity (Brígido et al. 2021).

Despite being predicted as hepatotoxic (active), none of the tested compounds exhibited carcinogenicity, mutagenicity, or cytotoxicity activities (all predicted as inactive) (Benfenati et al. 2009). These findings suggest that the compounds have a relatively safe toxicity profile, with minimal risk of genotoxic or carcinogenic effects (Nohmi 2018). However, the hepatotoxic potential indicates the need for cautious evaluation in further in vivo and clinical studies.

Pharmacokinetics Analysis

The Pharmacokinetic analysis of the selected compounds from *M. paniculata* was carried out using the

Table 1. PASS data of Alkaloid, Flavonoid, and Tannin. Pa > 0.7 are considered to have high predicted activity, 0.3 < Pa < 0.7 moderate activity, and Pa ≤ 0.3 insignificant activity

Target system	Pa	Pi
Alkaloid		
DNA polymerase I inhibitor	0.279	0.091
RNA synthesis inhibitor	0.222	0.138
Transactivator transcription protein inhibitor	0.223	0.118
Microtubule formation inhibitor	0.255	0.024
Flavonoid		
Membrane integrity agonist	0.960	0.003
Free radical scavenger	0.889	0.002
Ubiquinol-cytochrome-c reductase inhibitor	0.856	0.015
RNA directed DNA polymerase inhibitor	0.282	0.036
Proteasome endopeptidase complex inhibitor	0.252	0.037
Tannin		
Ubiquinol-cytochrome-c reductase inhibitor	0.924	0,004
Thioredoxin inhibitor	0.879	0,002
Methylenetetrahydrofolate reductase (NADPH) inhibitor	0.893	0,007
Fatty-acyl-CoA synthase inhibitor	0.869	0.003
Membrane permeability inhibitor	0.832	0.006

Table 2. Toxicity analysis summary

Compounds	LD50 (mg/kg)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
Alkaloid	1190	Class IV	Active	Inactive	Inactive	Inactive
Flavonoid	1190	Class IV	Active	Inactive	Inactive	Inactive
Tannin	1190	Class IV	Active	Inactive	Inactive	Inactive

SwissADME web tool (Table 3). The alkaloid compounds (CID: 73073) demonstrated favorable drug-likeness properties, with a molecular weight of 348.40 g/mol, no hydrogen bond donors, four hydrogen bond acceptors, and two rotatable bonds. Its lipophilicity (Log P = 3.32) and water solubility (Log S = –3.86) indicated moderate solubility, while a high gastrointestinal (GI) absorption and no Lipinski's rule violations suggest good oral bioavailability (score = 0.85) (Ahmad et al. 2025) (Figure 2).

The flavonoid compounds (CID: 44258292) had a higher molecular weight of 436.50 g/mol, three hydrogen bond donors, six hydrogen bond acceptors, and six rotatable bonds. It exhibited strong lipophilicity (Log P = 4.08) but relatively low solubility (Log S = –6.22). Despite these characteristics, the compounds showed high GI absorption and no violations of Lipinski's rule, with a bioavailability score of 0.55, supporting its potential as an orally active molecule (Liu et al. 2022) (Figure 3).

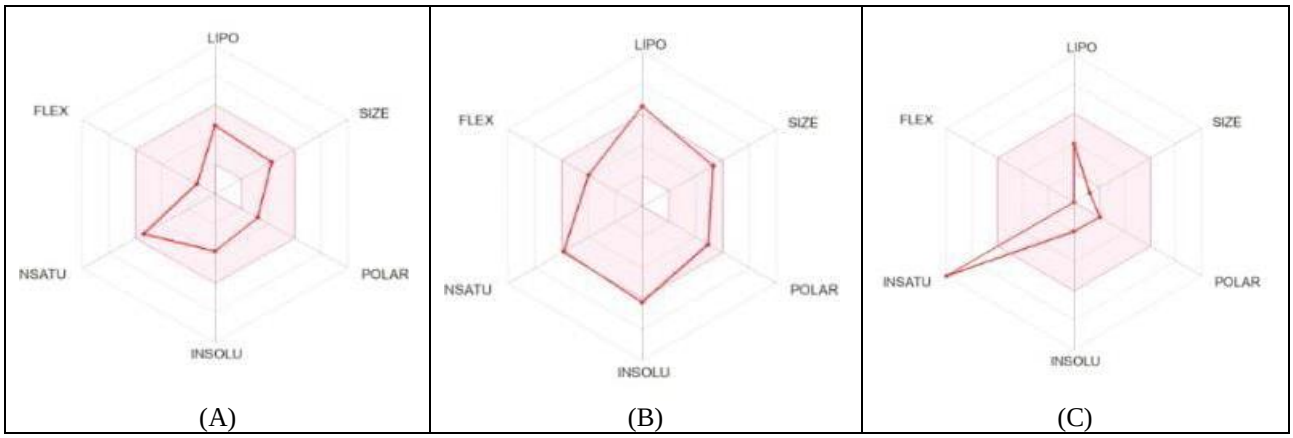


Fig. 2. Bioavaibility radar of alkaloid (A), flavonoid (B), and Tannin (C)

Table 3. Pharmacokinetics properties of alkaloid, flavonoid and tannin

Parameter	Alkaloid	Flavonoid	Tannin
Molecular weight (g/mol)	348.40 g/mol	436.50 g/mol	94.11 g/mol
Hydrogen bond donors	0	3	1
Hydrogen bond acceptors	4	6	1
Rotatable bonds	2	6	0
Total polar surface area	53.35 Å²	100.13 Å²	20.23 Å²
Log P (iLOGP)	3.32	4.08	1.24
Log S (ESOL)	-3.86	-6.22	-1.98
GI Absorption	High	High	High
Lipinski's (Ro5)	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation
Bioavailability score	0.85	0.55	0.55

The tannin compounds (CID: 996), on the other hand, presented a much smaller molecular weight of 94.11 g/mol, with one hydrogen bond donor and one hydrogen bond acceptor. The compounds had no rotatable bonds and a polar surface area of 20.23 Å², suggesting favorable permeability. Its Log P (1.24) and Log S (–1.98) values indicated good solubility and acceptable lipophilicity. Moreover, tannin showed high GI absorption, no Lipinski's rule violations, and a bioavailability score of 0.55, highlighting its potential for oral administration (Figure 3).

Collectively, these results suggest that the alkaloid and flavonoid compounds demonstrate stronger drug-likeness and binding potential, while tannin exhibits excellent solubility and permeability but comparatively weaker binding affinity (Didigwu and Nnadi 2024). Therefore, alkaloids and flavonoids may serve as promising lead candidates for the development of *M. paniculata*-derived DDI1 inhibitors in malaria drug discovery (Ali-Seyed and Vijayaraghavan 2020).

Molecular Docking

To validate the docking protocol, the native ligand of the target protein DDI1 (*P. falciparum*, PDB ID: 2i1a) was re-docked into its binding site (PORTAKAL 2023). Validation was assessed using the Root Mean Square Deviation (RMSD) value (Bagaria et al. 2012), which measures the positional deviation of the ligand in the crystal structure before and after docking (Bagaria et al. 2012). An RMSD value below 2 Å indicates that the docking method can reliably reproduce the ligand's binding pose within the protein's active site. In this study (Ramírez and Caballero 2018), the RMSD values obtained for all compounds were 0.000 Å, confirming the high accuracy and reliability of the docking procedure (Ramírez and Caballero 2018).

The docking simulations of selected *M. paniculata* phytoconstituents revealed varying binding affinities against the DDI1 protein (Saini 2021). The alkaloid compounds (CID: 73073) exhibited the strongest binding affinity at -7.2 kcal/mol (Bagaria et al. 2012), followed by the flavonoid compounds (CID: 44258292) at -6.9 kcal/mol. The tannin compounds (CID: 996) showed the weakest binding interaction, with a binding affinity of -4.2

kcal/mol (Table 5). These findings suggest that alkaloid and flavonoid classes have greater potential as DDI1 inhibitors compared to tannins, possibly due to more favorable hydrogen bonding, hydrophobic interactions, and steric complementarity within the active site.

Table 4. Molecular docking results of M. paniculata compounds against DDI1

Protein (PDB ID)	Ligand	RMSD (Å)	Rerank Score (kcal/mol)
DDI1 (2i1a)	Alkaloid	0.000	-7.2
DDI1 (2i1a)	Flavonoid	0.000	-6.9
DDI1 (2i1a)	Tannin	0.000	-4.2

The docking simulations of *M. paniculata* phytochemicals against DDI1 (PDB ID: 2i1a) revealed distinct binding affinities and specific amino acid interactions (Table 5). Among the tested compounds, the alkaloid demonstrated the strongest binding affinity (-7.2 kcal/mol) and formed stable interactions with several residues in the active site. Notably, hydrogen bonding was observed with Tyr 205 and Lys 325, while additional stabilization was provided by van der Waals interactions (Asp 284, Val 215, Val 287) and hydrophobic contacts such as alkyl Pi-alkyl (Pro 324, Lys 216, Ile 285). A Pi-anion interaction with Asp 286 further contributed to its binding stability (Figure 3a).

The flavonoid compounds exhibited a binding affinity of -6.9 kcal/mol, forming hydrogen bonds with Asp 243 and Arg 261, as well as conventional hydrogen bonding with Ser 277. It also displayed van der Waals interactions with residues Asn 211, Gln 266, His 263, Asp 292, Arg 296, and Thr 199, in addition to hydrophobic alkyl Pi-alkyl interactions with Tyr 273, Arg 245, and Pro 275 (Figure 3b).

The tannin compounds showed the weakest interaction with DDI1, with a binding affinity of -4.2 kcal/mol. Its interactions included hydrogen bonds with Phe 218, Lys 216, Ala 217, Asp 284, and Val 287. Additional interactions were characterized by Pi-Pi T-shaped bonding with Tyr 205 and Pi-alkyl interactions with Pro 324 and Ile 285. Interestingly, an unfavorable donor-donor interaction was also noted with Asp 286, which may explain its relatively lower binding affinity compared to alkaloid and flavonoid compounds (Figure 3c).

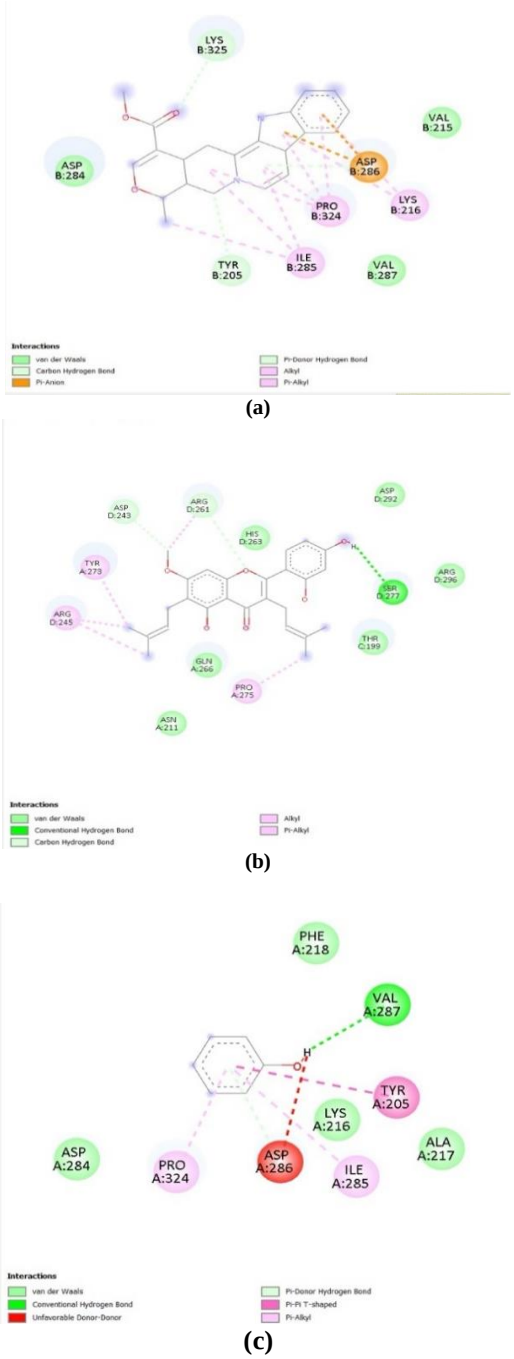


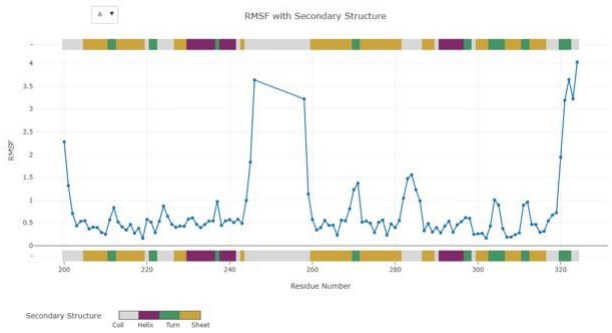
Fig. 3. Visualization of the interaction between DDI1 (2i1a) and alkaloid (a), flavonoid (b), and tannin (c)

Table 5. Molecular docking results and amino acid interactions

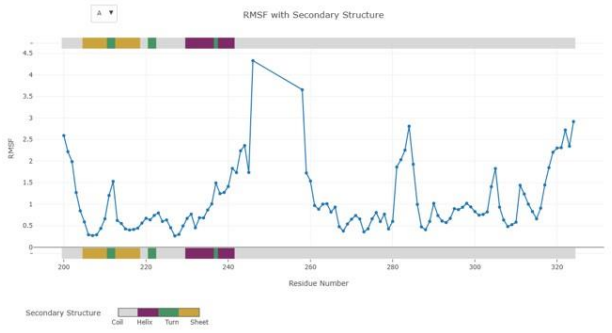
Compound	Binding Affinity	Protein Target	Residual Amino Acid Interactions
Alkaloid	-7.2 kcal/mol	DDI1	Hydrogen bonds: Tyr 205, Lys 325
			Van der Waals: Asp 284, Val 215, Val 287
			Alkyl Pi-Alkyl: Pro 324, Lys 216, Ile 285
			Pi-Anion: Asp 286
Flavonoid	-6.9 kcal/mol	DDI1	Hydrogen bonds: Asp 243, Arg 261
			Van der Waals: Asn 211, Gln 266, His 263, Asp 292, Arg 296, Thr 199
			Conventional hydrogen bonds: Ser 277
			Alkyl Pi-Alkyl: Tyr 273, Arg 245, Pro 275
Tannin	-4.2 kcal/mol	DDI1	Hydrogen bonds: Phe 218, Lys 216, Ala 217, Asp 284, Val 287
			Pi-Pi T shaped: Tyr 205
			Pi-Alkyl: Pro 324, Ile 285
			Unfavorable Donor-Donor: Asp 286

Molecular Dynamics Simulations

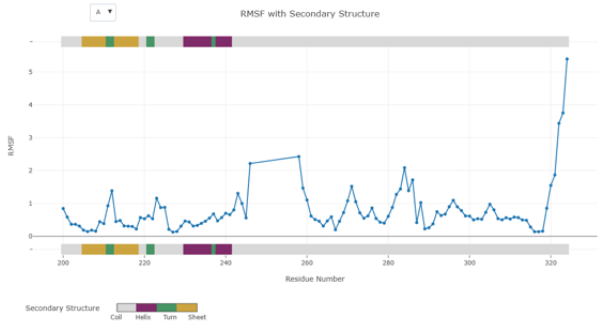
To estimate the stability of the DDI1-ligand complexes, MD simulations were performed using the CABS-flex 3.0 server. These simulations assess ligand-induced alterations in the protein structure and evaluate the persistence of the binding. The RMSF profiles of the DDI1 protein in complex with the lead compounds from *M. paniculata* show the flexibility of the amino acid residues throughout the 10 ns simulation trajectory (Figure 4). A higher RMSF value indicates greater local flexibility, typically in loop regions, while lower values suggest limited motion and high stability, often observed in the binding pocket or core secondary structures.



(a)



(b)



(c)

Fig. 4. RMSF profiles of DDI1 protein complexes with (A) Alkaloid; (B) Flavonoid; and (C) Tannin obtained from CABS-flex 3.0 simulation

After submitting the docked protein-ligand structures to CABS-flex 3.0, the server generated root-mean-square fluctuation (RMSF) profiles to calculate the per-residue fluctuations (Figure 3).

The DDI1-Alkaloid Complex showed a maximum fluctuation of 4.03 Å at the C-terminal region (residue no. 324:PRO Coil) and a minimum fluctuation of 0.161 Å at residue no. 219:VAL Sheet.

While, for the DDI1-Flavonoid complex, the maximum fluctuation was observed at 4.331 Å at residue no. 246:PHE Coi l, andthe minimum fluctuation was 0.264 Å at residue no. ILE Coil. The stability of the flavonoid within the pocket is supported by the minimal fluctuations recorded in the core catalytic domain of DDI1.

Furthermore, the DDI1-Tannin complex demonstrated a maximum fluctuation of 5.394 Å at residue no. 324:PRO Coil, whereas the minimum fluctuation occurred at residue no. 227:ILE Coil with a value of 0.128 Å.

Overall, all three complexes shared a similar fluctuation pattern, with the highest peaks localized at the flexible terminal loops and the lowest fluctuations concentrated in the regions where the ligands were docked. The consistently low RMSF values (well below 2.0 Å) in the internal residues and the binding site confirm that the DDI1 protein maintains its structural integrity and remains stable when bound to the bioactive compounds from *M. paniculata*.

Discussion

This study investigated the antimalarial potential of bioactive compounds from *M. paniculata*, specifically alkaloids, flavonoids, and tannins, using PASS prediction, toxicity prediction, pharmacokinetics analysis and molecular docking.

The PASS prediction results elucidate a sophisticated multi-targeted inhibitory mechanism where the alkaloid, flavonoid, and tannin constituents from *M. paniculata* act in synergy to compromise *P. falciparum* viability. The primary inhibitory mechanism of alkaloids involves a direct assault on the parasite's genetic and structural integrity; by inhibiting DNA polymerase I and RNA synthesis, these compounds arrest the replication and transcription processes, while the inhibition of microtubule formation effectively prevents mitotic division. Parallel to this, both tannins and flavonoids exert a potent attack on the parasite's bioenergetics by targeting ubiquinol-cytochrome-c reductase (Complex III), a validated antimalarial mechanism—similar to the drug atovaquone—that collapses the mitochondrial membrane potential and halts essential pyrimidine biosynthesis. Tannins further escalate metabolic failure by inhibiting methylenetetrahydrofolate reductase, thereby crippling the folate pathway necessary for nucleic acid synthesis, and blocking the thioredoxin system to induce lethal oxidative stress within the parasite. Interestingly, flavonoids offer a dual-action approach; while acting as membrane integrity agonists to potentially protect host erythrocytes, they also inhibit the proteasome endopeptidase complex. This specific proteasomal inhibition strongly complements the molecular docking findings regarding DDI1, as DDI1 is a critical shuttle protein in the ubiquitin-proteasome degradation pathway. Collectively, these results suggest that the antimalarial efficacy of *M. paniculata* arises from a coordinated biochemical assault that simultaneously cripples the

parasite's energy production, genetic replication, and protein homeostasis (Dwivedi et al., 2021).

The toxicity prediction revealed that all three compounds belong to Toxicity Class IV, with an LD50 value of 1190 mg/kg (Kyhoiesh et al. 2021). Notably, while hepatotoxicity was predicted to be active for all compounds, they were classified as inactive for carcinogenicity, mutagenicity, and cytotoxicity (Greene et al. 2010). This profile suggests a relatively safe margin for their development as therapeutic candidates, although hepatotoxicity remains a concern and requires further in vivo validation (Farghali et al. 2016).

Pharmacokinetics profiling further supported the drug-likeness of these compounds (Alam et al. 2016). Alkaloids displayed favorable properties, including high GI absorption, no violations of Lipinski's Rule of Five, and a relatively high bioavailability score (0.85), making them strong candidates for oral drug development (Peng et al. 2019). Flavonoids and tannins, while also showing high GI absorption and no Lipinski violations, had lower bioavailability scores (0.55), indicating limitations in systemic exposure. The radar plots for these compounds revealed suboptimal solubility and polarity profiles, which may hinder their absorption and distribution, especially for tannins with higher polarity.

The docking results revealed that all compounds demonstrated favorable binding affinities with the DDI1 protein (PDB ID: 2i1a), with alkaloids exhibiting the strongest interaction (-7.2 kcal/mol), followed by flavonoids (-6.9 kcal/mol), and tannins (-4.2 kcal/mol). These values suggest that alkaloids and flavonoids in particular could serve as potential inhibitors of DDI1 (Nuryady et al. 2024), a protease that plays an essential role in the survival of *P. falciparum* (Sulyman et al. 2023).

The docking interactions depicted that alkaloids formed multiple hydrogen bonds (Tyr 205, Lys 325), van der Waals interactions (Asp 284, Val 215, Val 287), and hydrophobic contacts such as Alkyl Pi-Alkyl and Pi-Anion interactions (Costa et al. 2015). These interactions contribute to the strong anchoring of the ligand within the binding pocket, enhancing stability and increasing the likelihood of effective enzyme inhibition (Varma et al. 2010). Similarly, flavonoids formed hydrogen bonds (Asp 243, Arg 261, Ser 277), van der Waals interactions (Asn 211, Gln 266, His 263, Asp 292, Arg 296, Thr 199), and multiple Alkyl Pi-Alkyl interactions, supporting their role as stabilizers within the hydrophobic core of the active site (Varma et al. 2010). In contrast, tannins demonstrated relatively weaker interactions, forming hydrogen bonds (Phe 218, Lys 216, Ala 217, Asp 284, Val 287), π-π interactions (Tyr 205), and unfavorable donor-donor interactions (Asp 286), which may account for their lower binding affinity compared to alkaloids and flavonoids.

These findings highlight that both alkaloids and flavonoids have the capacity to inhibit DDI1 by mimicking the interactions of native ligands, potentially disrupting proteolytic processes that are critical for parasite protein degradation and survival (Zhou et al. 2021). Tannins, although weaker in binding, may still contribute synergistically within a phytochemical mixture, but are less promising as single therapeutic agents (Ananda et al. 2024).

Molecular dynamics (MD) simulations via the CABS-flex 3.0 server provided critical insights into the structural stability of DDI1-ligand complexes within a dynamic environment. The Root-Mean-Square Fluctuation (RMSF) analysis revealed that while higher fluctuations were localized in the flexible coil and terminal regions, the core structural integrity of the protein remained robust, with fluctuations in the binding site staying consistently below 2.0 Å. The exceptional stability observed at internal residues, particularly the minimum fluctuations recorded for the alkaloid (0.161 Å) and tannin (0.128 Å) complexes, suggests that these phytoconstituents effectively restrict conformational mobility within the DDI1 catalytic domain. These findings demonstrate that bioactive compounds from *M. paniculata* possess not only high binding affinity but also the capacity to form persistent and stable complexes, a vital attribute for potential *P. falciparum* protease inhibitors aimed at disrupting parasitic protein degradation pathways.

Taken together, these results suggest that alkaloids from *M. paniculata* hold the strongest potential as lead compounds for antimalarial drug development targeting DDI1, followed by flavonoids, with tannins showing limited promise due to weaker binding and lower bioavailability. The hepatotoxicity signals observed highlight the need for structural optimization or formulation strategies to reduce toxicity while preserving efficacy. Future studies should focus on in vivo validation, analog development to improve Pharmacokineticss, and synergistic evaluations of combined phytochemicals to enhance antimalarial potency while minimizing toxicity.

Conclusion

Alkaloids and flavonoids from *M. paniculata* show promise as potential DDI1 inhibitors for antimalarial drug development, with binding affinities of -7.2 and -6.9 kcal/mol, respectively. Both compounds demonstrated favorable Pharmacokinetics profiles, although hepatotoxicity remains a concern. Tannins, with a weaker binding affinity (-4.2 kcal/mol) and limited bioavailability, appear less suitable as candidates. Further optimization and experimental validation are needed to improve efficacy and safety before clinical application.

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