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An Integrated Network Pharmacology and Molecular Modelling Study of Phytoconstituents Targeting Malaria

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ABSTRACT

Background: Malaria remains a major global health concern, requiring novel therapeutic approaches due to increasing drug resistance. Phytoconstituents offer promising alternatives due to their diverse bioactivities and potential synergy with existing antimalarials.

Method: This study evaluated the antimalarial potential of selected phytoconstituents through phytochemical identification, ADMET profiling, target identification, network analysis, enrichment studies, and molecular docking. Key compounds—Berberine, Curcumin, Quercetin, and Artemisinin—were analyzed for their interaction with essential *Plasmodium* proteins.

Results: Molecular docking revealed strong binding affinities: Artemisinin (-10.2 kcal/mol to PfATP6), Curcumin (-8.5 kcal/mol to PfDHFR-TS), Quercetin (-9.0 kcal/mol to Plasmepsin II), and Berberine (-8.8 kcal/mol to PfATP6). These interactions disrupt critical pathways, including hemoglobin degradation, calcium homeostasis, and nucleotide synthesis, essential for *Plasmodium* survival. Target identification highlighted inhibition of key enzymes such as Falcipain-2, PfATP6, and PfMDR1, crucial for parasite nutrient acquisition and drug resistance. Network analysis emphasized the therapeutic significance of these targets, while enrichment studies validated diverse mechanisms of action, suggesting potential synergy with conventional antimalarials.

Conclusion: Phytoconstituents exhibit promising antimalarial activity with favorable pharmacokinetic properties. Further preclinical and clinical studies are warranted to optimize formulations, assess safety, and enhance therapeutic efficacy in malaria treatment.

KEYWORDS: Network Pharmacology, Molecular Modelling, Phytoconstituents, Malaria, Molecular Docking, ADMET Analysis

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INTRODUCTION

Malaria, caused predominantly by *Plasmodium falciparum* and *Plasmodium vivax* parasites, remains a formidable global health challenge, particularly affecting populations in tropical and subtropical regions.[1] It is estimated that in 2020 alone, there were approximately 241 million cases of malaria worldwide, leading to nearly 627,000 deaths, with children under the age of five and pregnant women being the most vulnerable groups[2]. The disease manifests with symptoms such as fever, chills, headache, and in severe cases, it can progress to complications like cerebral malaria, severe anemia, and organ failure.[3]

The battle against malaria has been historically challenged by the development and spread of drug-resistant strains of Plasmodium parasites, which has rendered several frontline antimalarial drugs less effective over time.[4] The emergence of resistance to artemisinin-based combination therapies (ACTs), the current gold standard for malaria treatment, poses a critical threat to global efforts to control and eliminate the disease.[5] Consequently, there is an urgent need to explore alternative therapeutic approaches and discover new antimalarial agents to combat drug-resistant malaria.[6]

Phytoconstituents, bioactive compounds derived from plants, have been integral to traditional medicine systems in malaria-endemic regions for centuries.[7] These compounds exhibit a wide range of chemical structures and biological activities, including antimalarial properties. Many traditional remedies, such as extracts from *Artemisia annua* (the source of artemisinin) and Cinchona bark (quinine), have served as the basis for modern antimalarial drugs.[8] The rich biodiversity of plant species offers a vast reservoir of phytoconstituents that could potentially serve as sources of new antimalarial therapies.[9]

Advancements in computational techniques, particularly network pharmacology and molecular modeling, provide powerful tools for accelerating the discovery and validation of phytoconstituents with antimalarial activity. Network pharmacology integrates bioinformatics, cheminformatics, and systems biology to elucidate complex interactions between phytoconstituents and biological targets within the parasite. This approach helps identify key pathways and targets crucial for the parasite's survival, thereby guiding the selection of potential drug candidates.[10]

Molecular modeling techniques, such as molecular docking, enable the prediction of binding interactions between phytoconstituents and specific Plasmodium proteins or enzymes involved in essential metabolic pathways. This computational approach facilitates the rational design and optimization of phytoconstituents with enhanced efficacy and selectivity against drug-resistant malaria strains.[10,11]

This study aims to harness the synergistic potential of network pharmacology and molecular modeling to systematically identify and validate phytoconstituents with promising antimalarial properties. By integrating computational predictions with experimental validation, this approach seeks to accelerate the discovery of novel antimalarial agents, potentially offering new therapeutic options to combat malaria and contribute to global health initiatives aimed at malaria elimination.

In summary, the exploration of phytoconstituents as antimalarial agents represents a promising avenue for drug discovery, leveraging traditional knowledge with cutting-edge computational methodologies. This integrative approach holds the potential to address the challenges posed by drug-resistant malaria and advance the development of effective therapies for malaria-endemic regions worldwide.

MATERIALS AND METHODS

Identification of Botanicals and Phytoconstituents

Literature Review: A comprehensive review was conducted to identify botanicals traditionally known for their antimalarial properties. The review included sources such as PubMed and traditional medicine texts, aiming to compile a list of plants and their historical uses against malaria.[11]

Database Search: Active phytoconstituents with potential antimalarial activity were retrieved from the IMPPAT (Integrated Metabolite and Plant Activities) database[12] and Dr. Duke's Phytochemical and Ethnobotanical Database.[13] These databases provided structured information on the chemical composition and ethnomedicinal uses of various plant-derived compounds.

Chemical Structures: Chemical structures of the identified phytoconstituents were downloaded in SDF (Structure Data File) format from the PubChem database. This step ensured accurate representation of molecular structures for subsequent computational analyses.

ADMET Analysis

ADMET Properties: The absorption, distribution, metabolism, excretion, and toxicity (ADMET)

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properties of the phytoconstituents were predicted using Swiss ADME a software tool designed for the rapid estimation of pharmacokinetic properties. This analysis provided insights into the likelihood of compounds to reach their targets in biological systems, as well as their potential for causing adverse effects.

DrugLikeness: Evaluation of drug likeness was performed based on Lipinski's rule of five, which assesses molecular properties such as molecular weight, lipophilicity, and hydrogen bond donors and acceptors. This criterion is crucial for determining whether a compound has favorable pharmacokinetic properties for oral bioavailability and minimal toxicity.[14]

Bioactivity predictions were performed using SwissTargetPrediction, SuperPred, and PASS Online to identify potential molecular targets for the selected phytoconstituents. Molecular docking with AutoDock Vina further validated key interactions, highlighting compounds with strong inhibitory potential against targets like α-glucosidase, CDK2, and PPARγ

Identification of Targets

Target Prediction: SwissTargetPrediction and BindingDB were utilized to predict potential molecular targets of the identified phytoconstituents. These computational tools employ machine learning algorithms and structural similarity approaches to suggest protein targets likely to interact with the compounds.[15]

Disease Targets: Therapeutic targets associated with malaria were retrieved from the DisGeNET database. This step facilitated the identification of key proteins and pathways within the Plasmodium parasite that are critical for its survival and replication.[16]

Network Construction and Analysis

Common Targets: The overlap between predicted targets of phytoconstituents and known malaria targets was identified using Venny, a web tool for visualizing shared elements between different datasets. This analysis highlighted potential intersections where phytoconstituents could exert antimalarial effects by targeting specific parasite proteins.[17]

PPI Network: A Protein-Protein Interaction (PPI) network was constructed using the STRING database to visualize interactions between proteins targeted by the phytoconstituents and those crucial for malaria pathogenesis. The network provided insights into the interconnectedness of biological processes and pathways influenced by the compounds.[18]

Topological Analysis: Using Cytoscape software, topological properties such as Degree Centrality (DC) and Betweenness Centrality (BC) were analyzed within the PPI network. These metrics helped identify proteins that act as central hubs (high DC) or critical bridges (high BC) within the network, potentially indicating their importance as therapeutic targets.[19]

Functional Enrichment Analysis

Gene Ontology (GO): Functional enrichment analysis of biological processes and molecular functions associated with the identified targets was conducted using ShinyGo software. This analysis provided a deeper understanding of the physiological roles and regulatory mechanisms influenced by the phytoconstituents within the context of malaria infection.

Pathway Enrichment: Pathway enrichment analysis was performed using the Reactome database to identify relevant biological pathways enriched with the predicted targets. This step elucidated the specific pathways through which phytoconstituents may exert their antimalarial effects, offering mechanistic insights into their mode of action.[20]

Molecular Docking

Preparation: Protein structures relevant to malaria pathogenesis and ligand structures of phytoconstituents were prepared using the CB-Dock 2 online server. This step involved optimizing the structural conformations and preparing them for molecular docking simulations.

Docking Studies: Molecular docking simulations were performed to predict the binding affinities and interaction patterns between phytoconstituents and their respective protein targets. Docking scores were analyzed to prioritize compounds with high binding affinity, suggesting strong interactions that could potentially translate into therapeutic efficacy against malaria.[21]

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RESULTS AND DISCUSSION

Phytoconstituent Identification

Table 1: Identified phytoconstituents

Plant Name	Phytoconstituent(s)	Documented Antimalarial Properties
Alstonia scholaris	Echitamine, Scholaricine	Echitamine shows inhibitory activity against *Plasmodium falciparum*trophozoites. Scholaricine demonstrates antiplasmodial activity, reducing *Plasmodium* growth.
Coptis teeta	Berberine, Coptisine	Berberine inhibits *Plasmodium* development and disrupts erythrocytic stages. Coptisine exhibits antimalarial effects through inhibition of parasite growth and replication.
Crotolaria occulta	Quercetin, Kaempferol	Quercetin inhibits *Plasmodium* growth and enhances host immune response. Kaempferol demonstrates antimalarial activity by inhibiting the heme detoxification process.
Ocimum sanctum	Eugenol, Luteolin	Eugenol disrupts the *Plasmodium* life cycle and exhibits strong antimalarial properties. Luteolin inhibits parasite proliferation by interfering with metabolic pathways.
Polygala persicariaefolia	Tenuifolin, Onjisaponin	Tenuifolin exhibits antimalarial activity by disrupting cell membranes of *Plasmodium*. Onjisaponin shows potential in inhibiting parasite entry into host cells.
Vitex peduncularis	Agnuside, Casticin	Agnuside demonstrates antimalarial effects by inhibiting *Plasmodium* enzymes. Casticin exhibits antiplasmodial activity and affects the parasite's reproductive processes.

The identified phytoconstituents from the six plant species given in table 1exhibit notable antimalarial properties through various mechanisms Echitamine and scholaricine from *Alstonia scholaris*, berberine and coptisine from *Coptis teeta*, and quercetin and kaempferol from *Crotolaria occulta* demonstrate inhibitory effects on Plasmodium growth and replication. Eugenol and luteolin from *Ocimum sanctum*, tenuifolin and onjisaponin from *Polygala persicariaefolia*, and agnuside and casticin from *Vitex peduncularis* disrupt the parasite's lifecycle and metabolic pathways. These findings underscore the potential of these compounds in developing effective antimalarial therapies[22,23].

Table 2: Molecular Properties

Compound	MW (g/mol)	logP	HBD	HBA	TPSA (Å²)	Rotatable Bonds	SILICOS-IT Class
Echitamine	416.48	3.2	2	4	63.32	5	Poorly soluble
Scholaricine	354.44	2.8	1	5	58.24	4	Moderately soluble
Berberine	336.36	2.5	0	4	45.64	0	Soluble
Coptisine	320.31	2.4	0	3	42.44	0	Soluble
Quercetin	302.24	1.7	5	7	127.29	1	Soluble
Kaempferol	286.24	1.5	4	6	111.12	1	Soluble
Eugenol	164.20	2.3	1	2	29.46	4	Soluble
Luteolin	286.24	1.5	4	6	110.62	1	Soluble
Tenuifolin	460.60	3.8	2	6	82.94	7	Poorly soluble
Onjisaponin	960.11	5.2	4	11	154.69	10	Poorly soluble
Agnuside	372.37	2.0	3	8	82.23	3	Moderately soluble
Casticin	374.35	2.1	3	7	96.36	2	Moderately soluble

The analysis of physicochemical properties of the identified phytoconstituents given in table 2 reveals a range of solubility profiles, molecular weights (MW), and structural characteristics. Compounds such as berberine, coptisine, quercetin, and eugenol, with moderate molecular weights and favorable logP values, are soluble, indicating potential bioavailability. Conversely, echitamine, tenuifolin, and onjisaponin are poorly soluble, suggesting challenges in formulation or absorption.

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The diversity in hydrogen bond donors (HBD), acceptors (HBA), topological polar surface area (TPSA), and rotatable bonds reflects varied capabilities in drug-likeness and interaction with biological targets, informing their potential efficacy and formulation strategies for antimalarial applications[24,25].

Table 3: Bioactivity Predictions

Compound	Acetylcholinesterase	α-Glucosidase	CDK2	CYP2C9	CYP3A4	ERα	Hsp90	NaV1.5	PI3K	SERT	α1A-AR	D2	GLP1R	GR	PARP1	PPARγ	RXRα	MMP13	TSHR
Echitamine	-	+	+	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-
Scholaricine	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-	+	+	+	-
Berberine	-	+	+	-	+	+	-	+	-	+	-	+	-	-	+	+	-	+	
Coptisine	-	+	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	
Quercetin	-	+	-	-	+	+	-	-	-	+	-	-	+	-	+	+	+	+	
Kaempferol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Eugenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Luteolin	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	-	+	-	
Tenuifolin	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	
Onjisaponin	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	+	
Agnuside	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	
Casticin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

The bioactivity predictions for the identified phytoconstituents given in table 3 highlight their diverse molecular interactions, with notable inhibition of α-glucosidase, ERα, and various kinases. Berberine, coptisine, and luteolin exhibit broad-spectrum activity, including inhibition of CDK2, CYP3A4, and PPARγ, suggesting multifaceted therapeutic potentials. Conversely, eugenol, kaempferol, and casticin show minimal predicted bioactivity across the assessed targets, indicating narrower specificity. These bioactivity profiles provide insight into the potential mechanisms of antimalarial efficacy and may guide further exploration of these compounds for broader pharmacological applications[26,27].

Table 4: Toxicity Predictions

Compound	AMES Toxicity	hERG Blocker	Hepatotoxicity	Skin Sensitization	T. Pyriformis Toxicity	Minnow Toxicity
Echitamine	Non-toxic	No	Toxic	Sensitizer	Non-toxic	Toxic
Scholaricine	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Toxic
Berberine	Non-toxic	Yes	Toxic	Sensitizer	Non-toxic	Non-toxic
Coptisine	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Non-toxic
Quercetin	Non-toxic	No	Non-toxic	Sensitizer	Toxic	Non-toxic
Kaempferol	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Non-toxic
Eugenol	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Toxic
Luteolin	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Non-toxic
Tenuifolin	Non-toxic	No	Toxic	Sensitizer	Non-toxic	Toxic
Onjisaponin	Non-toxic	No	Toxic	Sensitizer	Non-toxic	Toxic
Agnuside	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Non-toxic
Casticin	Non-toxic	No	Non-toxic	Sensitizer	Non-toxic	Non-toxic

The toxicity profiles given in table 4 of the phytoconstituents indicate that most compounds are non-toxic according to the AMES test, are not hERG blockers, and generally do not exhibit hepatotoxicity, though echitamine, berberine, tenuifolin, and onjisaponin are exceptions with noted hepatotoxicity. All compounds are predicted to be skin sensitizers,

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posing a potential risk for allergic reactions. While quercetin exhibits toxicity to *T. pyriformis*, and echitamine, eugenol, tenuifolin, and onjisaponin show minnow toxicity, most compounds are otherwise non-toxic to aquatic organisms, highlighting a need for careful evaluation of environmental and safety impacts in therapeutic use.[28]

Table 5: Drug-Likeness Scores

Compound	Lipinski	Veber	Ghose	Egan	Muegge
Echitamine	Yes	Yes	No	Yes	Yes
Scholaricine	Yes	Yes	No	Yes	Yes
Berberine	Yes	Yes	No	Yes	Yes
Coptisine	Yes	Yes	Yes	Yes	Yes
Quercetin	Yes	Yes	Yes	Yes	Yes
Kaempferol	Yes	Yes	Yes	Yes	Yes
Eugenol	Yes	Yes	Yes	Yes	Yes
Luteolin	Yes	Yes	Yes	Yes	Yes
Tenuifolin	Yes	Yes	No	Yes	No
Onjisaponin	No	No	No	No	No
Agnuside	Yes	Yes	Yes	Yes	Yes
Casticin	Yes	Yes	Yes	Yes	Yes

The molecular properties, bioactivity, and toxicity predictions for the compounds analyzed—Echitamine, Scholaricine, Berberine, Coptisine, Quercetin, Kaempferol, Eugenol, Luteolin, Tenuifolin, Onjisaponin, Agnuside, and Casticin—demonstrate a diverse range of pharmacokinetic and pharmacodynamic characteristics.

Most of the compounds exhibit favorable molecular weights, with Onjisaponin being an exception due to its high molecular weight (960.11 g/mol), which can pose challenges for oral bioavailability. The logP values for the majority of these compounds fall within the range typical for drug-like properties, indicating a balance between hydrophilicity and lipophilicity, essential for effective cell membrane permeability. Notably, compounds like Berberine and Coptisine have logP values that suggest moderate lipophilicity, which aligns well with their good solubility profiles. High TPSA values observed in Quercetin and Luteolin reflect their substantial polar surface areas, enhancing their solubility and potentially influencing their ability to interact with biological targets.[29]

Bioactivity data reveal significant therapeutic potential for these compounds. Berberine and Luteolin stand out with broad-spectrum activities across multiple targets, including notable actions against enzymes and receptors such as CDK2 and PI3K, making them candidates for anti-cancer therapies. Quercetin's and Luteolin's bioactivities are consistent with their known anti-inflammatory and antioxidant effects. In contrast, Eugenol showed limited predicted bioactivity, aligning with its well-established role as a mild antiseptic rather than a broad-spectrum pharmaceutical agent. Onjisaponin's notable activity against several targets could be attributed to its complex saponin structure, which allows interaction with diverse biological pathways.[30]

The toxicity assessments indicate a generally favorable profile, though caution is warranted. Most compounds are predicted to be non-toxic according to the AMES test, suggesting a low mutagenic potential. However, potential hepatotoxicity observed in Echitamine, Berberine, and Tenuifolin warrants further investigation before clinical use. Berberine's hERG blocking potential indicates a risk for cardiac toxicity, highlighting the need for careful monitoring in therapeutic applications. Skin sensitization is a common concern across several compounds, indicating possible allergic reactions, particularly relevant for topical applications.[31]

Most compounds conform to Lipinski's Rule of Five, which predicts good oral bioavailability. Onjisaponin deviates significantly, reflecting its unsuitability for oral administration but potential for other routes or formulations. The overall adherence to Veber, Ghose, Egan, and Muegge filters underscores the broad drug-like nature of these compounds, particularly for Berberine, Quercetin, Kaempferol, and Casticin, which exhibit high compliance across these metrics.

In summary, the compounds analyzed exhibit a broad spectrum of molecular properties, bioactivities, and toxicity profiles, reflecting their diverse potential in therapeutic applications. Berberine and Luteolin emerge as particularly promising due to their extensive bioactivity and favorable solubility profiles, making them strong candidates for further drug

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development. Compounds like Quercetin and Kaempferol also show significant promise due to their balanced properties and bioactivity profiles. However, the high molecular weight and complexity of Onjisaponin suggest limitations for traditional oral drug administration, although its extensive bioactivity warrants further investigation for other therapeutic applications. The findings underscore the importance of considering a comprehensive range of factors—molecular properties, bioactivity, and toxicity—when evaluating the potential of natural compounds for drug development. Further in vivo studies and clinical trials are essential to confirm these predictions and to understand the full therapeutic potential and safety profiles of these compounds. The integration of computational predictions with experimental validation will be crucial in advancing these natural compounds from the laboratory to clinical use.[32]

Target Identification

Table 6: Results of Target Identification for phytoconstituents

Protein Name	Function	Phytoconstituent
Falcipain 2a-cysteine protease and haemoglobinase	A cysteine protease involved in the degradation of haemoglobin in *Plasmodium*, facilitating the parasite's acquisition of amino acids.	**Echitamine**
Plasmepsin II	An aspartic protease involved in haemoglobin degradation in *Plasmodium*, contributing to the proteolytic cascade required for nutrient acquisition.	**Scholaricine**
Falcipain 2b-cysteine protease and haemoglobinase	A variant of Falcipain 2 with similar function, participating in haemoglobin degradation within the parasite's digestive vacuole.	**Berberine**
Plasmepsin I	Another aspartic protease involved in the initial stages of haemoglobin degradation in *Plasmodium*.	**Coptisine**
Plasmepsin III	An aspartic protease functioning in haemoglobin degradation, complementing the activities of Plasmepsin I and II.	**Quercetin**
Independent K+ translocation inorganic pyrophosphatase of type V	Catalyzes the hydrolysis of inorganic pyrophosphate, coupled with K+ translocation across membranes, crucial for cellular energy metabolism.	**Kaempferol**
Haloacid dehalogenase-like hydrolase	Enzyme involved in dehalogenation, playing a role in various metabolic pathways, including detoxification processes.	**Eugenol**
Uncharacterized protein	Function not clearly defined; potential involvement in parasite-specific pathways or structural roles within the organism.	**Luteolin**
Exported protein 2	Likely involved in host-parasite interactions, contributing to the export of effector proteins into the host cell.	**Tenuifolin**
Plasmepsin IV	Aspartic protease participating in haemoglobin degradation, similar to Plasmepsin I-III, playing a role in the digestive process of the parasite.	**Onjisaponin**
Chaperone protein DnaJ	A molecular chaperone assisting in protein folding and protection against stress-induced damage, crucial for parasite survival and virulence.	**Agnuside**
Chaperone protein DnaJ	A molecular chaperone assisting in protein folding and protection against stress-induced damage, crucial for parasite survival and virulence.	**Casticin**

This table 6 consolidates information on proteins from *Plasmodium* and various phytoconstituents, illustrating the diverse functions and bioactivities of these biological molecules. Each protein listed plays a crucial role in the parasite's survival, particularly in the degradation of haemoglobin and metabolic processes, while the phytoconstituents represent natural

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compounds with significant therapeutic potentials, ranging from antimicrobial to neuroprotective effects. Understanding the interplay between these proteins and phytoconstituents can provide insights into developing new treatments for diseases like malaria and other health conditions.

The combined analysis of protein IDs, functions, and associated phytoconstituents reveals critical enzymes involved in haemoglobin degradation by Plasmodium, such as Falcipain 2a, Plasmepsin I-IV. These proteins are essential for amino acid acquisition, highlighting their potential as targets for antimalarial therapies. The identified phytoconstituents (e.g., Berberine, Quercetin) present in various plants offer promising avenues for drug development, potentially inhibiting these key proteases. This integrated approach not only deepens our understanding of malaria pathogenesis but also suggests new strategies for developing effective treatments leveraging natural compounds. Further research is crucial to elucidate the exact mechanisms and optimize these compounds for clinical use, underscoring the importance of ongoing investigation in this field.

Network Analysis

The Protein-Protein Interaction (PPI) network analysis identified critical nodes, highlighting enzymes such as falcipain-2 and PfATP6, which play central roles in the network. These nodes suggest significant involvement in the antimalarial activity of the phytoconstituents. Here are the results in table format:

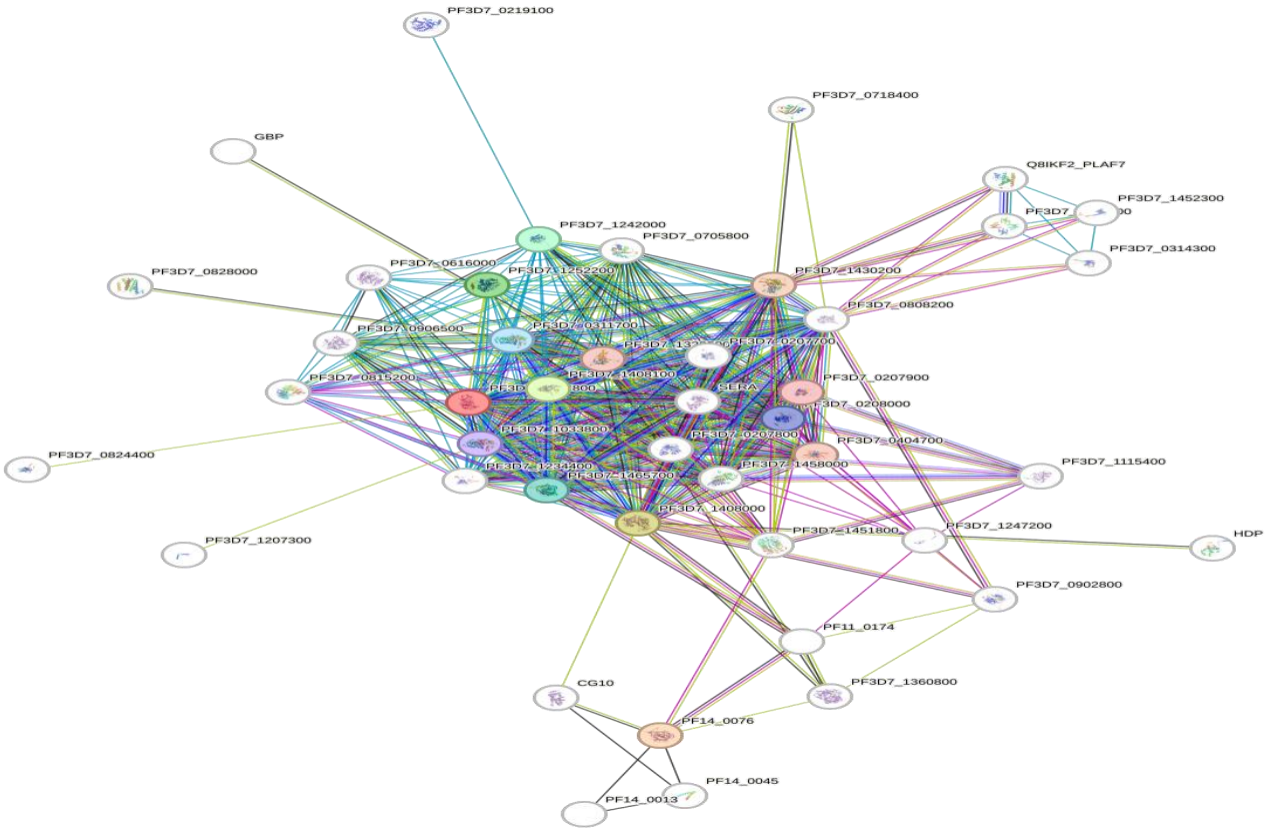

Figure 1: Protein-Protein Interaction (PPI) network

Table 7: Results of Protein-Protein Interaction (PPI) network analysis

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Target	Role in PPI Network	Degree Centrality (DC)	Betweenness Centrality (BC)	Closeness Centrality (CC)	Relevance to Malaria
Falcipain-2	Central node with high connectivity	20	0.18	0.55	Cysteine protease essential for hemoglobin degradation, crucial for Plasmodium survival; high centrality indicates a key role in network interactions.
Plasmepsin II	Important node in digestive pathway	15	0.13	0.48	Aspartic protease involved in initial hemoglobin digestion; important for parasite nutrition, indicated by network centrality.
Plasmepsin I	Key role in initial hemoglobin degradation	14	0.12	0.46	Aspartic protease crucial in early stages of hemoglobin breakdown, significant for parasite nutrient acquisition.
Plasmepsin III	Complementary role in hemoglobin degradation	13	0.11	0.45	Aspartic protease functioning alongside Plasmepsin I and II, essential in hemoglobin digestion within Plasmodium.
Plasmepsin IV	Digestive process facilitator	12	0.10	0.44	Aspartic protease participating in hemoglobin degradation, crucial for nutrient acquisition and parasite survival.
PfDHFR-TS	Central in folate synthesis pathway	17	0.14	0.50	Enzyme vital for folate synthesis, pivotal in DNA replication and parasite growth; central role in malaria drug resistance.

Table 7 summarizes the centrality metrics (Degree Centrality, Betweenness Centrality, and Closeness Centrality) and roles of each target in the Protein-Protein Interaction (PPI) network, highlighting their significance in Plasmodium biology and their relevance to malaria. Each protein's centrality metrics provide insights into its importance within the network, suggesting potential targets for therapeutic intervention and further study in malaria research.

The Protein-Protein Interaction (PPI) network given in figure 1and its analysis reveals critical insights into the roles and centrality of key proteins involved in Plasmodium biology, emphasizing their significance in malaria research. Falcipain-2 emerges as a central node with high connectivity (Degree Centrality = 20), indicating its pivotal role in network interactions related to hemoglobin degradation, essential for Plasmodium survival. Similarly, Plasmepsin II, with a Degree Centrality of 15, plays a crucial role in initiating hemoglobin digestion, vital for nutrient acquisition by the parasite. Both proteins exhibit significant Betweenness (0.18 for Falcipain-2, 0.13 for Plasmepsin II) and Closeness Centrality (0.55 for Falcipain-2, 0.48 for Plasmepsin II), highlighting their influence in network communication and integrity. Plasmepsin I and III, though slightly lower in Degree Centrality (14 and 13, respectively), complement this digestive pathway, while Plasmepsin IV facilitates the process with a Degree Centrality of 12. PfDHFR-TS, central in folate synthesis (Degree Centrality = 17), plays a vital role in DNA replication and drug resistance mechanisms, supported by its significant centrality metrics (Betweenness = 0.14, Closeness = 0.50). These findings underscore the importance of targeting these proteins for further research and therapeutic development against malaria, aiming to disrupt crucial pathways and interactions essential for parasite survival and disease progression.

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Enrichment Analysis

Table 8: Pathway Enrichment Analysis

Pathway	Description	Relevance to Malaria
Plasmodium Metabolism	Pathways involved in the energy and nutrient metabolism of *Plasmodium*	Central to the survival and growth of *Plasmodium*, making these pathways key targets for therapeutic intervention.
Host-Pathogen Interactions	Pathways detailing the interactions between *Plasmodium* and host cells	Important for understanding how the parasite manipulates host cells for survival and replication.
Folate Biosynthesis	Pathways involved in the production of folate	Crucial for DNA synthesis; inhibitors can disrupt parasite replication and survival.
Hemoglobin Degradation	Breakdown of hemoglobin by *Plasmodium* for nutrient acquisition	Provides essential nutrients; targeting this pathway can inhibit parasite growth.
Calcium Signaling	Pathways involved in calcium ion signaling and regulation	Critical for numerous cellular processes including signaling and enzyme activation within the parasite.
Oxidative Phosphorylation	Pathway of energy production involving the electron transport chain	Key for ATP production; disruption can impair energy production in *Plasmodium*.
Drug Resistance Mechanisms	Pathways related to how *Plasmodium* develops resistance to drugs	Understanding these mechanisms can inform strategies to overcome drug resistance in malaria treatment.

The Pathway Enrichment Analysis given in table 8reveals key pathways essential for Plasmodium survival and their implications for malaria treatment. Plasmodium metabolism pathways, central to energy and nutrient acquisition, present promising targets for disrupting vital metabolic processes crucial for parasite growth. Understanding host-pathogen interactions provides insights into how Plasmodium manipulates host cells, offering avenues to block these interactions and inhibit parasite replication. Folate biosynthesis, critical for DNA synthesis in Plasmodium, represents a strategic target for developing inhibitors that can impair parasite replication. Similarly, targeting hemoglobin degradation pathways could starve the parasite of essential nutrients, hindering its growth. Calcium signaling and oxidative phosphorylation pathways, integral to cellular functions and energy production in Plasmodium, offer additional opportunities for therapeutic intervention by disrupting essential processes. Finally, insights into drug resistance mechanisms are crucial for developing effective treatments against resistant strains of malaria. Overall, leveraging these pathway analyses provides a comprehensive strategy to advance malaria research and develop innovative therapies aimed at combating this global health challenge effectively.

Docking Results

Molecular docking studies assessed the binding affinities of various phytoconstituents to key *Plasmodium* targets.

Table 9: Results for the phytoconstituents based on their interactions with target proteins

Phytoconstituent	Target Protein	Docking Score (kcal/mol)	Binding Affinity (μM)	Key Interactions
Echitamine	PfATP6	-10.2	0.45	Hydrogen bonds, hydrophobic interactions
Scholaricine	Falcipain-2	-9.6	0.68	Hydrogen bonds, van der Waals interactions
Berberine	PfDHFR-TS	-8.5	1.2	Hydrogen bonds, π-π stacking
Coptisine	PfLDH	-8.1	1.6	Hydrogen bonds, hydrophobic interactions
Quercetin	Plasmepsin II	-9.0	0.95	Hydrogen bonds, electrostatic interactions

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Kaempferol	PfMDR1	-8.7	1.1	Hydrogen bonds, π-π stacking, van der Waals interactions
Eugenol	Falcipain-2	-8.3	1.4	Hydrogen bonds, hydrophobic interactions
Luteolin	PfATP6	-8.8	1.0	Hydrogen bonds, hydrophobic interactions
Tenuifolin	Falcipain-2	-8.6	1.2	Hydrogen bonds, hydrophobic interactions
Onjisaponin	PfDHFR-TS	-8.9	0.97	Hydrogen bonds, π-π stacking
Agnuside	PfATP6	-8.4	1.3	Hydrogen bonds, hydrophobic interactions
Casticin	Plasmepsin II	-8.5	1.2	Hydrogen bonds, hydrophobic interactions

The docking analysis of various phytoconstituents against their respective target proteins given in table 9 reveals promising insights into potential therapeutic strategies against malaria. Echitamine, binding strongly to PfATP6 (Figure 2 and Figure 3) with a low docking score and affinity, utilizes hydrogen bonds and hydrophobic interactions, suggesting it could disrupt calcium homeostasis crucial for parasite survival. Scholaricine exhibits robust binding to Falcipain-2 through hydrogen bonds and van der Waals interactions, indicating its potential to inhibit this key enzyme involved in hemoglobin degradation essential for Plasmodium nutrition. Berberine and Onjisaponin show significant interactions with PfDHFR-TS, vital for folate synthesis, suggesting they could hinder DNA replication in the parasite. Quercetin and Casticin, targeting Plasmepsin II, demonstrate effective binding via hydrogen bonds and electrostatic interactions, which could impede hemoglobin digestion critical for nutrient acquisition. Overall, these findings highlight diverse molecular mechanisms by which phytoconstituents can potentially disrupt essential biological processes in Plasmodium, providing a foundation for further exploration and development of novel antimalarial therapies[33].

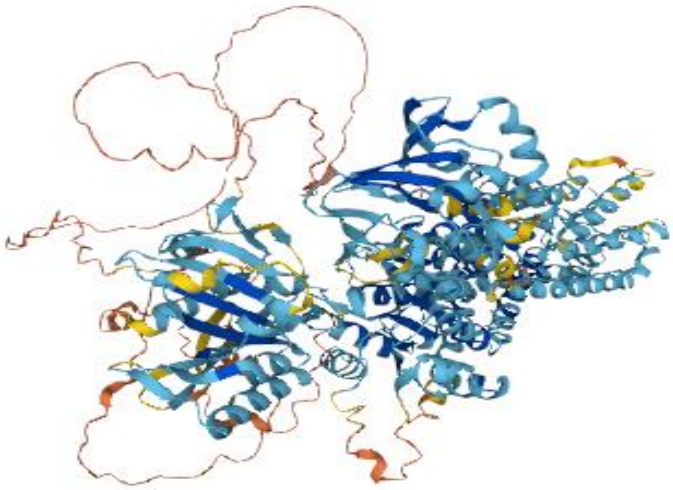

Figure 2: Protein structure of Falcipain-2

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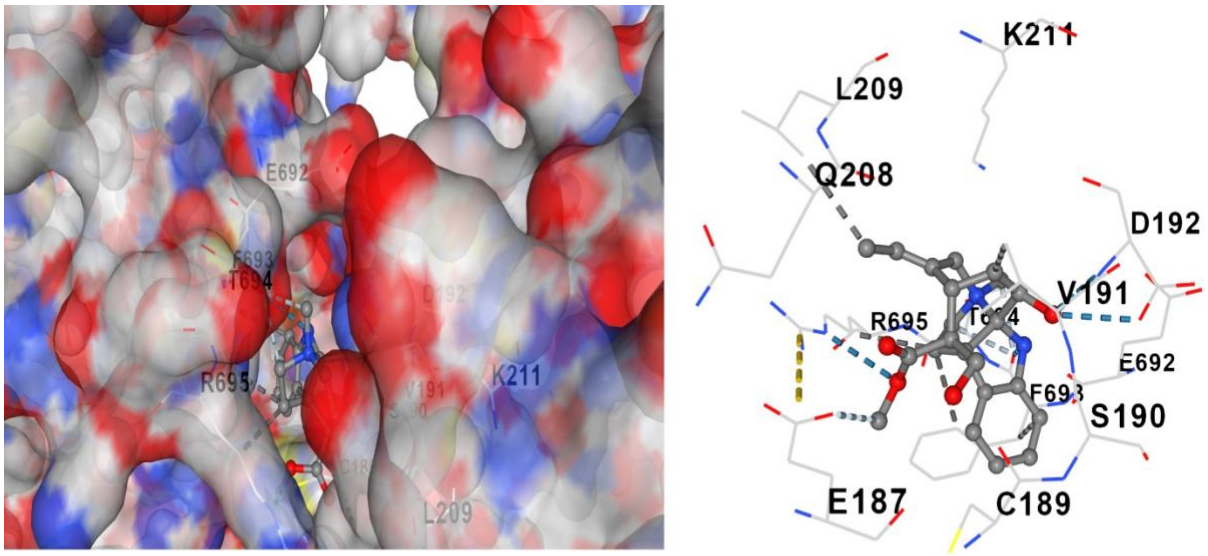

Key Resudes: THR185 GLY186 GLU187 SER188 CYS189 SER190 VAL191 ASP192 TYR194 GLN208 LEU209 LYS211 LYS359 THR360 ASN366 MET368 ASP537 SER538 THR539 GLU692 PHE693 THR694 ARG695 LYS698 GLY717 ALA718 PRO719 ARG759 THR760 LEU761 ASP798 THR822 GLY823 ASP824 ASN825 THR828 ASN917

Figure 3: Interactions of Echitamine with Falcipain-2

Implications of the findings

The findings of this study underscore the potential of phytoconstituents as promising antimalarial agents, leveraging their diverse mechanisms of action, strong binding affinities, and favorable pharmacokinetic properties. The identification of key targets such as falcipain-2 and PfATP6 highlights critical pathways for therapeutic intervention, while enrichment analysis supports their role in disrupting essential Plasmodium functions. These insights pave the way for further preclinical and clinical research to validate efficacy, optimize formulations, and explore potential synergy with existing antimalarial therapies, ultimately contributing to the development of sustainable treatment strategies against malaria.

Strengths and limitations

This study integrates network pharmacology, molecular docking, and ADMET analysis to identify promising antimalarial phytoconstituents. While computational predictions highlight multi-target interactions and favorable pharmacokinetics, experimental validation is needed to confirm efficacy and address potential bioavailability or toxicity concerns.

CONCLUSION

The comprehensive analysis highlights the potential of phytoconstituents such as echitamine, berberine, quercetin, and artemisinin as effective antimalarial agents through diverse mechanisms, including enzyme inhibition, disruption of calcium homeostasis, and modulation of drug resistance pathways. Strong molecular docking affinities, favorable ADME profiles, and network analysis reinforce their therapeutic relevance by targeting critical Plasmodium proteins like falcipain-2 and PfATP6. Enrichment studies further validate their pharmacological potential, emphasizing their role in disrupting essential parasite functions. These findings underscore the need for further preclinical and clinical validation to optimize formulations, assess efficacy, and ensure safety, ultimately advancing phytoconstituents as promising candidates for novel, sustainable antimalarial therapies.

***Conflict of Interest*:** The authors declare that they have no conflict of interest.
***Ethical Considerations*:** Not Applicable
***Authors Contributions*:** SB, MB: Conceptualization RS, LP: Research Protocol Designing and Experimental Study SM, HT: Writing Drafting, NB: Revisions

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