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Molecular Docking and Dynamics Simulation Assessment of Multiple Bioactive Compounds from the Zingiberaceae Family as anti-malarial Drug Candidates through Inhibiting Duffy Antigen Receptor for Chemokines (DARC) Protein

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Abstract

Malaria is a major threat to public health, particularly in tropical and subtropical regions. Malaria affects over half of the global population and killed approximately 597,000 people in 2023. Notably, malaria elimination is a major global challenge and has become a top priority worldwide. Thus, in the present study, we aimed to evaluate the antimalarial effects of bioactive compounds from the *Zingiberaceae* family by inhibiting the Duffy antigen receptor for chemokines (DARC) protein using an in silico approach. Several bioactive compounds collected from fifteen *Zingiberaceae* species were collected in the present study. Prior to molecular docking, the structure validation of the DARC protein was performed. Furthermore, molecular docking, membrane permeability prediction, molecular dynamics simulation, and toxicity prediction were performed to further assessing the antimalarial potency of *Zingiberaceae* bioactive compounds. To a greater extent, molecular docking results demonstrated that isocoronarin D and 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (BHPHTO), compounds commonly found in *Alpinia galanga,* exhibited binding affinity scores comparable to those of the control drugs, Chloroquine. Interestingly, the chemical interaction of both compounds showed the favorable number compared to the control, especially at the initial and terminal stages of the molecular dynamics simulation. Furthermore, molecular dynamics simulation revealed that BHPHTO exhibited the most stable interaction with the DARC protein and maintained protein structure stability better than both the isocoronarin D and Chloroquine complexes. Together these findings suggest that BHPHTO could be developed as a potential anti-malarial drug candidate. However, further studies are needed to validate BHPHTO toxicity via in vitro or in vivo, and to clarify its detailed mechanism of action against DARC protein.

Keywords: DARC, drug discovery, in silico, malaria, *Zingiberaceae*.

1. Introduction

Up to date, malaria is still considered as a major public health problem, especially in tropical and subtropical areas. It has been recorded that almost half of the world population is susceptible to Malaria (Liu *et al*., 2021; Cowman *et al*., 2016). In 2023, approximately 597,000 deaths worldwide were attributed to malaria (Daily and Parikh, 2025). Despite that, children and pregnant women in underdeveloped countries have a major risk of malaria. Additionally, several factors contribute to the high incidence of malaria, which include biological and societal challenges such as other viral outbreaks, armed conflicts, limited access to healthcare, inadequate infrastructure, and natural disasters (Pattanshetty *et al*., 2024; Monroe *et al*., 2022). Up till now, based on World health Organization deadline (WHO) guidelines, there are several basic interventions which could be applied for malaria control and management such as Artemisinin-based combination therapy, indoor residual spraying, education and communication campaigns, intermittent preventive treatment for infants, intermittent preventive treatment in school-aged children, intermittent preventive treatment of pregnant women, larval source management, long lasting insecticidal nets, mass drug administration, perennial malaria chemoprevention, post-discharge malaria chemoprevention, rapid diagnostic tests, seasonal malaria chemoprevention, and targeted drug administration (Pattanshetty *et al*., 2024). Finally, eliminating malaria, particularly reducing its mortality and morbidity, has emerged as a significant priority and challenge, dependent on strategies such as insecticide-treated nets, antimalarial medications, and mosquito control programs.

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Malaria is caused by *Plasmodium* parasite which is transmitted via infected female *Anopheles* mosquitoes. It has been reported that *P. falciparum* and *P. vivax* cause the most serious infections which remain as major risk of malaria cases in underdeveloped countries. The life cycle of *Plasmodium* infecting humans consists of two stages, the initial development stage found in the liver, and the second proliferation stage found in the blood (Talapko *et al*., 2019). Understanding the parasite's life cycle has become a unique challenge to develop strategy to overcome malaria. On the other hand, several drugs such as quinine, chloroquine, and artemisinin have been used to treat malaria; however, the parasite has developed resistance to each, making effective control increasingly difficult. Therefore, the current challenge in malarial disease control requires a novel drug which could effectively inhibit the activities of the malarial parasites.

The DARC is a receptor protein that plays a critical role in *Plasmodium* invasion. To a greater extent, the DARC specifically serves as a receptor for *P. vivax* to invade red blood cell (Dean, 2005). Compared to the other *Plasmodium* species, *P. vivax* has a wider distribution and could survive in colder climates. It has been reported that the high expression of the DARC in malaria patients is correlated to the severe outcome (Kaur *et al*., 2019; Dean, 2005). Additionally, another investigation has demonstrated that the DARC polymorphisms affect susceptibility to clinical *P. vivax* and impact the immune response to malaria blood antigens, thereby compromising the effectiveness of future malaria vaccines (Oliveira *et al*., 2012). Targeting DARC is a promising therapeutic approach, as interfering with this protein can prevent the parasite from entering red blood cells and thus impair its ability to cause infection (Moskovitz et al., 2023).

Plant-derived natural products have been recognized for their therapeutic effects since ancient times, particularly in treating pain, wound healing, gut health, and ameliorating other ailments (Putra *et al*., 2023; Davila and Papada, 2023; Nasim *et al.*, 2022; Putra and Rifa'i, 2020; Putra et al., 2020; Putra and Rifa'i, 2019). *Zingiberaceae* family is a group of plants that exhibit adaptations to diverse ecological conditions, which are mostly found in tropical rainforests and moist environments. In addition to their economic value, *Zingiberaceae* plants exhibit significant therapeutic properties including antioxidant, anti-inflammatory, antimicrobial, anticancer, and antiemetic activities (Boonma *et al*., 2023). Additionally, some species such as *Zingiber nimmonii*, *Zingiber officinale*, and *Zingiber cernuum* demonstrated anti-malarial effects (Biruksew *et al*., 2018; Rajeswary *et al*., 2018; Govindarajan *et al*., 2016). In addition to their therapeutic potencies, *Zingiberaceae* plants serve as abundant sources of chemical compounds, including alkaloids, carbohydrates, proteins, phenolic acids, flavonoids, and diarylheptanoids which are known as a source of natural biopharmaceuticals (Ballester *et al*., 2023). Therefore, in the present study we aimed to evaluate the anti-malarial effect of various bioactive compounds from *Zingiberaceae* family by targeting the DARC protein.

2. Materials and Methods

2.1. Ligand structure preparation

The present study evaluated multiple bioactive compounds from fifteen species of Zingiberaceae family including Aframomum arundiaceum, Aframomum escapum, Aframomum latifolium, Aframomum zambesiacum, Alpinia galanga, Boesenbergia rotunda, Curcuma longa, Curcuma zanthorriza, Kaempferia galanga, Kaempferia rotunda, Renealmia cincinnata, Siphonochilus aethiopicus, Siphonochilus aethiopicus, Zingiber officinale, and Zingiber zerumbet (Heikal et al., 2024). Before molecular docking, the chemical structures of the ligands were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Each compound and control drug used in this study was identified with a unique PubChem Compound ID (CID), including Isocoronarin D (CID: 71550939), BHPHTO (CID: 71346280), and Chloroquine (CID: 2719). Then, each chemical structure was saved in sdf. file for further molecular docking analysis.

2.2. Target protein preparation

The target protein used in this study was human DARC, which was known as potential target for anti-malarial. The 3D structure of the protein was collected from the RSCB PDB database (https://www.rcsb.org/), with PDB ID. 4NUU. Furthermore, the protein optimization was performed to remove the native molecule and water, and at the same time, the polarity of the structure was added. The optimization was performed by using PyMOL software (https://www.pymol.org/) and Discovery Studio software (https://www.3ds.com/).

2.3. Protein structure validation

Structure validation server or SAVESv6.1. (https://saves.mbi.ucla.edu/) was used to perform the DARC protein validation. In this analysis, the Ramachandran plot and ERRAT analysis were conducted to evaluate the quality of the structure (Colovos *et al*., 1993). Moreover, protein structure analysis or ProSA-Web (https://prosa.services.came.sbg.ac.at/prosa.php) was utilized to correctly check the structure errors of DARC protein (Wiederstein and Sippl, 2007; Sippl, 1993).

2.4. Molecular docking and visualization and membrane permeability prediction

After finishing protein and ligand preparation, the PyRx 0.8 software (https://pyrx.sourceforge.io/) was used to perform molecular docking. The grid of molecular docking was set to cover all the surface of the DARC protein with specification grid box center (Å): X: 47.7956, Y:-45.000, Z:96.0143 and grid box dimensions (Å): X: 15.9225, Y: 24.8906, Z:14.8393. Binding affinity scores were analyzed as a result of molecular docking. Furthermore, the visualization of chemical interaction and the 3D structure of the complex were performed by using Discovery Studio software (Ruswanto *et al*., 2024; Hidayatullah *et al*., 2021; Putra and Rifa'i, 2020). In the present study, the PerMM webserver (https://permm.phar.umich.edu/) was used to estimate the potency of ligands to penetrate the plasma membrane (Lomize and Pogozheva, 2019; Lomize *et al*., 2019).

Additionally, the temperature was set to 310 K and the pH to 7.4 to mimic physiological conditions.

2.5. Molecular dynamics simulation and toxicity prediction analysis

YASARA software (http://www.yasara.org/) was used to perform molecular dynamic simulation to evaluate the stability of protein-ligand complex. The simulation was run for 1000 ps. Multiple parameters were analyzed such as potential energy, RMSD, RMSF, ligand movement, ligand conformation, solvent accessible surface, Hydrogen bonds, and DCCM (Hidayatullah *et al*., 2023; Hidayatullah *et al*., 2022). Finally, ProTox 3.0. web server (https://tox.charite.de/protox3/) was used to validate the toxicity prediction and the probability of the compounds to induce toxicity.

3. 3. Results and Discussion

In the present study, we were able to validated, and then discovered that the 3D structure of DARC is qualified and showed the high score value for its quality according to some parameters such as the Ramachandran plot, ProSA-web evaluation, and the ERRAT quality assessment (Fig. 1). According to the Ramachandran plot results, the DARC protein structure has a dominant number of residues occupying in most favored regions of 92.5%, in additional allowed regions of 7.3%, and in generously allowed regions of 0.2%. Moreover, ProSA-web demonstrated that the DARC protein structure has *Z*-score of -5.57. Additionally, the ProSA-web analysis demonstrated that the DARC protein has minimal errors which indicated by the window size mostly has negative value. Based on the ERRAT analysis, the DARC protein has an overall quality factor of 98.201, which indicates DARC structure has good quality. The study of protein structures is crucial for understanding the fundamental principles of protein function, which play a significant role in drug design (Ghosh *et al*., 2017). Protein structures are elucidated via experimental techniques such as X-ray crystallography and nuclear magnetic resonance. The protein structure may encounter global errors as a result from the misinterpretation of the polypeptide chain (Bagaria *et al*., 2012; Pugalenthi *et al*., 2006). Therefore, accurate structural validation of proteins is extremely crucial in a drug discovery study.

DARC is known as a potential target for malaria treatment. DARC becomes a key receptor for *P. vivax* to infect the red blood cells (Tobin *et al*., 2023). Interfering with *P. vivax* antigen-DARC interaction and avoiding its downstream activation are considered a novel anti-malarial strategy (Moskovitz *et al*., 2023). In this study, several bioactive compounds from multiple *Zingiberaceae* family have been evaluated for their anti-malarial activities by inhibiting the DARC protein. According to the docking result, we found that two hit-to-lead compounds namely BHPHTO and isocoronarin D have closely binding affinity scores to the control drug, Chloroquine (Fig. 2). BHPHTO and Chloroquine share a close value of binding energy due to their similar interaction characteristics, including several hydrogen bonds and hydrophobic interactions with critical residues in Chain C of DARC. Both compounds have advantageous docking poses and occupy similar binding site areas, which may explain their expected binding energies. Despite its structural differences and bioactivity, isocoronarin D has less interactions, such as hydrogen bonding or hydrophobic contacts, which may explain its reduced binding affinity.

Furthermore, we evaluated the penetration potency of these compounds by performing membrane permeability prediction. Interestingly, we found that both isocoronarin D and BHPHTO can easily penetrate the plasma membrane (Fig. 3). Isocoronarin D can penetrate cell membranes more easily because it is more hydrophobic, more compact, and less polar structure, which helps it pass through the lipid layer without needing any transport. Conversely, BHPHTO, although also including three oxygen atoms and many cyclic moieties, exhibits a wider array of polar functional groups, possibly enhancing its overall polarity and diminishing its lipophilicity.

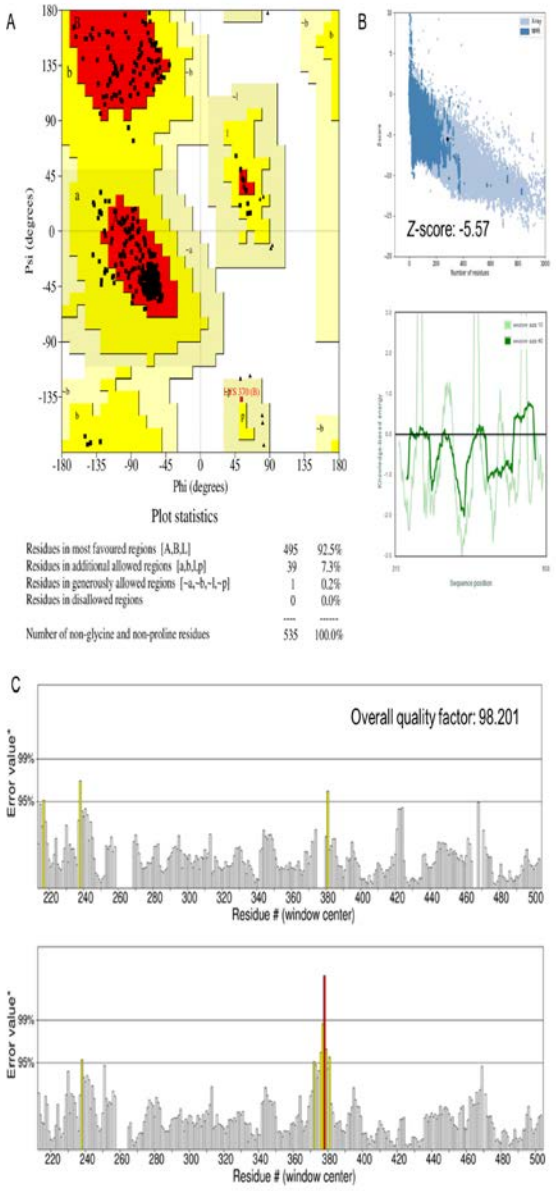

Figure 1. DARC protein structure validation. A). Procheck. B) the z-score plot of ProSA – Web. C). ERRAT analysis.

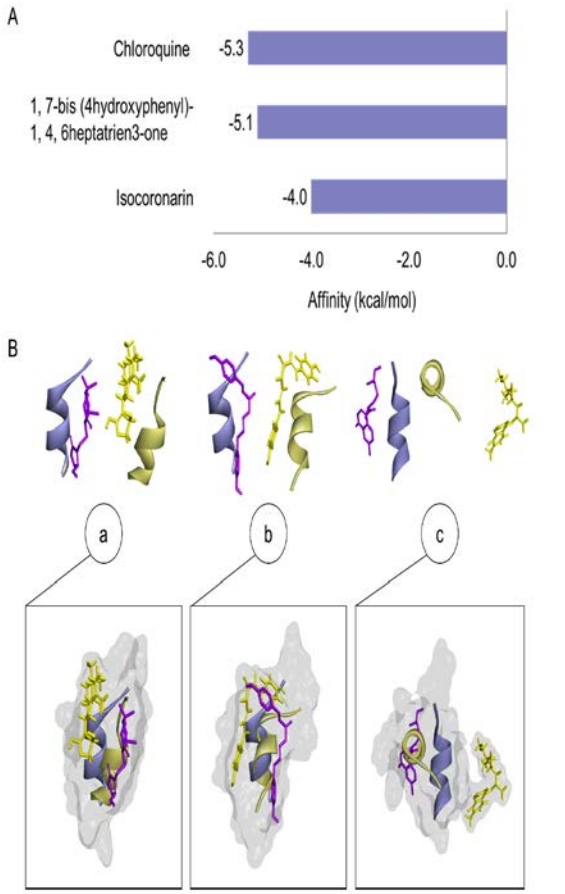

Figure 2. Molecular docking and dynamics simulation. A). The binding affinity values of isocoronarin D, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and control drug (chloroquine) toward DARC protein. B). The 3D structure visualization of isocoronarin D (a), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (b), and control drug (c) toward the DARC protein at initial and the terminal stages of molecular dynamics simulation.

In molecular docking, scoring functions estimate binding affinity, with more negative values indicating a stronger, more stable interaction with the target protein. The binding affinity scores are also determined by H-bond composition. The strong H-bond could reflect the high-affinity of ligand against the target protein (Pantsar and Poso, 2018; Hidayatullah et al., 2021). Our results indicate that all three ligands were precisely docked to residues of Chain C, which is believed to be functionally relevant for ligand interaction. Besides the importance of binding affinity and H-bond composition in complex interaction, the ability of ligand to penetrate the plasma membrane is important. Importantly, membrane permeability prediction could provide physicochemical and pharmaceutical information about drug–membrane thermodynamics (Menichetti *et al*., 2019). Permeability is crucial for assessing a drug's ability to pass through gastrointestinal membranes, and it plays a vital role in ensuring effective distribution to pharmacological target organs and cells. (Wang and Skolnik, 2013).

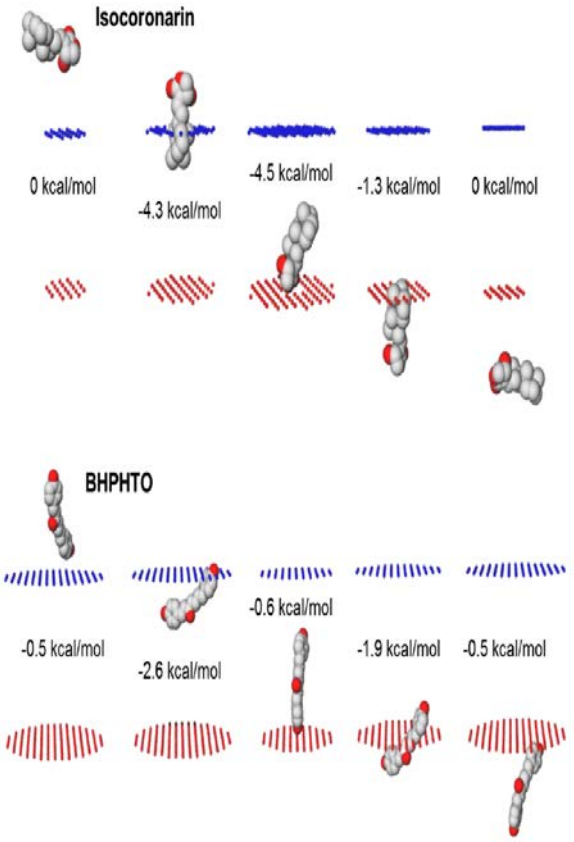

Figure 3. The prediction of membrane permeability penetration of isocoronarin D and 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one.

Molecular dynamics simulations predict the movements of each atom in a protein or other molecular systems. These simulations reveal the positions of atoms over time, capturing a wide range of significant biomolecular processes, including conformational changes, ligand binding, and protein folding (Hollingsworth and Dror, 2018). Interestingly, we noticed that there are changes on the ligands and protein position during molecular dynamics simulation (Fig. 2). We then compared the initial and the terminal stage of molecular dynamics simulation at each complex. Interestingly, we found that there are changes in the composition of chemical interaction which showed H-bond and hydrophobic interaction relatively constant on both isocoronarin D and BHPHTO. However, on the control drug, we found that in the terminal stage of dynamics simulation, the ligand is disassociated with the target protein, indicating that there is no chemical interaction found in terminal stage of DARC - Chloroquine complex (Fig. 4, Table 1). This finding suggests that both hit-to-lead compounds, especially BHPHTO, are stable enough to interact with the DARC compared to the control drug.

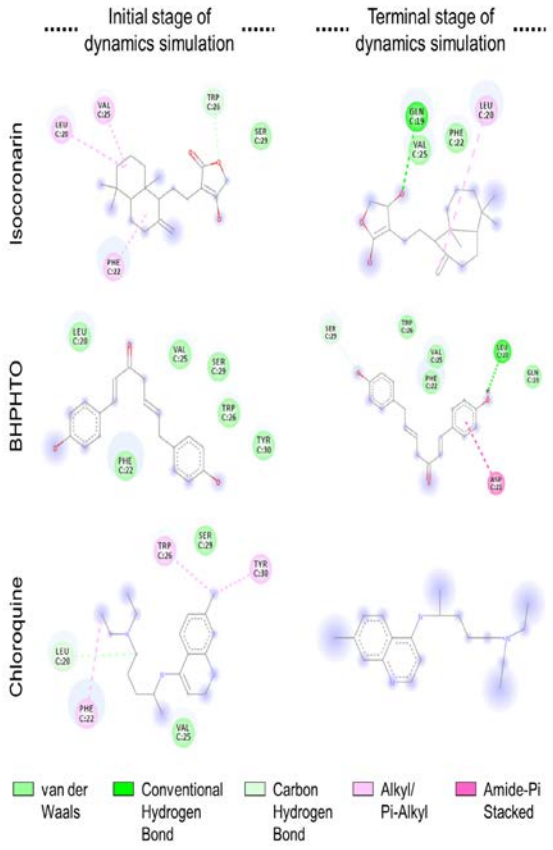

Figure 4. The 2D structure and interaction visualization of isocoronarin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and control drug toward the DARC protein at the initial and terminal stage of molecular dynamics simulation.

Table 1. List of amino acids residue and chemical interaction of isocoronarin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and control drug toward the DARC protein at initial and terminal stage of molecular dynamics simulation.

Compound	Amino Acids Residue		Interaction
	Initial stage	Terminal stage	
Isocoronarin D CID. 71550939	Trp26(C)	Gln19(C)	Hydrogen Bond
	Ser29(C)	Phe22(C); Val25(C)	Van der Waals
BHPHTO CID. 71346280	-	Leu20(C); Ser29(C)	Hydrogen Bond
	Leu20(C); Val25(C); Ser29(C); Trp26(C); Tyr30(C); Phe22(C)	Gln19(C); Phe22(C); Val25(C); Trp26(C)	Van der Waals
Chloroquine CID. 2719	Leu20(C)	-	Hydrogen Bond
	Ser29(C); Val25(C)	-	Van der Waals

To the greater extent, we evaluated eight parameters on molecular dynamics simulation such as potential energy, RMSD, RMSF, ligand movement, ligand conformation, solvent accessible surface, and Hydrogen bonds (Fig. 5). Some parameters such as potential energy, RMSF, ligand conformation, solvent accessible surface, and Hydrogen bonds showed similar pattern among the complexes. However in parameter of RMSD backbone, isocoronarin D and Chloroquine shared a similar pattern which showed a higher value compared to the BHPHTO (Fig. 5B). This showed that DARC – isocoronarin D structure and of DARC – Chloroquine complex during dynamic simulation are less stable compared to BHPHTO. The lower number of RMSD backbone indicates the stability and compactness of complex structure (Hidayatullah *et al*., 2023). Moreover, in the ligand movement parameters, we found that isocoronarin D and BHPHTO are relatively stable against the target protein, but there is extreme fluctuation on Chloroquine which demonstrated that this control drug is not stable against the DARC. This result is supported by the previous finding related to absent of chemical interaction in the DARC – Chloroquine complex at the terminal stage of molecular dynamic simulation (Fig. 4). DCCM analysis is one of important parameters to evaluate the structure movement whether it's parallel (1) or anti-parallel (-1). According to the DCCM results, the protein structures of both isocoronarin D and BHPHTO complexes are moving in parallel as indicated by the thicker yellow's diagonal line compared to the Chloroquine complex (Fig. 6).

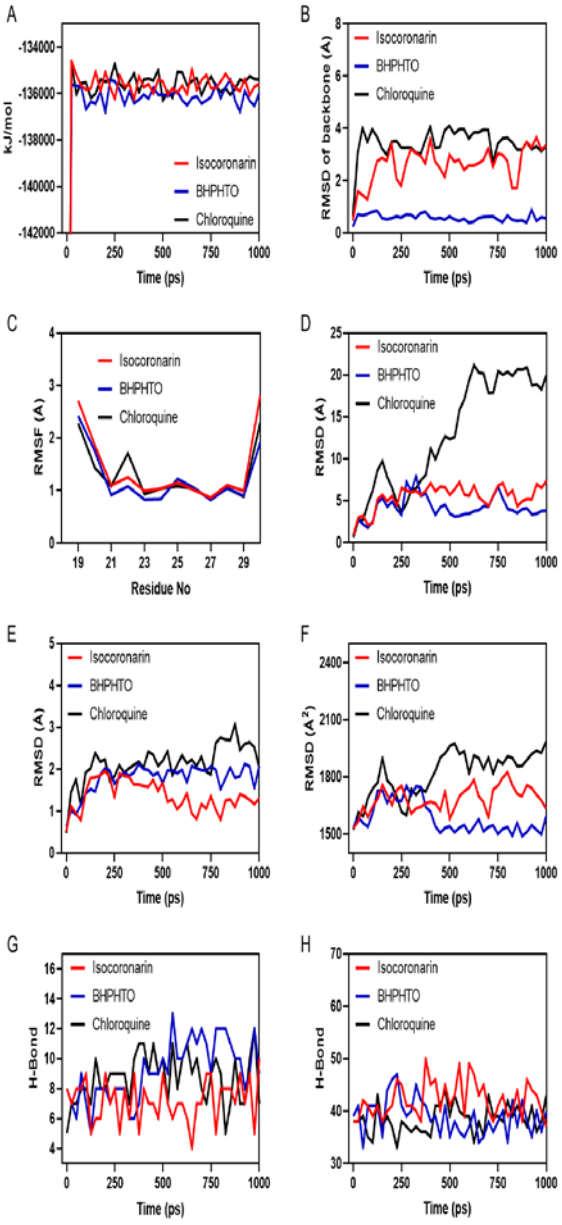

Figure 5. Molecular dynamics simulation. (A). Potential energy plot; (B). RMSD plot; (C). RMSF plot; (D). Ligand movement plot; (E). Ligand conformation plot; (F). Solvent accessible surface plot; (G). Hydrogen bonds in the solute plot; and (H). Hydrogen bonds between solute and solvent plot for isocoronarin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, or control drug - protein complexes over a 1000 ps of dynamics simulation.

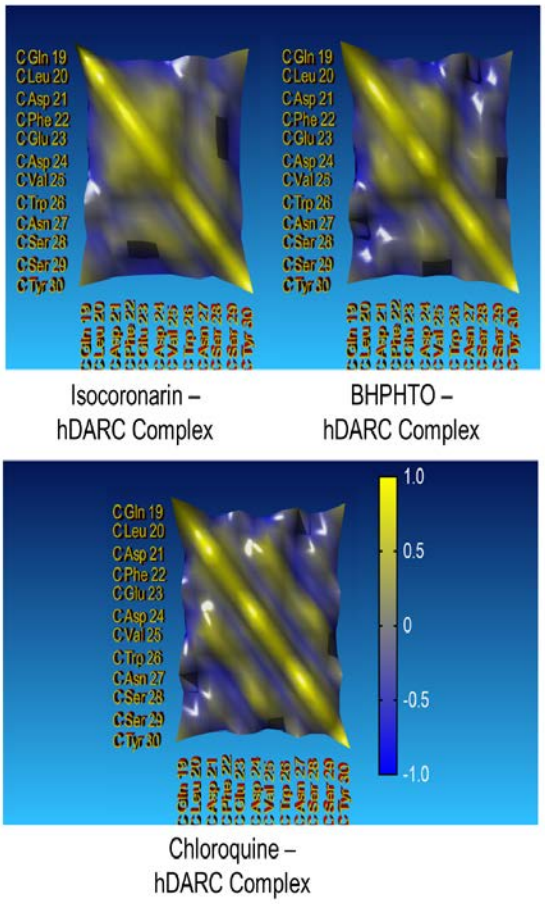

Figure 6. Dynamic cross-correlation matrix (DCCM) for isocoronarin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, or control drug - protein complexes. Yellow represents correlated motions, and blue represents anticorrelated motions.

Together, these findings indicate that the hit-to-lead compound namely 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one has better stability compared to the isocoronarin D and Chloroquine. Molecular dynamics simulation could determine the stability of protein structure during interaction with ligand, and at the same time it could indicate the behavior of ligand, whether it has a strong binding mode or it disassociated with target proteins (Choi *et al*., 2022; Gill *et al*., 2019; Liu *et al*., 2017). Some parameters, such as RMSD backbone, H-bond, ligand conformation, and DCCM, explicitly demonstrated the stability and compactness of the structure. Additionally, ligand movement parameter showed how the ligands behave with the target proteins. To a greater extent, BHPHTO forms more stable and frequent hydrogen bonds with the DARC protein compared to isocoronarin D and Chloroquine, supporting its stronger binding affinity. In contrast, isocoronarin D forms the highest number of hydrogen bonds with solvent molecules, indicating greater solvation and exposure to the aqueous environment. This suggests that isocoronarin D is less embedded in the binding pocket, which may explain its lower binding affinity.

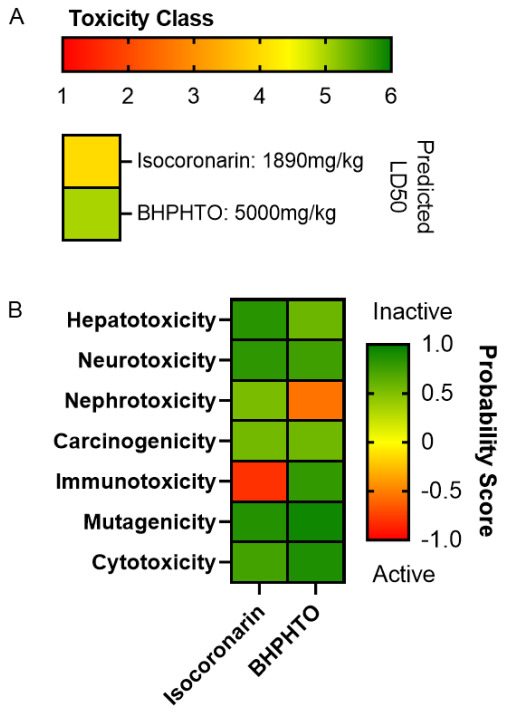

Figure 7. Toxicity evaluation of isocoronarin D and 1, 7-bis (4hydroxy phenyl)-1, 4, 6 heptatrien3-one. A). Toxicity class and LD50 prediction. B). The probability of bioactive compounds to induce toxicity in organs.

Finally, to support these findings, the toxicity prediction of hit-to-lead compounds was examined (Figure 7). The result showed that isocoronarin D was more toxic compare than BHPHTO with toxicity classes 4 and 5, and predicted LD50 1890 mg/kg and 5000 mg/kg respectively. Interestingly, we found that isocoronarin D and BHPHTO is relatively safe to human body because these compounds do not cause hepatotoxicity, neurotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. However, certain alerts should be noted that isocoronarin D may cause immunotoxicity, and BHPHTO may pose a risk of nephrotoxicity. As with other in silico approaches, toxicity prediction plays an important role in accelerating drug discovery research. Considering its benefit that can save several drug assessments including cellular, animal, and clinical test, toxicity prediction is crucial in order to reduce the cost and labour involved drug's preclinical and clinical trials (Füzi *et al*., 2023; Wu and Wang, 2018).

4. Conclusion

The present study validates two bioactive compounds collected from fifteen members of *Zingiberaceae* family as anti-malarial drug candidates against the DARC protein. Molecular docking results demonstrated that isocoronarin D and BHPHTO from *A. galanga* have close binding affinity scores to the control drug. Interestingly, the chemical interaction within both compounds demonstrated the favorable number compared to the control, especially at initial and terminal stages of the molecular dynamics simulation. Furthermore, molecular dynamics simulation demonstrated that BHPHTO has the most stability movement to the DARC protein and stability of protein structure compared to isocoronarin D and Chloroquine complexes. Together, these findings suggest that BHPHTO might be developed as drug candidates for anti-malaria. However, further study on validating the toxicity and detailed mechanism against DARC is badly needed.

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