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Research Article

IN SILICO EVALUATION OF ANTIMALARIAL POTENTIAL OF *ARTEMISIA ANNUA* PHYTOCONSTITUENTS TARGETING PLASMODIUM PROTEINS

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ABSTRACT

Malaria remains a major health threat in tropical and subtropical regions. Resistance to existing antimalarial drugs, especially artemisinin-based therapies, has driven the urgent search for new therapeutic agents[1,2]. *Artemisia annua*, a traditional antimalarial herb, is rich in bioactive phytochemicals with potential multi-target antimalarial effects[3,7] . This study aims to evaluate the antimalarial potential of selected phytoconstituents from *Artemisia annua* using an *in silico* approach. Key goals included molecular docking against malaria-related protein targets, ADME (Absorption, Distribution, Metabolism, and Excretion) analysis, and target prediction to identify promising lead compounds for future drug development.

Methods

Molecular docking was performed using AutoDock Vina[5] against four *Plasmodium*-associated proteins (1O5X, 4DP3, 4GAE, 7SXZ). ADME properties were analyzed using SwissADME, and target prediction was carried out using SwissTargetPrediction[11]. Ligands were chosen from literature-reported constituents of *A. annua.*[3,14]

Results

Artesunate exhibited the highest binding affinity (-9.3 kcal/mol) with protein 4DP3, followed by other artemisinin derivatives. Most compounds showed zero violations of Lipinski's rule[9] and acceptable pharmacokinetics. Predicted targets included carbonic anhydrase isoforms, PfDHFR, and PfMDR1, indicating multi-target action against *Plasmodium.*

Conclusion

The *in silico* analysis highlights several potent phytochemicals from *Artemisia annua,* especially artesunate

and artemisinin derivatives, as promising antimalarial agents[2,7,13] . Their favorable ADME profiles and strong interactions with malaria targets support further investigation. This study reinforces the potential of *A. annua* in antimalarial drug discovery using computational screening strategies[3,12,14] .

KEYWORDS

ADME, Artemisia annua, Carbonic Anhydrase, Molecular Docking, PfMDR1

Introduction

Malaria remains one of the most devastating infectious diseases worldwide, with an estimated 247 million cases and over 600,000 deaths reported annually, according to the World Health Organization[1] . The disease is caused by protozoan parasites belonging to the genus Plasmodium, with *Plasmodium falciparum* and *Plasmodium vivax* being the most prevalent and deadly species. Transmission occurs through the bite of female Anopheles mosquitoes, which act as vectors for the parasite. While global efforts have led to a significant reduction in malaria incidence and mortality over the past two decades, the disease continues to exert a heavy toll on health systems in sub-Saharan Africa, South Asia, and parts of Latin America. Chemotherapeutic intervention remains the cornerstone of malaria management, supported by vector control measures such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). However, the growing emergence of resistance to frontline antimalarial drugs—including chloroquine, sulfadoxine-pyrimethamine, and more alarmingly, artemisinin-based combination therapies (ACTs)—has raised serious concerns. Resistance mechanisms such as mutations in the PfKelch13 gene and enhanced drug efflux via PfMDR1 compromise treatment outcomes and threaten to reverse progress in malaria control and elimination[12] . Given the urgency of the situation, there is a critical need to explore alternative therapeutic agents that are effective, affordable, and capable of overcoming current resistance mechanisms. Innovative strategies, including natural product-based drug discovery and computational screening approaches, are gaining momentum as potential solutions to this global health challenge.

Historically, plants have served as an invaluable source of medicinal compounds, particularly in the treatment of parasitic diseases such as malaria. Among the most notable herbal remedies is *Artemisia annua L.*, commonly referred to as sweet wormwood or Qinghao, which has been used in traditional Chinese medicine for centuries to treat intermittent fevers. The landmark discovery of artemisinin, a sesquiterpene lactone endoperoxide derived from *A. annua*, revolutionized malaria therapy and earned Dr. Tu Youyou the Nobel Prize in Physiology or Medicine in 2015. Artemisinin and its derivatives—dihydroartemisinin, artesunate, and artemether—have become essential components of ACTs due to their potent and rapid action against blood-stage Plasmodium parasites[7,13] . Beyond artemisinin, *A. annua* contains a rich array of secondary metabolites, including flavonoids (e.g., quercetin, luteolin), coumarins (e.g., scopoletin), terpenoids, and phenolic acids (e.g., chlorogenic and caffeic acids). These compounds have demonstrated various pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects[3,14] . Emerging research suggests that several of these constituents may exhibit intrinsic antiplasmodial activity or potentiate the effects of artemisinin through synergistic interactions, potentially delaying the onset of resistance. However, systematic investigations into their molecular mechanisms of action, drug-likeness, and target specificity are still limited. Advances in *in silico* tools, such as molecular docking, ADME profiling, and target prediction algorithms, now enable high-throughput screening and prioritization of bioactive molecules from complex herbal matrices. These methods offer a cost-effective and time-efficient platform for natural product-based drug discovery.

The present research focuses on investigating the antimalarial potential of phytochemicals isolated from *Artemisia annua* using comprehensive *in silico* methodologies. The aim is to screen and evaluate selected compounds for their interaction with malaria-relevant protein targets, predict their pharmacokinetic properties, and assess their suitability as drug candidates. Through molecular docking simulations, the study seeks to determine the binding affinity and interaction profiles of these compounds with four key protein targets associated with *Plasmodium* biology and drug resistance, namely: *Plasmodium falciparum* Dihydrofolate Reductase (PfDHFR), Carbonic Anhydrase isoforms, and the Multidrug Resistance Protein 1 (PfMDR1). To facilitate a systematic evaluation, the specific objectives of the study are outlined as follows. First, to perform molecular docking of phytochemical constituents from *Artemisia annua* against selected *Plasmodium*-associated protein targets in order to assess their potential inhibitory interactions. Second, to evaluate the ADME properties and drug-likeness of these compounds using Lipinski's Rule of Five and SwissADME

profiling tools. Third, to predict the possible protein targets of the selected compounds using SwissTargetPrediction, providing insights into their mechanism of action and selectivity. Finally, the study aims to identify and prioritize lead compounds that demonstrate strong binding affinity, favorable pharmacokinetic properties, and target specificity for further in vitro and in vivo validation studies.

Materials and Methods

2.1. Proteins / Macromolecules

Four malaria-related proteins were selected as macromolecular targets based on their crucial roles in Plasmodium falciparum survival and resistance. The crystal structures were retrieved from the Protein Data Bank (PDB) in .pdb format. The selected proteins were:

- 1O5X – Plasmodium falciparum Dihydrofolate Reductase (PfDHFR)
- 4DP3 – Carbonic Anhydrase
- 4GAE – Carbonic Anhydrase Isoform
- 7SXZ – Multidrug Resistance Protein (PfMDR1)

These proteins are involved in essential biological processes such as folate metabolism, pH regulation, and drug efflux, making them attractive targets for antimalarial drug design. Protein preparation involved removal of water molecules and heteroatoms, followed by energy minimization using PyRx tools.

2.2. Ligand Collection

Phytochemical constituents of Artemisia annua were selected based on literature reports of antimalarial activity. The 3D structures of 20 compounds, including artemisinin, artesunate, dihydroartemisinin, quercetin, and luteolin, were downloaded from the PubChem database in .sdf format. These compounds are listed in the observation table along with their binding affinities.

2.3. Molecular Docking

Molecular docking was performed using PyRx 0.8, integrating AutoDock Vina as the docking engine. Ligands were docked into the active sites of selected proteins to estimate their binding affinities and interactions. Grid boxes were defined around the active sites, and Lamarckian Genetic Algorithm was employed. The best-docked conformation based on binding energy (kcal/mol) was selected for further analysis.

2.4. ADME Analysis

ADME properties were predicted using SwissADME. Drug-likeness was assessed through Lipinski's Rule of Five, which stipulates that molecules with poor oral bioavailability tend to have a molecular weight >500 Da, more than 5 hydrogen bond donors, more than 10 hydrogen bond acceptors, and ClogP >5.

2.5. Target Prediction

SwissTargetPrediction was used to predict the most likely protein targets for the selected phytochemicals. The tool compares the chemical similarity of query molecules to known bioactives based on 2D and 3D fingerprints. This analysis provided insights into the potential mechanism of action by identifying relevant protein targets associated with malaria pathogenesis.

Observation and Results

TABLE 1 : *In Silico* study of *Artemisia annua*

Sr. No.	Phytochemical Constituents	Molecular Docking								ADME					Target Predicted
		1st protein 1O5X		2nd protein 4DP3		3rd protein 4GAE		4th protein 7SXZ		Mol. Wt.	#H-bond acceptors	#H-bond donors	iLOGP	Lipinski #violations	
		*B.A.	RMSD value	*B.A.	RMSD value	*B.A.	RMSD value	*B.A.	RMSD value						
1	Artemisinin	-7.3	33.038	-8.4	76.066	-7.5	50.254	-8.4	36.485	282.33	5	0	2.72	0	Carbonic anhydrase

2	Dihydroartemisinin	-7	5.524	-7.9	20.63	-7.3	28.377	-7.8	2.675	284.35	5	1	2.49	0	Carbonic anhydrase
3	Artesunate	-7.9	7.244	-9.3	2.07	-6.9	41.09	-8.5	30.866	384.42	8	1	2.92	0	Plasmodium falciparum dihydrofolate reductase (PfDHFR)
															Carbonic anhydrase
4	Dihydroartemisinic acid	-6.7	4.18	-7.6	4.203	-7.7	4.665	-7.3	51.412	236.35	2	1	2.67	0	Carbonic anhydrase
5	9-epi-artemisinin	-7	11.939	-8.5	2.269	-7.6	42.015	-7.7	65.402	282.33	5	0	2.71	0	Carbonic anhydrase
6	Artemisia ketone	-4.5	2.452	-4.9	3.797	-5.1	4.039	-4.9	41.446	152.23	1	0	2.46	0	Carbonic anhydrase
7	Thujone	-5.3	2.539	-5.5	76.061	-6	3.169	-5.4	2.588	152.23	1	0	2.28	0	Carbonic anhydrase
8	Camphor	-5.4	3.488	-5.5	21.986	-5.7	4.482	-5.2	55.803	152.23	1	0	2.12	0	Carbonic anhydrase
9	Artemetin	-6.8	9.497	-8.1	70.097	-7.4	45.675	-6.6	30.691	388.37	8	1	3.59	0	Carbonic anhydrase
															Multidrug resistance protein 1 (PfMDR1)
10	Casticin	-6.9	3.366	-7.8	8.96	-7	2.345	-7.3	54.053	374.34	8	2	3.44	0	Carbonic

															anhydrase
															Multidrug resistance protein 1 (PfMDR1)
11	Luteoline	-7.5	8.393	-8.7	8.489	-8.1	7.014	-8	65.24	286.24	6	4	1.86	0	Carbonic anhydrase
															Multidrug resistance protein 1 (PfMDR1)
12	Kaempferol	-7.3	9.786	-8.1	9.061	-7.9	29.027	-7.8	2.759	286.24	6	4	1.07	0	Carbonic anhydrase
															Multidrug resistance protein 1 (PfMDR1)
13	Quercetin	-7.6	8.097	-8.3	6.55	-7.9	28.163	-8.2	2.741	302.24	7	5	1.63	0	Carbonic anhydrase
															Multidrug resistance protein 1 (PfMDR1)
14	Apigenin	-7.3	6.582	-8.4	75.478	-8	27.902	-7.6	22.163	270.24	5	3	1.89	0	Carbonic anhydrase
															Multidrug

															resistance protein 1 (PfMDR1)
15	Acacetin	-7.4	7.195	-8.5	70.027	-7.9	50.711	-7.9	59.462	284.26	5	2	2.56	0	Carbonic anhydrase
															Multidrug resistance protein 1 (PfMDR1)
16	Chlorogenic acid	-7.6	8.476	-7.9	29.519	-7.2	2.955	-8	2.881	354.31	5	8	0.87	0	Carbonic anhydrase
17	Caffeic acid	-7	6.016	-6.4	57.307	-6.4	47.845	-6.2	62.566	180.16	4	3	0.97	0	Carbonic anhydrase
18	Scopoletin	-6.6	14.443	-6.6	27.605	-6.6	67.554	-6.4	44.297	192.17	4	1	1.86	0	Carbonic anhydrase

*B.A.=Binding Affinity/ Energy

Discussion

The *in silico* evaluation of phytochemical constituents derived from *Artemisia annua* provided compelling insights into their antimalarial potential. Among the diverse range of compounds analyzed, artemisinin derivatives and plant flavonoids emerged as the most promising candidates. Notably, artesunate exhibited the highest binding affinity with the 4DP3 protein (Carbonic Anhydrase) at -9.3 kcal/mol, indicating a strong and stable interaction[2,13] . This suggests its significant inhibitory potential against key enzymes in *Plasmodium falciparum*, particularly *Plasmodium* Dihydrofolate Reductase (PfDHFR), a central enzyme involved in the folate biosynthesis pathway critical for parasite DNA synthesis and cell division.

Other artemisinin derivatives, such as artemisinin itself, dihydroartemisinin, and 9-epi-artemisinin, also demonstrated effective binding interactions with multiple protein targets including 1O5X, 4GAE, and 7SXZ. These proteins correspond to isoforms of carbonic anhydrase and multidrug resistance-related transporters[12,13] . The ability of these compounds to bind efficiently to various macromolecular targets indicates a potential **multi-target mechanism of action**, which is highly desirable in modern antimalarial drug development. Multi-targeting can enhance therapeutic efficacy, minimize the chances of resistance development, and provide broader protection against different stages or strains of the *Plasmodium* parasite[3,14] .

The ADME profiling of the compounds further reinforced their drug-likeness. Most of the phytochemicals complied well with **Lipinski's Rule of Five**, which predicts good oral bioavailability[4,9] . Molecular weights remained below 500 Daltons, the number of hydrogen bond donors and acceptors were within acceptable limits, and lipophilicity (iLOGP) values supported adequate membrane permeability. Such favorable

pharmacokinetic parameters are crucial for compounds to be considered viable drug candidates for oral administration[4,10] .

Target prediction analysis using SwissTargetPrediction identified multiple malaria-relevant proteins, including PfDHFR and Multidrug Resistance Protein 1 (PfMDR1), which are both crucial for parasite survival and drug resistance modulation. The identification of PfMDR1 as a predicted target is especially noteworthy, given its role in mediating resistance to a wide range of antimalarial agents through drug efflux mechanisms[11,12] . The ability of certain *A. annua* compounds to interact with this protein suggests a potential to inhibit resistance pathways and restore sensitivity to existing therapies[12] .

These findings are consistent with the established antimalarial properties of artemisinin and extend the therapeutic potential to other non-endoperoxide phytochemicals found in *Artemisia annua*[3,14] . Compounds like quercetin, kaempferol, and chlorogenic acid—traditionally regarded for their antioxidant activity—demonstrated respectable binding affinities and ADME profiles, indicating they may contribute synergistically or serve as novel scaffolds for future antimalarial agents[6,14] .

Conclusion

The present in silico investigation successfully identified a range of bioactive phytochemicals from *Artemisia annua* with notable potential for antimalarial activity. Through detailed molecular docking studies, compounds such as artesunate, artemisinin, dihydroartemisinin, and 9-epi-artemisinin demonstrated strong binding affinities with key *Plasmodium falciparum* proteins, including dihydrofolate reductase (PfDHFR), various isoforms of carbonic anhydrase, and the multidrug resistance protein PfMDR1[2,5,13] . Among these, **artesunate** stood out as the most promising candidate due to its exceptionally high binding energy of -9.3 kcal/mol, indicating a strong and stable interaction with the target site and supporting its known clinical efficacy.

The ADME profiling of all tested compounds further confirmed their potential suitability as oral drugs. Most phytochemicals adhered strictly to Lipinski's Rule of Five, reflecting good pharmacokinetic properties, low toxicity risks, and favorable bioavailability[4,9,10] . These characteristics enhance their drug-likeness and suggest their potential for development into viable antimalarial agents.

This study extends the therapeutic promise of *A. annua* beyond artemisinin alone. Several non-endoperoxide compounds, particularly flavonoids such as quercetin, luteolin, and kaempferol, showed favorable interactions with parasite targets and satisfactory drug-like profiles. These findings suggest that the synergistic effects of multiple phytoconstituents in *A. annua* may contribute to its traditional effectiveness and could be harnessed in novel polyherbal or combinatorial drug formulations[3,14] .

The integration of molecular docking, pharmacokinetic analysis, and target prediction provides a powerful approach to prioritize compounds for further research. However, these computational predictions must be complemented by rigorous **in vitro** and **in vivo** studies to confirm the biological efficacy, safety, and potential synergism of these compounds[8,14,15] . The outcomes of this study lay a strong foundation for future drug discovery efforts aimed at combating drug-resistant malaria through nature-inspired therapeutic strategies.

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