Unlocking Nature's Arsenal: Drug design with *Zanthoxylum armatum* against Malaria

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Abstract:

Malaria remains a significant global health challenge, necessitating innovative approaches in drug development to address the growing resistance of the Plasmodium parasite. In this study, we explored the potential of the herb Zanthoxylum armatum as a treatment for malaria, given its rich composition of phytochemicals. Using molecular docking techniques, we focused on the interactions between these phytochemicals and key malarial receptor proteins: PfAMA1, 1F9-3D7, PvRON2, and PfAMA1. Among the various compounds analyzed, α-Amyrins exhibited the strongest docking efficiency, suggesting its potential as a promising anti-malarial candidate. PfAMA1 and PvRON2 are crucial receptor proteins involved in the invasion mechanisms of Plasmodium vivax and Plasmodium falciparum, making them attractive targets for drug development. The successful binding of α-Amyrins to these proteins highlights its potential to disrupt the parasite's invasion process. Gaining insight into the molecular interactions between α-Amyrins and malarial proteins could provide the structural foundation for designing new anti-malarial therapies. These findings contribute to the growing body of evidence supporting the use of natural products in combating malaria. With its diverse array of bioactive compounds, Zanthoxylum armatum serves as a valuable resource for drug discovery. However, further experimental studies are required to validate the efficacy, safety, and potential of α-Amyrins and its derivatives as a novel class of anti-malarial agents. This research marks an important step in leveraging nature's resources for the development of effective and sustainable anti-malarial treatments.

Keywords:

Malaria, Drug development, Zanthoxylum armatum, Phytoconstituents, Molecular docking

1. Introduction:

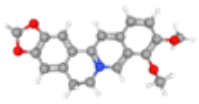
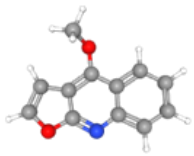
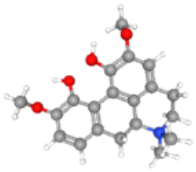
In 2017, malaria affected cases were 219 million, leading to 435,000 deaths (W. H. Organization, 2023). Over three billion people are suffering from malaria, which is estimated to impact 97 nations, and 600,000 deaths yearly worldwide (A. F. Cowman, J. Healer, D. Marapana, & K. Marsh, 2016) . Malaria, the most widespread vector borne disease in Africa and certain Asian countries, has the highest number of native cases (Alert, 2018; P. A. H.

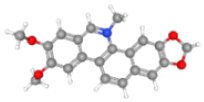
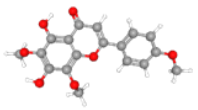
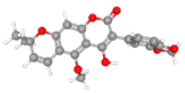
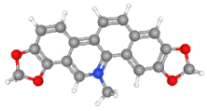
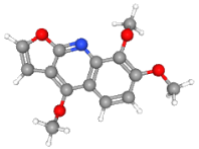
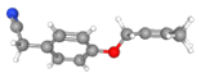
Organization, 2017; PAHO, 2017). Various studies have indicated that the prevalence of malaria parasite infection has risen since 2015. The causative agent of malaria is a small protozoan from the Plasmodium species group, comprising several subspecies. Some Plasmodium species can lead to illness in humans (Escalante & Pacheco, 2019; P. A. H. Organization & Organization, 2018). The Plasmodium genus is an amoeboid intracellular parasite that accumulates malaria pigment, an insoluble hemoglobin metabolite (Hilgenfeld, Nagarajan, Alterio, Hogg, & Schmidt, 2007; Roepe, 2008). These parasites can be found in various vertebrates, including red blood cells and tissue. Out of the 172 Plasmodium species, five can infect humans: *P. malariae, P. falciparum, P. vivax, P. ovale,* and *P. knowlesi* (Antinori, Galimberti, Milazzo, & Corbellino, 2012; Singh & Daneshvar, 2013). The deadliest and most highly pathogenic parasite, *P. falciparum(*Prugnolle et al., 2011*)*, is the cause of malaria in humans (Ogony, 2021, Daxon, 2019). The symptoms included chills, fever, and a headache. The clinical symptoms of malaria are primarily due to rupture and the destruction of erythrocytes. The genome sequencing serves as the basis for upcoming research on this organism and is being used to develop novel anti-malarial medications and vaccines (Vanheer et al., 2023). Malaria mortality has decreased because of increased antimalarial drug accessibility that is now safe, efficient, practical, and cost-effective. Antimalarial medications target specific stages of the parasite's life cycle. Prophylactic drugs stop the parasite from establishing itself in the liver, while schizontocidal drugs act on the parasite in the red blood cells to prevent or stop the clinical attack (Warrell, Watkins, & Winstanley, 2017; White, 1988).

The plant named as Indian prickly ash, Nepal pepper and toothache tree, scientific name is *Zanthoxylum armatum* DC, belongs to Rutaceae family (Sikarwar, Tiwari, Shukla, & Sikarwar, 2023). Local names of this plant are Tejphal (Hindi), Tejowati (sansikrit), Mukhtrubi (Manipuri) and Timur (Nepal) ("TIMUR KO ACHAAR," 2022). It can be found all over Northeast India and is extensively dispersed throughout India, from Kashmir to Bhutan at altitudes up to 2,500 m. At elevations between 1,300 and 1,500 m, it is also present in most of the areas of China, Taiwan, Nepal, the Philippines, Malaysia, Pakistan, and Japan. The species typically inhabits valleys and thickets in mountains, wastelands, and the understory of mixed forests. It is a sizable spiky shrub or small tree. In the family Rutaceae, there are 250 species native to temperate and subtropical regions of the world and used as the source of pharmaceuticals and cosmetics. Different species of *Z. armatum* are commonly used nowadays(Muthaura et al., 2007). In traditional systems of medical treatment, *Z. armatum's* tree bark, fruits, and seeds are widely employed as carminatives, stomachic, and anthelmintics and the dried fruit serves as a spice(Barkatullah, Ibrar, & Hussain, 2009). The fruit and seeds are used to treat dyspepsia and fever with an aromatic tonic. Round worms can be eliminated using a substance extracted from the fruit. The fruits' deodorizing, sanitizing, and antibacterial qualities make them useful for dental issues, and their ointment is used to treat infestations (Adam & Mitchell, 2024). In addition, they are employed to deter houseflies as well. When suffering from cholera and indigestion, people consume a powdered combination of its dried fruit, dried *Mentha longifolia* leaves, *Trachyspermum ammi* seeds, and black salt with water. The leaves can be utilized as an alternative toothbrush for toothaches and gums conditions(Abbasi et al., 2010). Alkaloids, sterols, phenolics, lignins, terpenoids, flavonoids and their glycosides, benzenoids, fatty acids, alkenic acids, and amino acids are only a few of the phytochemical components that have been identified from this plant. The *Z. armatum* extracts are also used as a leech repellent, anti-skin sensitivity(Guglielmini, Cristoni, & applications, 2006), lousicidal, anti-inflammatory (linalool and linalyl acetate)(Peana et al., 2002), antibacterial, antifungal and cytotoxic activities(A. Khan, Rahman, & Islam, 2008).

Malaria mortality has decreased as a result of increased antimalarial drug accessibility that is now safe, efficient, practical, and cost-effective(Kincaid et al., 2022). The asexual form of the parasite in human erythrocytes has been inhibited or killed by many medications, including quinine, chloroquine, pyrimethamine, sulfonamides, sulfones, and artemisinin derivatives (Rathmes et al., 2020). Due to parasites that have developed drug resistance, these medications have largely failed to aid in the recovery process (Narwal et al., 2024). Due to toxicities, poor compliance, and the introduction of more effective and efficient modern treatments, quinine, the first medication used to treat malaria about 400 years ago, should no longer be used to treat simple malaria (Achan et al., 2011). In this research endeavor, the primary objective is to design a pharmaceutical agent with exceptional efficacy while also conferring resistance against parasitic activities. The present investigation has been undertaken to assess the inhibitory capabilities of alkaloid phytochemical compounds, all sourced from *Z. armatum*. In our studies we computationally assessed these compounds for their effectiveness against *P. falciparum*, the parasite responsible for malaria. Molecular docking and molecular dynamic simulations were carried out to guarantee the reliability of interactions between these compounds and their target proteins. The stability of the complex formed between proteins and ligands was confirmed through these simulations. The approach of network pharmacology has become increasingly important, providing a comprehensive method for creating 'protein-compound/disease-gene' networks to identify simultaneous treatment pathways (Wei, Zhang, Liang, Piao, & Zhu, 2022; Zhou et al., 2020) .These approaches are also useful for anticipating compound toxicity, classifying drugs, and bioactivity. Scientists commonly integrate network pharmacology with molecular docking to comprehend drug-target interactions, predict potential drug candidates more efficiently, and hasten the drug discovery process (Ibrahim et al., 2023). Furthermore, different Pharmacokinetic analyses were performed to assess the drug-like properties and toxic potential of these phytochemicals. These evaluations are crucial for identifying potential candidates for the development of new anti-malarial drugs.

Table 1. The molecular weight, molecular formula, and structure of specific phytochemicals from *Z. armatum.*

Ligand name	PubChem CID	Molecular formula	Molecular weight	Structure
Berberine	CID2353	$C_{20}H_{18}NO_4^+$	336.4g/mol	
Dictamnine	CID 68085	$C_{12}H_9NO_2$	199.20g/mol	
Haplopine	CID5281846	$C_{13}H_{11}NO_4$	245.13g/mol	
Magnoflorine	CID 73337	$C_{20}H_{24}NO_4^+$	342.4g/mol	

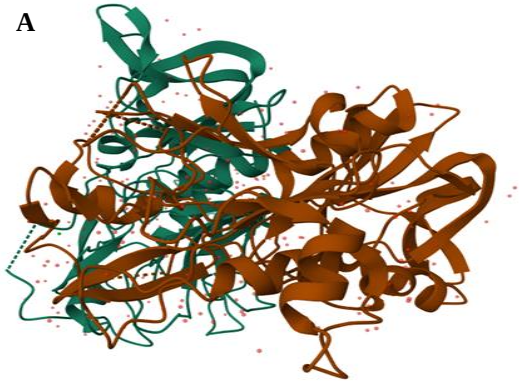
Nitidine	CID 4501	$C_{20}H_{24}NO_4^+$	342.4g/mol	
Nevadensin	CID160921	$C_{18}H_{16}O_7$	344.3g/mol	
Robustinee	CID164950	$C_{12}H_9NO_3$	215.20g/mol	
Sanguinarine	CID5154	$C_{20}H_{14}NO_4^+$	332.3g/mol	
Skimmianine	CID6760	$C_{14}H_{13}NO_4$	259.26 g/mol	
Zanthonitrile	CID 5315425	$C_{13}H_{15}NO$	201.26g/mol	

2. Methodology:

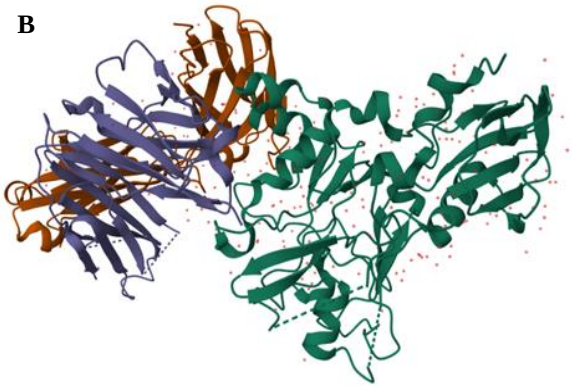
Receptor selection

The human body contains the AMA 1 receptor, known as Apical Membrane Antigen 1, and three different antimalarial receptor proteins, which are P *f* AMA 1 (PDB ID: 1Z40), 1F9-3D7 (PDB ID 2Q8A), and PvRON2 and P *f* AMA 1 (PDB ID:5NQF), specifically for malaria(Kocken et al., 2000; Tonkin et al., 2011). The protein structures in 3D format were obtained from RCSB PDB. Subsequently, using the Discovery Studio software, the ligands and other small molecules or atoms were eliminated from the receptors. Further refinements were made by removing water molecules, adding polar hydrogen atoms, and assigning charges to the receptors. The final refined receptor structures were then saved in PDBQT format, for docking experiments. Figures. 1 (A), (B) and (C) are receptor proteins.

A



B



C

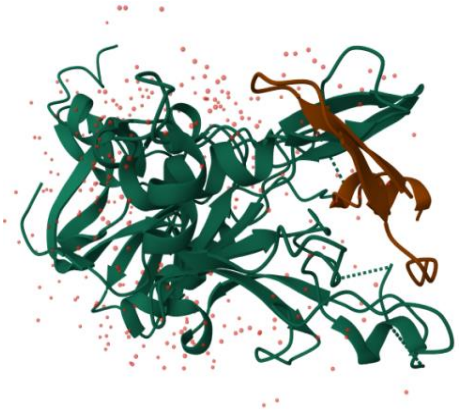


Figure 1. Representation of the 3-Dimensional structure of Malarial receptor proteins at the cell surface (A) P *f* AMA1, (B) 1F9-3D7 and (C) PvRON2 and P *f* AMA1

Prediction of activity spectra for substances (PASS) analysis

The outlook of Activity the Spectra for Substances (PASS) analysis program *i.e.*, this analytical tool is designed to predict a range of important properties of studied phytochemicals, including their biological properties, pharmacological characteristics, drug-likeness, potential side effects, and likely mode of action. These predictions are made based on the structural and active relationships observed with established chemical entities(T. Khan et al., 2017). The PASS analysis was executed through a combination of both online and offline resources, as outlined below.

Lipinski's rule of five

This study examines the molecular attributes of drug compounds, with a specific focus on their pharmacokinetic properties such as absorption, distribution, excretion, and metabolism. This investigation plays a pivotal role in guiding the design and development of pharmaceutical agents. The research specifically assessed the drug-likeness of 52 compounds derived from *Z. armatum*. To estimate Lipinski's Rule of Five an online tool known as Molinspirationv2021, (https://www.molinspiration.com/cgi-bin/properties) was employed for calculations and comparative analyses. The determination of Lipinski's Rule of Five was based on certain predefined criteria, including the logarithm of the partition coefficient between n-octanol and water (log P $\leq$ 5), molecular weight (MW $\leq$ 500), the number of hydrogen bond donors (NOHNH $\leq$ 5), hydrogen bond acceptor sites (NON $\leq$ 10), topological polar surface area (TPSA $\leq$ 140 Å^2), and the number of rotatable bonds ($\leq$ 10). It is crucial to note that, to maintain bioavailability, an orally active drug should not exceed a single Lipinski violation(Santos, Ganesan, & Emery, 2016).

Bioactivity score

The bioactivity score indicates the likelihood that the potential complex will be developed into a drug. The bioactivity score of phytochemicals against human receptors, such as receptors associated with G proteins, kinase, protease, ion channels, enzymes, and nuclear receptors, is determined by a computational tool called Molinspiration version 2021.13. If the score is higher than 0.0, the compound is active; if it is between -0.5 and 0.0, it is moderately active; and if it is less than -0.5, it is inactive(Jindal, Rani, & Biotechnology, 2023).

Toxicity risk assessment

The study provides an assessment of the anticipated adverse effects of a pharmaceutical compound on human health. The Protox-II server was employed to evaluate the toxicological properties of phytochemicals found in *Z. armatum*, encompassing aspects such as

hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and tumorigenicity. Toxicity categorizations were established in alignment with the Globally Harmonized System (GHS) criteria for the classification and labeling of chemical compounds(Banerjee, Eckert, Schrey, & Preissner, 2018).

Pharmacokinetic activity prediction

Utilizing the online tool Swiss-ADME software, the study employed predictive modeling to assess the absorption, distribution, metabolism, excretion, and toxicity (ADMET) attributes of all phytochemical compounds under investigation. This analytical approach focuses on key pharmacokinetic parameters, encompassing considerations such as blood-brain barrier (BBB) permeability, tissue distribution, gastrointestinal (GI) absorption, metabolism of P-glycoprotein (P-gp) substrate interactions, metabolic effects as an inhibitor of CYP3A4, and the fluidity pertinent to plasma membrane absorption(Muhammed et al., 2021).

AutoDock Vina 1.5.6

The ligands were selected to incorporate into rigid receptors by the AutoDock version 1.5.6. due to great speed and processing (Huey, Morris, & Forli, 2012). AutoDock vina is used in which grid values are calculated automatically. In this research, the Grid box size was 40×40×40 (x, y and z) and spacing was 0.375 for 3 receptors. For 1Z40 the center of the grid box was set at the position of 11.657 ,12.407 and 63.734 for x, y and z respectively. For2Q8A the center was set at the position of -8.789, -26.494 and 20.909 for x, y and z. For 5NQF the center of Grid was set at -24.064, -4.509 and -13.838 for x, y and z respectively. for each ligand, the configuration file containing Grid box values was calculated and saved in txt. format. The next step was docking analysis for which the command or code was generated in the command prompt and output was obtained. The output was in PDBQT format. The result displayed the binding affinity of ligand with receptor that was Gibbs free energy in kcal mol^{-1} ·in these energy values the negative highest value was considered as the highest binding affinity for receptor proteins. For the above 50 values the best fit one was selected. All the profiles were displayed in Discovery Studio and 2D output image having ligand interactions with amino acid residues was selected(Vong, Hwang, Chee, Sim, & Biotechnology, 2022).

Molecular Dynamic Simulation

For the study of interactions and movement, molecular dynamic simulations were performed(Karplus & McCammon, 2002). To predict binding rearrangements, simulations can be used to locate the most important interactions a ligand has with the binding pocket(Spahn et al., 2017)Using the CABS-Fex 2.0 server, molecular dynamic simulations were used to confirm the protein elasticity of the best docked structures. The resulting RMSF values (root mean square fluctuation) were then shown. The default parameters for Cabsflex simulations were as follows: temperature range of 1.40, number of cycles: 50, number of cycles between trajectories: 50, protein rigidity: 1.0, and random number of generators seed 432. Fast, high-resolution (10 ns) protein flexibility simulation with drastically decreased system limitations is provided by Cabsflex (Srivastava, 2022; Yuniwati, Syaban, Anoraga, & Sabila, 2022).

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3. Results:

Lipinski's rule of five

The Lipinski's rule of five contain the PASS analysis of selected phytoconstituent of *Z. armatum* (Table 2). None of the compounds have shown any violations as per standards of ideal lead compounds.

Table 2. Lipinski's rule of five of selected phytochemicals from *Z. armatum*

Ligands	**M logP (<4.15)**	**MW (<500g/mol)**	**NOHNH (<5)**	**NON (<10)**	**TPSA(A^2) (<140°A)**	**Number of rotatable bonds (<10)**	**Lipinski's Violation**
Berberine	2.53	336.36	4	0	40.80	2	Yes'0 violation
Dictamnine	2.63	199.21	3	0	35.26	1	Yes'0 violation
Haplopine	2.24	245.23	5	1	64.72	2	Yes'0violation
Magnoflorine	0.65	342.41	4	2	58.92	2	Yes'0 violation
Nitidine	2.95	348.37	4	0	40.80	2	Yes'0 violation
Nevadensin	2.42	344.32	7	2	98.36	4	Yes'0 violation
Robustine	2.27	215.20	4	1	55.49	1	Yes'0 violation
Sanguinarine	2.88	332.33	4	0	40.80	0	Yes' 0 violation
Skimmiamine	2.52	259.26	5	0	53.72	3	Yes' 0 violation
Zanthonitrile	2.88	332.33	4	0	40.80	0	Yes'0 violation

Bioactivity prediction

The bioactivity score prediction showed that Magnoflorine is active in GCPR ligand, Ion channel modulators and enzyme inhibitors but moderately active in kinase inhibitors, nuclear receptor ligands and protease inhibitors. Nevadensin are active Kinase inhibitor, nuclear receptor ligands and enzyme inhibitors and moderately active in GCPR Ligand, ion channel modulators and protease inhibitors.

Nitidine and Sanguinarine are active Ion channel modulators, kinase and enzyme inhibitors and moderately active GCPR Ligand, protease inhibitors and inactive in nuclear receptor ligands. Haplopine, Robustine, Skimmiamine and Berberine are active Ion channel modulator and enzyme inhibitor. Dictamnine is active Ion channel modulators. The highest values of enzyme inhibitor were shown by Berberine (i.e. 0.8) and sanguinarine (i.e. 0.5) and ion channel modulator by Magnoflorine (i.e. 0.7), Berberine (i.e. 0.7) and Sanguinarine (i.e. 0.5). Other details are shown in Table 3.

Table 3. Bioactivity score of selected phytochemicals from *Z. armatum*

Phytoconstituents	**GPCR ligand**	**Ion channel modulator**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibitor**	**Enzyme Inhibitor**
Zanthonitrile	-0.52	-0.49	-0.79	-0.24	-0.68	-0.28
Skimmiamine	-0.26	0.25	-.0.01	-0.77	-0.62	0.15
Sanguinarine	-0.03	0.50	0.17	-0.76	-0.14	0.54

Robustine	-0.41	0.32	-0.14	-0.97	-0.82	0.15
Nitidine	-0.12	0.33	0.01	-0.83	-0.17	0.36
Nevadensin	-0.16	-0.12	0.12	0.03	-0.28	0.17
Magnoflorine	0.37	0.78	-0.20	-0.26	-0.19	0.26
Haplopine	-0.24	0.35	-0.01	-0.72	-0.66	0.23
Dictamnine	-0.61	0.31	-0.23	-1.27	-0.93	-0.01
Berberine	-0.11	0.71	-0.27	-0.78	-0.35	0.82

Pharmacokinetic properties

The studies show that the Nevadensin does not cross the blood-brain barrier and remains cross the BBB barrier and show positive results**.** Berberine, Magnoflorine, Nitidine, Sanguinarine and Tambuletin show positive responses of P-gp substrate, and the remaining were negative. These results show that the negative will be persistent in the cell and show the activity in the cell. The understudy substances were anticipated to inhibit the various classes of cytochromes for continuous plasma concentrations and increased bioavailability P450, i.e.,CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. All compounds show inhibitory effect on CYP1A2 inhibitor. Berberine, Haplopine, Nevadensin, Robustine, Skimmiamine inhibits the CYP2D6 and berberine, Dictamnine, Haplopine, Magnoflorine, nitidine, Nevadensin , Robustine and Skimmiamine inhibits CYP3A4 inhibitors. All compounds show high GI Absorption. The other information is given below in Table 4.

Table 4. Pharmacokinetic study of phytochemicals of *Z. armatum***.**

Ligands	**GI absorption**	**BBB permeant**	**P-gp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**Log Kp**
Berberine	High	Yes	Yes	Yes	No	No	Yes	Yes	-5.78cm/s
Dictamnine	High	Yes	No	Yes	No	No	No	Yes	-5.46cm/s
Haplopine	High	Yes	No	Yes	Yes	No	Yes	Yes	-.6.01cm/s
Magnoflorine	High	Yes	Yes	Yes	No	No	No	Yes	-6.44cm/s
nitidine	High	Yes	Yes	Yes	Yes	No	No	Yes	-5.17cm/s
Nevadensin	High	No	No	Yes	No	Yes	Yes	Yes	-6.32cm/s
Robustine	High	Yes	No	Yes	Yes	No	Yes	Yes	-5.81cm/s
Sanguinarine	High	Yes	Yes	Yes	Yes	No	No	No	-5.17cm/s
Skimmiamine	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-5.87cm/s
Zanthonitrile	High	Yes	No	Yes	No	No	No	No	-5.26cm/s

Toxicity assessment

Using the Protox-II server the drug-likeliness and toxicity potential of different ligands were estimated. Robustine, Nitidine, Nevadensin and berberine has High Immunotoxin effects while Nevadensin and Robustine are mutagenic and berberine has cytotoxic effects.

Berberine and Robustine are slightly carcinogenic, lupeol is slightly Immunotoxin, berberine is slightly mutagenic and Nevadensine is slightly cytotoxic. The toxicity assessment result is given in Table 5. For the LD50 potential value of ligands LD 50 potential of all classes belong to Class 3 and 4. Other results are given below in Table 6.

Table 5. Predicted toxicity of risk assessment of phytoconstituents of *Z. armatum*.

Phytoconstituents	Mutagenicity	Hepatoxicity	Immunotoxicity	Cytotoxicity	Carcinogenicity
Berberine					
Dictamnine					
Haplopine					
Magnoflorine					
Nitidine					
Nevadensin					
Robustine					
Sanguinarine					
Skimmiamine					
Zanthonitrile					

Table 6. LD_{50} potential of drugs.

Ligands	LD50 Value	Toxicity Class
Berberine	200mg/kg	Class 3
Dictamnine	1000mg/kg	Class 4
Haplopine	1000mg/kg	Class 4
Magnoflorine	401mg/kg	Class 4
Nitidine	1190mg/kg	Class 4
Nevadensin	1190mg/kg	Class 4
Robustine	1000mg/kg	Class 4
Sanguinarine	1190mg/kg	Class 4
Skimmiamine	1190mg/kg	Class 4
Zanthonitrile	2000 mg/kg	Class 4

According to the GHS (Globally Harmonized System of Classification and Labeling of Chemicals), toxicity classifications are established. Values for LD50 are provided in [mg/kg] Class I: if swallowed, deadly (LD50 5) Class 2: If ingested, lethal (5 LD50 50) Class 3: poisonous if ingested (50 LD50 300) Class 4: dangerous if ingested (LD50: 300–2000) Class 5: May be dangerous if ingested (LD50: 2000–5000) Class 6: nontoxic (LD50 more than 5000)

Predicting the Druglikeness of Phytochemicals

Lipinski rule of five is stated above. All compound follows all laws except Dictamnine that violates Muegge law i.e. its weight is more than 200. All the compounds show positive bioavailability score.

Table 7. Druglikeness properties of phytochemicals of *Z. armatum*

Ligand	Lipinski	Ghose	Veber	Egen	Muegge	Bioavailability Score
Berberine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Dictamnine	Yes; 0 violation	yes	yes	Yes	No. 1violation:MW<200	0.55
Haplopine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Magnoflorine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Nitidine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Nevadensin	Yes; 0 violation	yes	yes	Yes	yes	0.55
Robustine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Sanguinarine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Skimmiamine	Yes; 0 violation	yes	yes	Yes	yes	0.55
Zanthonitrile	Yes; 0 violation	yes	yes	Yes	yes	0.55

Molecular Docking

A total of 10 Phytoligands from *Z. armatum* were docked with target proteins with the help of AutoDock vina v1.5.6. They show different ligand affinities with targets. For 1Z40 the affinities range from -9.3 Kcal/mol to -6.2 Kcal/mol. The highest binding affinity is shown by Nevadensin and Sanguinarine (i.e., -9.3 Kcal/mol) lowest energy. The lowest binding affinity was shown by Magnoflorine(i.e., -6.2 Kcal/mol). Sanguinarine shows Asn413, Lys395, Ser347, Lys351, pro330 and Nevadensin shows Gln349, Ser347, Lys351, pro330 .The lowest interacting amino acids residue is shown by berberine. Dictamnine and Haplopine don't show any interactions. The affinity value and amino acids are given below in a Table 7. Chimera displayed the docking postures, while Discovery Studio displayed the interacting amino acid residues.

For 2Q8A receptor proteins the affinity ranges from -8.5 Kcal/mol to -6.5 Kcal/mol. The highest affinity shown by -8.5 Kcal/mol i.e., by Sanguinarine and Nevadensin. Sanguinarine shows Val85, Lys103, Lys59 and Nevadensin shows Val85, Lys103, Lys9 .Lowest binding affinity shown by Zanthonitrile i.e., -6.5 Kcal/mol.

The receptor 5NQF the affinity ranges from -7.9 Kcal/mol to -5.6 Kcal/mol. The binding affinity shown by -7.9Kcal/mol by Nevadensin and the lowest shown by Dictamnine -5.6 Kcal/mol. The highest interacting amino acids residue shown by Nevadensin are Gln407, Arg404, Glu308, Val437, Ile435, Ile426, Asp333, Asn332, Tyr402.

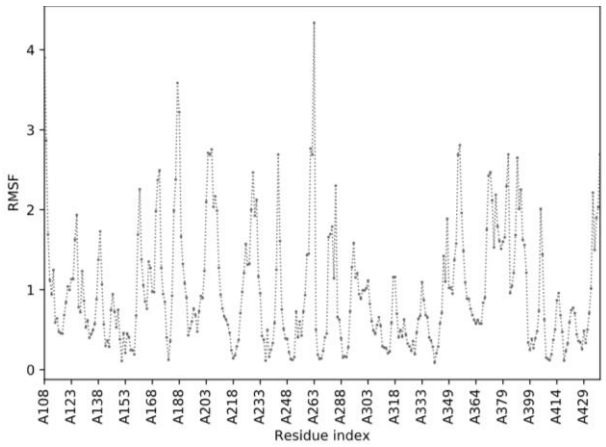
Table 8. Docking of selected phytochemicals from *Z. armatum* with receptor proteins of Malaria.

Ligands	Binding Affinity (Kcal/mol) with Receptor proteins

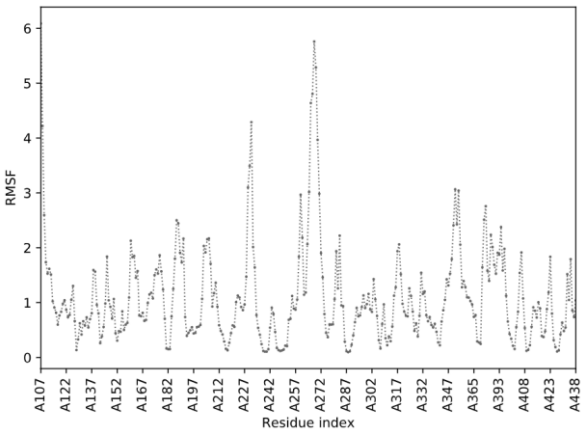
	1Z40	2Q8A	5NQF
Berberine	-6.4	-6.9	-6. 0
Dictamnine	-6.5	-6.6	-5. 6
Haplopine	-6.4	-6.9	-6. 0
Magnoflorine	-7.9	-7.3	-6. 9
Nitidine	-6.9	-8.2	-6. 8
Nevadensin	-9.3	-8.5	-7. 9
Robustine	-6.9	-6.8	-5. 9
Sanguinarine	-9.3	-8.5	-7. 8
Skimmiamine	-6.2	-6.8	-5. 8
Zanthonitrile	-6.4	-6.5	-6. 0

MD Simulations

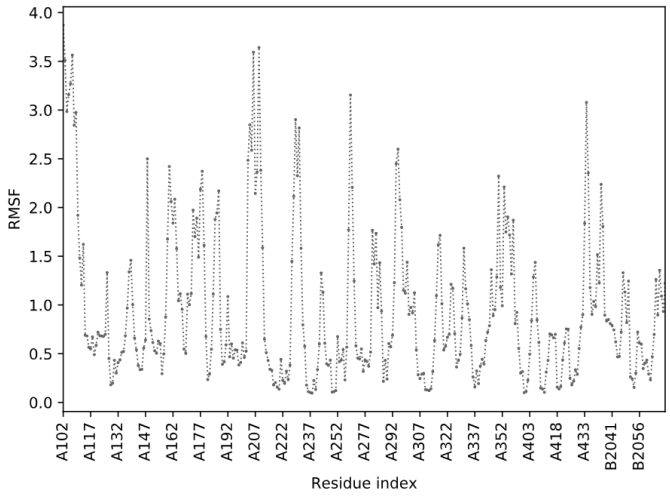
The structural flexibility of the top docking complexes was evaluated by Cabs-flex 2.0. Our results indicated that the RMSF value of 1Z40-Nevadensin complex was recorded below 4.8Å (Figure. 2A). Similarly, the RMSF value of the 2Q8A-Sanguinarine 5NQF-Nevadensin protein complexes were also as reduced as 5.6Å and 3.6Å (Figure 2B,2C). Since the RMSF value recorded for selected complexes was closer to the ideal value, i.e., 3.8Å, the complexes were said to have stable interactions. According to the RMSF graph, it does not show that when ligands bind to protein it alters the configuration and stability of protein.



1Z40-Nevadensin



2Q8A-Sanguinarine



5NQF-Nevadensin

Figure 3. The RMSF plot of the top docking complexes; (A) 1Z40-Nevadensin, (B) 2Q8A-Sanguinarine and (C) 5NQF-Nevadensin

5. Discussion:

The drug resistance continues to challenge the effectiveness of conventional malaria treatments, the exploration of medicinal plants rooted in traditional anti-malarial medicine represents a promising avenue for mitigating the impact of this deadly disease. The synthesis of indigenous knowledge with rigorous scientific inquiry can pave the way for the development of novel and effective anti-malarial interventions, offering hope for improved healthcare outcomes in malaria endemic regions of the world (Alonso, Chitnis, Hall, & Alliance, 2014; Karunamoorthi, 2014). Finding ligands that can bind successfully with the target receptors is the goal of the search for ligand-based medicines (Yang et al., 2021). This does not necessarily imply that the chemical would be effective if taken orally. Pharmacokinetic events, such as ADME, take drugs from their starting points to their goals and drug interactions in the body. Consequently, pharmacokinetics must be taken into account when developing new medications**.** Designing an oral active medication should adhere to Lipinski's rule of 5**.** This rule determines if specific chemical compounds have the necessary physical and chemical properties to be employed as active pharmaceutical ingredients that can be consumed by humans, as well as to compare different drugs.

According to Lipinski's Ro5 study, the five likely compounds with the highest BFE have excellent bioavailability since they follow the requirements. Therefore, it is anticipated that these chemicals will function when taken orally. This indicates that the understudy substances attaches to the receptor with ease and that the ligand can pass across the cell membrane with ease(Sadhana, Gupta, Amita, & Sciences, 2013).

The toxicity prediction of possible drug candidates is one of the most crucial elements in contemporary drug discovery. This comprises the most crucial considerations while looking for novel medications with potentially advantageous features, such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. A compound's acute toxicity is given as its median lethal dose (LD50). The studied ranged from 2000 (class 4 toxicity) to 5000 (class 5 toxicity) mg/kg of LD_{50} of the compounds. In general, the lower the LD_{50}, the more toxic the substance(Ruiz, Begluitti, Tincher, Wheeler, & Mumtaz, 2012). Toxicity potential suggests that none of the compounds is more toxic, but LD 50 potential of the bergapten and umbelliferon is more than 5000 and belongs to class 6. The current studies M log P us the effective treatment of malaria by *Z. armatum*. Magnoflorine has 0.65 low value of M log P without any violations (Daina, Michielin, & Zoete, 2014).

A pharmacoinformatic investigation of possible *Z. armatum* components revealed that these chemicals have a big impact on the development of antimalarial medications. In silico techniques will become more widely used in regulatory decision-making as public confidence in their applicability and dependability increases(N. Gellatly & F. Sewell, 2019) The three parameters that are usually considered when calculating molecular docking results are binding affinity, the interaction of the amino acid residuals involved, and the hydrogen bond energy. Molecular dynamics simulation studies in drug active ingredient designs are frequently used in predictive studies of potential ligand–receptor interactions(Salmaso & Moro, 2018). In an in silico physiological environment, simulations are accepted as a rational approach for evaluating the molecular dynamics and interactions between the ligand and the protein (Binder, Horbach, Kob, Paul, & Varnik, 2004)A molecular dynamics simulation of 50 ns duration was performed using CHARMM force fields, and RMSD, RMSF analysis were measured for both target proteins.

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6. Conclusion:

In conclusion, our research underscores the potential of natural compounds like alkaloids as crucial candidates for novel drug development. This research involved an exploration of the possible mechanisms involved in utilizing phytochemicals from *Zanthoxylum armatum* for the treatment of Malaria. Natural compounds offer a vast repertoire of bioactive molecules with diverse mechanisms of action, often exhibiting reduced toxicity and mutagenicity compared to synthetic counterparts. Harnessing the predictive potential of natural compounds in drug discovery not only expands our pharmacological arsenal but also holds promise for developing safer and more efficacious therapies. Thus, the inclusion of α-amyrins and other phytochemicals in drug development pipelines represents a strategic approach towards addressing global health challenges, including malaria, while also tapping into nature's vast reservoir of therapeutic compounds.

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