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In-Silico Study of Black Sea Cucumber (*Holothuria atra*) Active Compounds against *Plasmepsin 2* Protein of *Plasmodium falciparum*

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Abstract

The existence of evidence of resistance to artemisinin derivatives as first-line malaria therapy is a problem that must be resolved. One of them is a marine biota, which has the potential to act as an anti-malarial, namely the black sea cucumber (*Holothuria atra/ H.atra*). The aim of this research is to analyze *H. atra* antimalarial activity against *P. falciparum* - plasmepsin 2 protein used in-silico approach. This type of research is experimental, using in-silico tests (computerized tests) on bioactive compounds and target proteins. This research analyzes several aspects: the preparation of materials and tools, the preparation of *P. falciparum* protein, the preparation of active compounds for *H. atra*, the prediction of compound and pathway potential, the prediction of interactions between plasmepsin protein and bioactive compounds, and the prediction of antimalarial inhibitors on active compounds. The results of Quantitative Structure–Activity Relationship/ QSAR analysis show that the biologically active compounds of sea cucumbers have anti-parasitic properties. Furthermore, the docking results show that 3 biologically active compounds from sea cucumbers have quite good inhibitory activity against *Plasmepsin2* proteins, especially the compounds chlorogenic acid, catechin, and rutin. Chlorogenic acid compounds also have high PA values as antiparasitic agents, especially greater than 0.4. ADME/T demonstrated that chlorogenic acid and catechin conform to Lipinski's rule, but rutin fails to satisfy Lipinski's rule. Toxicity analysis showed that catechin (level 6) has lower toxicity than chlorogenic acid (level 4), and rutin (level 5). Therefore, it can be predicted that chlorogenic acid is the most potent compound in sea cucumbers with anti-malarial effects.

Keywords: *Holothuria atra, In silico, molecular docking, antimalarial*

INTRODUCTION

One of the many diseases that affects a large portion of the global population is malaria. In 2023, global malaria prevalence reached 263 million cases with 597,000 deaths, a 4% case increase from 2022. Africa bore 94% of cases and 95% of deaths, with 76% of fatalities among children under five (1). Indonesia reported 418,546 cases (down from 443,530 in 2022) and 120 deaths in 2023, contributing to 94% of Southeast Asia's malaria deaths alongside India. By April 2024, Indonesia's cases surged to 543,965 (93% in Papua), highlighting persistent regional disparities. Morbidity in Indonesia showed an Annual Parasite Incidence (API) of 1.6 per 1,000 nationally in 2022, while Papua's API soared to 113.07 per 1,000 (2,3). Globally, 2024 trends indicate rising risks from climate change and drug resistance, with Africa remaining the epicenter (4). Despite progress (389 Indonesian districts malaria-free by 2023), challenges persist in high-transmission areas like Papua (5).

Malaria infection triggers various clinical abnormalities, and without adequate therapy, it can trigger severe complications, such as acute kidney failure, coma or cerebral malaria, hypovolemic shock, anemia, hypoglycemia, black water fever, or hemoglobinuria malaria(6,7).

WHO and the Indonesian Ministry of Health recommended ACT (Artemisinin Combination Therapy) therapy in 2004. (8,9). Resistance to artemisinin-based com-

bination therapy (ACT) is posing a growing threat to malaria treatment effectiveness. Mutations in the kelch13 gene (such as C580Y) in Plasmodium falciparum are the primary cause of parasite sensitivity to ACT, particularly in Southeast Asia (10,11). According to the WHO, artemisinin resistance is characterized by slow parasite clearance after treatment, which increases the risk of therapeutic failure when combined with resistance to the partner drug (12,13).

P. falciparum contains an aspartic protease called Plasmepsin 2, which is essential to the parasite's digestion of host haemoglobin in the acidic food vacuole. This enzyme starts the cleavage of native hemoglobin, denaturing it and supplying vital amino acids for the parasite's development and protein synthesis(14). This process is essential to the survival of the parasite because it helps control the osmolarity in the infected erythrocyte in addition to providing nutrients. Plasmepsin 2 is regarded as a promising target for antimalarial drug development due to its crucial function in hemoglobin degradation(15).

The black sea cucumber (Holothuria atra) can be one of the options for the treatment of malaria. Several researchers have previously researched the content of active compounds of H.atra. The study's results with HPLC measurements showed that H.atra contains several active ingredients, such as chlorogenic acid, pyrogallol, rutin, coumaric acid, and catechin (Febrianti, and Utami, 2022). Meanwhile, chlorogenic acid is the most abundant in black sea cucumber, which is 90% (17,18). These materials will later become the focus of this research. In-silico method, a method that uses computerization in the process of selecting and extracting active compounds and target proteins. This method predicts the bonds between receptors and proteins using computerization (19) .

The aim of this research was to analyze H. atra antimalarial activity against P. falciparum - plasmepsin 2 protein using an in-silico approach. There are 4 parameters in this study: prediction of antiparasitic activity of H.atra using way; molecular docking between plasmpesin 2 P.falciparum and active compound of H.atra; ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction; and toxicity prediction analysis.of H.atra chemical compounds

METHODS

This study was conducted at Laboratorium Biomolekuler & Bioinformatika INBIO-Malang using the experimental study type and in silico as the method. Several steps of this research are as follows:

1. Prepare potential materials from H.atra and P. falciparum.
2. Data mining process (data collection) from various sources.
3. Predict antiparasitic activity.
4. Molecular docking
5. ADME Prediction
6. Predicting toxicity

Preparation

Based on a literature study, there are six active ingredients in H. atra—chlorogenic acid, pyrogallol, rutin, coumaric acid, catechin, and ascorbic acid—that are thought to have antimalarial properties (20).

Data mining

The initial step entails extracting bioactive compounds from H.atra via the database maintained by PubChem (https://pubchem.ncbi.nlm.nih.gov/) to acquire the SMILES notation for each compound (21). The three-dimensional crystal structure of the target protein, Plasmepsin 2 (5YIB), is obtained from the Protein Data Bank, or PDB (https://www.rcsb.org/) (22).

Predicting Antiparasit activity and Pathway of H. Atra Active Compound

The WAY2DRUG PASS Online Prediction web server (http://www.pharmaexpert.ru/passonline/predict.php) will be utilized in order to conduct an analysis of the antimalarial potential of active compounds derived from H.atra (23,24). The Quantitative Structure-Activity Relationship (QSAR) method is a computational approach that correlates mo-

lecular characteristics (structural and physicochemical) with biological activity. This enables us to predict the efficacy of a drug. QSAR analysis, which ultimately results in a Pa (Probability to Be Active). Pa is a value ranging from 0 to 1 that assesses the probability of a compound demonstrating a biological activity, predicated on its structural resemblance to established active molecules within a training dataset (25).

Molecular Docking Analysis

Docking analysis elucidates the interaction between receptors and ligands, as well as the affinity energy, to determine the potential of compounds as antimalarials. It also enables you to infer weak and strong affinities. A greater value indicates a more robust relationship(26).

After identifying the target protein structure and bioactive compounds, Discovery Studio 2019 eliminates the water molecules from the protein to prepare it (27), while PyRx v. 0.9.8 optimizes the energy of the ligands. Autodock Vina, in conjunction with PyRx version 0.9.8, is employed for docking purposes (28). If the value of the tested bioactive compound is similar to that of the control, it functions analogously to a protein target inhibitor. Docking targets the plasmepsin-2 of P. falciparum and the inhibitory compound KNI-10743. BioVia Discovery Studio 2019 is utilized to analyze their interactions.

ADMET Prediction Analysis

The ADMET prediction for each ligand is conducted in accordance with Lipinski's Rule of Five and analyzed utilizing AdmetLab2.0. An integrated, complementary online platform for precise and comprehensive ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction of chemical compounds. It assists pharmaceutical developers in optimizing lead compounds and minimizing late-stage failures by assessing drug-likeness, pharmacokinetics, and toxicity early in the development process (29).

Toxicity Prediction Analysis

A vital first step to guarantee that protox inhibitors are not only safe for human use but also efficient against the malaria parasite is toxicity testing of these molecules. ProTox-II is employed to evaluate the toxicity of the drug-likeness character, which demonstrates inhibitory effects on parasite cultures(https://toxnew.charite.de/protox_II/index.php?site=compound_input) (30).

The LD50 measure is employed to evaluate toxicity prediction, which indicates lethal doses at which 50% of the tested animal population dies because of exposure to the tested compound. LD50 values are used by ProTox-II to classify toxicity into six categories, ranging from highly toxic to non-toxic when ingested. According to the Globally Harmonized System (GHS), ProTox-II is classified into six toxicity levels based on the LD50 value (30), as follows:

1: fatal/ danger if ingested (≤ 5 mg/ Kg)
2: fatal if ingested (5 - 50 mg/ Kg)
3: toxic if ingested (50 – 300 mg/ Kg)
4: hazardous if ingested (300 – 2000 mg/ Kg).
5: may be harmful if ingested (2000 -5000 mg/ Kg). 6)
6: non-toxic if ingested (> 5000 mg/Kg)

The toxicity of a compound decreases as it is ingested, as its level of toxicity increases (31)

RESULT

Antimalarial Activity Prediction Analysis

Bioactive constituents in H. atra and their antiparasitic prediction analysis are represented by all of the active compounds, as shown in Figure 1. Contrary to coumaric acid, which has the highest Pa value of 0.446, catechin has a low potential as an antimalarial agent, according to the Probability to Be Active (Pa) assessment.

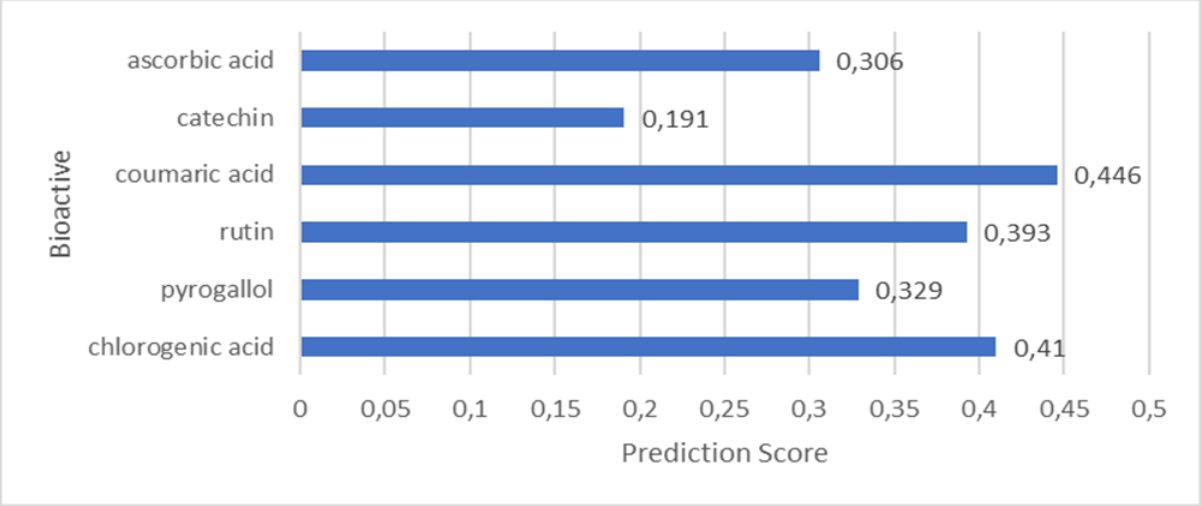

Figure 1. Antiparasitic Activity of *H. Atra* Active Compounds

The control molecule in this study was KNI -10743, which functions as a potent peptidomimetic inhibitor of plasmepsin-2 (PM2), an aspartic protease essential for the survival of *Plasmodium falciparum* through haemoglobin degradation. KNI-10743 exhibits no cytotoxic effects in human cell lines, establishing it as a dependable experimental control.

Binding affinity analysis is essential in pharmacology, facilitating rational drug design and assessment of therapeutic candidates.

Binding affinity quantifies the interaction strength between a biomolecule (e.g., protein) and its ligand (e.g., drug), as measured by the equilibrium dissociation constant. The binding affinity prediction of *H. atra* active compounds towards PM2, as presented in Table 1, indicates that all active compounds exhibit a greater value than the control. This indicates that control exhibits a stronger affinity for PM2 than for the active compound of *H. atra*.

Table 1. Affinity Binding Prediction Analysis Between Plasmepsin-2 And Ligands

Receptor	Ligand	PDB ID	Binding Affinity (Kcal/mol)
Plasmepsin 2/ PM2	KNI-10743	5YIB	-9,1
	Rutin	5280805	-7,9
	Chlorogenic acid	1794427	-7,4
	Catechin	9064	-7,1
	Ascorbic acid	54670067	-5,1
	Coumaric acid	637542	-5,3
	Pyrogallol	1057	-4,6

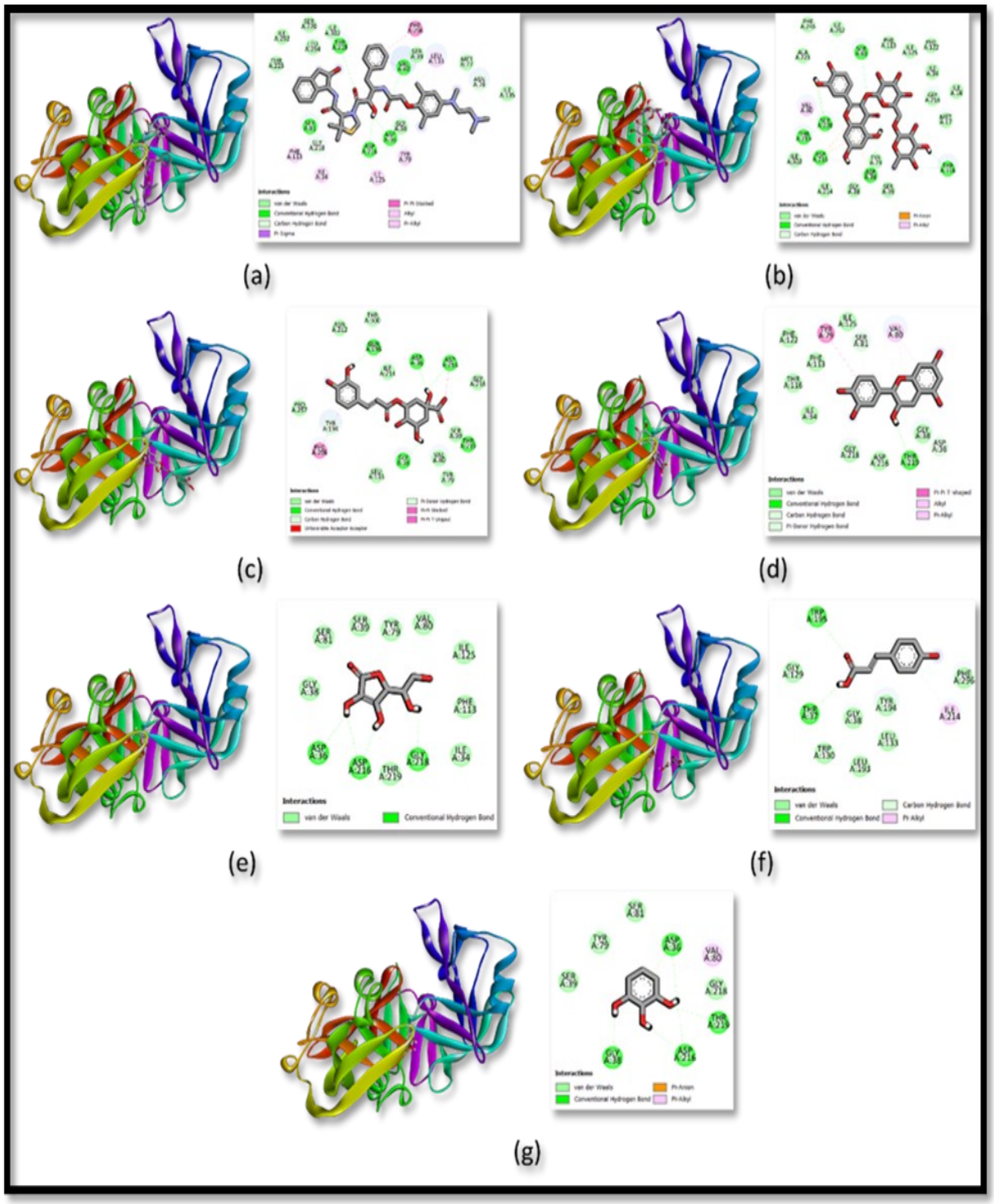

Figure 2. Visualization of docking outcomes: a) Plasmepsin 2 Protein/KNI-10743 (control), b) Plasmepsin 2 Protein/Rutin, c) Plasmepsin 2 Protein/Chlorogenic Acid, d) Plasmepsin 2 Protein/Catechin, e) Plasmepsin 2 Protein/Ascorbic Acid, f) Plasmepsin 2 Protein/Coumaric Acid, g) Plasmepsin 2 Protein/Pyrogallol. The left side displays a three-dimensional visualization, whereas the right side illustrates the type of bond established between the ligand and the protein.

Table 2. Amino Acid Residues Interaction between Plasmepsin-2 with Ligand and Control

Protein	Ligand	Amino Acid Residues Interaction					
		Van der Waals	**Hydrogen Bond**	**Hydrogen Karbon**	**Hydrophobic**	**Other**	**Electro static**
PM2/ Plasmepsin2	**PDB Control**	A: ILE135 A: SER134 A: PHE296 A: LEU294 A: THR223 A: THR116 A: GLY38 A: SER39	A: VAL80 A: SER81 A: THR219 A: SER220 A: GLY218 A: ASP36 A: ASP216	A: GLY218 A: LEU133 A: ASP132	A: VAL80 A: THR219 A: ILE302 A: MET77 A: ILE34 A: ILE125 A: TYR79 A: LEU133 A: ALA221 A: ILE292	A: PHE113	
	KNI-10743	A: THR223 A: ILE292 A: SER220 A: LEU294 A: ILE302 A: SER39 A: MET77 A: ILE135 A: GLY38	A:ASP36 A: ASP216 A: VAL80 A: SER81 A: THR219	A: ASN78 A: ASP36 A: GLY218	A: VAL80 A: PHE296 A: LEU133 A: ILE34 A: ILE125 A: TYR79		
	Rutin	*A: ALA221* A: PHE246 **A: ILE292** *A: PHE113* *A: ILE125* A: PHE122 *A: ILE34* *A: GLY218* A: ILE16 A: MET17 *A: TYR79* **A: SER39** **A: GLY38** A: ILE214 **A: ILE302**	**A:ASP36** **A:ASP216** *A: THR116* **A: SER81** **A: THR219** *A: SER220*	*A: SER220*	**A: VAL80**		*A: ASP36* *A:*

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Chloro-genic Acid	A: PRO297 A: ASN212 A: THR300 A: ILE214 *A: GLY218* **A: SER39** *A: VAL80* *A: TYR79* *A: LEU133*	A: GLN196 A: ASP36 **A: ASP216** *A: GLY38* **A: THR219**	*A: GLY38* A: TYR194	A: TYR194 **A: PHE296**	*A: ASP216*
Catechin	**A: GLY38** *A: ASP216* *A: GLY218* *A: ILE34* **A: THR116** *A: PHE113* A: PHE122 *A: ILE125*	**A:THR219**	**A:ASP36** *A: SER81*	**A:TYR79** **A: VAL80**	
Ascorbic Acid	**A: GLY38** *A: SER81* **A: SER39** *A: TYR79* *A: VAL80* *A: ILE125* *A: PHE113* *A: ILE34* *A: THR219*	*A: GLY218* **A: ASP216** **A: ASP36**			
Couma-ric Acid	*A: GLY129* A: TRP130 **A: GLY38** A: TYR194 *A: LEU133* A: LEU193 *A: PHE296*	A: THR37 A: TRP195	A: TRP195	A: ILE214	
Pyrogal-lol	**A: SER39** *A: TYR79* *A: SER81* *A: GLY218*	*A: GLY38* **A: ASP36** **A: ASP216** **A: THR219**		**A: VAL80**	*A: ASP36*

Subsequently, Table 2 illustrates the interactions established between each PM2 and ligand sample (control and active compound of *H. atra*). Hydrogen, van der Waals, and hydrophobic bonds are the most prevalent based on the types of bonds formed. Amino acid residues highlighted in bold represent those from the control ligand and that are preserved in the sample with identical bonding characteristics.

Meanwhile, residues in italics denote those preserved in the sample but with altered types and bond distances. Residues marked with a single underline

are those associated with the PDB control ligand retained in the sample.

According to the molecular docking results (Table 2; Figure 2) and binding affinity predictions (Table 1), three active compounds—rutin, chlorogenic acid, and catechin—exhibit high potency as antimalarial agents.

Table 3. ADME/T Prediction Analysis Of *H.atra* Bioactive Compound

ADME/T	Bioactive Compound of *H.atra*					
	Chlorogenic Acid	Pyrogallol	Rutin	Coumaric Acid	Catechin	Ascorbic Acid
MW	354,1	126,03	610,15	164,05	290,08	176,03
nHA	9	3	16	3	6	6
nHD	6	3	10	2	5	5
TPSA	164,75	60,69	269,43	57,53	110,38	114,29
LogP	0,331	0,458	-0,038	1,923	1,343	-1,42
Lipinski	Accepted	Accepted	Rejected	Accepted	Accepted	Accepted
Pgp-inh	0	0,001	0,005	0	0,008	0,001
Pgp-sub	0,992	0,002	0,997	0,017	0,01	0,089
HIA	0,455	0,046	0,876	0,009	0,035	0,069
F(20%)	0,991	0,982	0,038	0,004	0,998	0,918
F(30%)	0,998	0,959	0,999	0,194	1	0,978
BBB	0,264	0,043	0,041	0,29	0,029	0,073
H-HT	0,19	0,051	0,083	0,673	0,103	0,168
DILI	0,057	0,06	0,982	0,2	0,07	0,936
FDAMDD	0,558	0,028	0,01	0,031	0,136	0,009
Carcinogenicity	0,111	0,638	0,055	0,151	0,185	0,266

Note: Red indicates that the compound exhibits low druglikeness and bioavailability and may possess toxic potential as an isolated entity. (MW/molecular weight; nHA/hydrogen bond acceptors; nHD (hydrogen bond donors); TPSA (total polar surface area); LogP (octanol-water partition coefficient): Pgp-inh (P-glicoprotein inhibitor); Pgp-sub (P-glycoprotein substrate); HIA (Human Intestinal Absorption); F(20%)/ oral bioavailability ≥20%); F(30%)/ oral bioavailability ≥30%); BBB/blood brain barrier; H-HT/Human Hepatotoxicity; DILI/ Drug-Induced Liver Injury; and FDAMDD/FDA Maximum Recommended Daily Dose)

ADME/T Prediction Analysis

The ADMETlab database (Table 3) serves as a valuable resource for predicting

The Lipinski rule and the ADME/T characteristics of a compound. Lipinski's rule of five serves as a valuable guideline for differentiating drug-like molecules from their non-drug-like counterparts. Lipinski can forecast the chances of a compound's metabolic success or failure by analyzing its resemblance to existing drugs.

To comply with Lipinski's rule, compounds must demonstrate at least 2 of the 5 essential properties (Lipinski et al., 1997). The Lipinski rule of 5 outlines key criteria for drug-like properties, including a molecular weight under 500 Dalton, a Log P value below 5 indicating high lipophilicity, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a TPSA (Topological Polar Surface Area) ranging from 60 to 140 Å².

According to the ADME/T analysis presented in Table 1, nearly all active compounds comply with Lipinski's rules, except for rutin.

Chlorogenic acid, pyrogallol, rutin, catechin, and ascorbic acid exhibit lower bioavailability than coumaric acid. Rutin and ascorbic acid can induce drug-induced liver injury (DILI), whereas other compounds do not cause tissue damage; however, all ac-

tive compounds lack carcinogenic properties.

Toxicity Prediction Analysis

Figure 2 presents the results of toxicity tests, revealing that the bioactive compound in *H. atra* with the lowest hazard level is catechin, exhibiting an LD50 of 10,000 mg.kg-1 wt and categorised within toxicity class 6. In contrast, pyrogallol, which is classified as belonging to the third toxicity class, is the bioactive compound that poses the greatest risk.

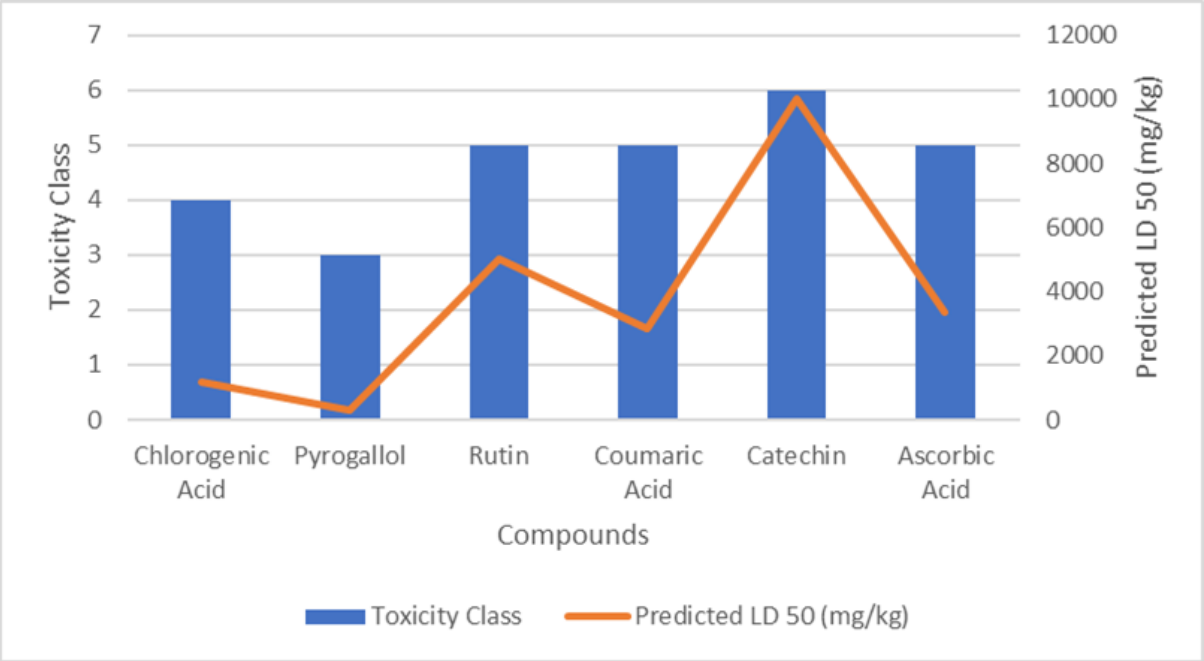

Figure 2. Toxicity Prediction of H. atra Extract

DISCUSSION

Antiparasite Activity Prediction Analysis

The extract of *H. atra* comprises several active compounds exhibiting antiparasitic properties. The compounds comprise chlorogenic acid, pyrogallol, rutin, catechin, coumaric acid, and ascorbic acid. Considering the antiparasitic value (Pa), five compounds exhibit potential as effective antimalarials. The compounds include chlorogenic acid, ascorbic acid, rutin, pyrogallol, and coumaric acid. The Pa values of these three compounds exceed 0.3. Conversely, the bioactive compound catechin possesses a Pa value below 0.3, signifying its diminished efficacy as an antimalarial agent, as depicted in Figure 1.

Despite a compound exhibiting robust binding affinity or favorable structural characteristics, various factors diminish its Pa (probability of activity) score in in silico analyses: (1) Structural characteristics and electronic attributes, compounds containing electron-withdrawing groups, such as nitro groups, or heterocyclic substitutions may exhibit diminished Pa scores owing to decreased bioactivity (32)); (2) Suboptimal ADME properties may result in diminished Pa scores, notwithstanding elevated docking or binding affinity (19) (3) Certain in silico software exhibit a lack of transparency regarding their foundational algorithms, and the precision of Pa predictions is contingent upon the quality and diversity of the training data. If a compound's structure is inadequately represented in the model's database, the Pa score may be inaccurately low (19). QSAR methode

Molecular Docking of *H. atra* Active Compounds to Plasmepsin 2

A higher binding affinity indicates that a ligand adheres more firmly and selectively to its target protein, thereby stabilizing the protein-ligand complex. Pharmaceutical design depends on strong binding to enhance potency and specificity for the intended biological effect (33,34).
Molecular docking analysis (Table 2 and Figure 2) demonstrated that all active compounds of *H. atra* in this study can bind to PM2 with the same amino acid residues as the control. But each active compound has different binding affinities (Table 1). The control substance (KNI-10743) has a higher binding affinity than *H. atra* active compounds. Nonetheless, there are three active constituents of *H. atra* that exhibit a binding affinity comparable to the control, specifically—rutin, chlorogenic acid , and catechin—that exhibit high potency as antimalarial agents.

Numerous prior in vivo and in vitro studies provide evidence supporting the antimalarial activity of active compounds from *H. atra*. In addition to their antimalarial effect, these active compounds exhibit another advantageous activity.

The first compound is rutin, which is a glycosylated flavonoid present in plants, renowned for its antioxidant, anticancer, and anti-inflammatory properties (35). Rutin demonstrates antiplasmodial activity against *P. falciparum*, with IC_{50} values ranging from 3.53 to 10.38 µM in both laboratory and field isolates, indicating moderate efficacy (36,37). Extracts from Achillea fragrantissima leaves (2024) have demonstrated the ability to inhibit the synthesis of β-hematin, a crucial process for the survival of malaria parasites (37). Synergistic effects that augment antimalarial potency have been observed when combined with other flavonoids, such as quercetin (36,37).

Coumaric acid is a derivative of hydroxycinnamic acid, commonly found in fruits and cereals (38). It possesses multiple activities, including antioxidant, anti-inflammatory, and anticancer properties. Coumaric acid interacts with PfOMPDC in silico but exhibits negligible inhibitory effects in functional assays(39)

Catechin, an exceptional flavan-3-ol from the flavonoid family, is prevalent in green tea and is recognized for its significant antioxidant, cardioprotective, and anticancer properties (40). Several studies have explored the antimalarial activity of catechin through in vivo studies. Catechin extracted from Osyris quadripartita leaves exhibited 64.26% chemo suppression against *Plasmodium berghei* in mice at a dosage of 400 mg/kg. The extract demonstrated a 70.61% suppression rate, substantiating its traditional application for malaria treatment (41). In vitro study (2020) demonstrated that catechin inhibited β-hematin formation, essential for heme detoxification in *Plasmodium*, with an efficacy of 85.7% at 0.4 mM, albeit less potent than morin. This indicates that catechin interferes with heme polymerization, a mechanism essential for parasite survival (42).

Chlorogenic acid, a polyphenol prevalent in green coffee beans, serves as a neuroprotective agent and metabolic modulator. This active compound exhibits multiple effects, including anti-inflammatory, antioxidant, and antidiabetic activities (43). Moelyadi et al. (2020) demonstrated a strong binding affinity between chlorogenic acid to *Plasmodium falciparum* Orotidine 5-Monophosphate Decarboxylase (PfOMPDC) through molecular docking studies; however, no direct antiprotozoal activity was experimentally confirmed. (20). Phytochemical profiling serves as a foundational approach in understanding the chemical constituents of plants. Identified in plant extracts exhibiting antiplasmodial activity; however, the specific contribution of each component remains ambiguous (44).

Ascorbic Acid (Vitamin C) is a water-soluble antioxidant crucial for collagen synthesis and immune function. It functions as an antioxidant; however, at elevated doses, it produces reactive oxygen species, thereby diminishing the efficacy of artemisinin in malaria treatment. It functions as an enzyme cofactor, facilitating

dopamine β-hydroxylase and peptidylglycine α-amidating monooxygenase (45). Another study showed that ascorbic acid exhibits negative drug interactions, diminishing the efficacy of artemether by scavenging free radicals essential for the antimalarial action of artemisinin, resulting in reduced plasma concentration and therapeutic failure (46).

Pyrogallol is a phenolic compound characterized by a trihydroxybenzene structure, which transforms into purpurogallin in alkaline conditions, thereby augmenting xanthine oxidase inhibition and interfering with reactive oxygen species generation (47). Similar to artemisinin's mechanism, pyrogallol demonstrates moderate antimalarial efficacy (IC_{50} = 2.84 μM) by elevating the pH of the parasite's digestive vacuole. This disrupts parasite metabolism and the degradation of hemoglobin. It eradicates parasites by inhibiting the V-type H^+-H-ATPase proton pump, which is crucial for maintaining acidic vacuolar pH (48,49).

ADME/T Prediction Analysis

Lipinski's Rule of Five is a widely utilized method for predicting the oral bioavailability of drugs (50). Five active compounds comprise chlorogenic acid, pyrogallol, coumaric acid, catechin, and ascorbic acid, which satisfy Lipinski's criteria except for rutin. The glycosylated flavonoid rutin possesses an increased number of hydrogen bond donors and acceptors attributable to its sugar moieties. It is less pharmacologically similar and less prone to oral absorption (20).

The therapeutic efficacy is fundamentally contingent upon bioavailability, which refers to the fraction of an administered dose that attains systemic circulation (51). Coumaric acid demonstrates the highest bioavailability. The favorable physicochemical properties—elevated water solubility, efficient gastrointestinal absorption, and metabolic stability—account for this phenomenon (52).

Chlorogenic acid, despite its high water solubility, is swiftly metabolized and inadequately absorbed in the intestine, resulting in a urinary excretion rate of merely 4.9%, in contrast to coumaric acid's 54.1% (53). Despite being water-soluble, pyrogallol and ascorbic acid exhibit restricted bioavailability due to rapid metabolism and excretion. The low bioavailability of rutin (<20%) primarily stems from its inadequate solubility and restricted permeability through intestinal membranes, which directly result from its non-adherence to Lipinski's rules. Despite being safer and relatively bioavailable (33% gastrointestinal absorption), catechin does not possess the efficacy of coumaric acid (20).

Drug-induced liver injury constitutes a significant challenge in drug development. Notwithstanding their intricate risk profile (Björnsson, 2021).Rutin and ascorbic acid influence liver function. Rutin at therapeutic doses safeguards the liver by diminishing oxidative stress and inhibiting profibrotic signaling pathways (55). Rutin alters liver enzyme levels at elevated concentrations or under specific pathological conditions (e.g., high-cholesterol diets), indicating potential hepatotoxicity (55,56).

Despite its recognized antioxidant properties, ascorbic acid can influence liver enzyme activity and induce hepatic damage, particularly when administered concurrently with other hepatotoxic medications. Both drugs induce drug-induced liver injury (DILI) at supratherapeutic or extended exposures, while providing protection at standard dosages (55–57).

Importantly, all these compounds lack carcinogenic properties and, in some cases, may even offer protective effects against cancer. These findings underscore the necessity of incorporating drug-likeness, pharmacokinetic, and safety profiles in the selection of phytochemicals for therapeutic development.

Toxicity Prediction Analysis

The prediction of toxicity is essential for assessing the therapeutic potential and safety of bioactive compounds. Compounds are categorized according to their lethal dose (LD_{50}) and mechanistic risks under the Globally Harmonized System (GHS)(58).

According to the data presented in Figure 2, catechin, a flavonoid prevalent in green tea, is categorized as non-toxic (LD_{50} >5,000 mg/kg) owing to its advantageous safety profile. Although catechin shares structural similarities with epigallocatechin gallate (EGCG), which demonstrates hepatotoxicity at elevated doses, catechin does not possess reactive functional groups that induce oxidative stress (59).

Artemisinin exhibits moderate toxicity (Category 4) to humans and presents considerable environmental hazards (60,61). Chlorogenic acid has the same level of toxicity as artemisinin. Coumaric acid, rutin, and ascorbic acid have 5 levels of toxicity. Class 4 and 5 toxic drugs are safe for human use when administered within therapeutic ranges and adequately monitored. Regulatory frameworks and advancements in predictive toxicology render risks manageable, highlighting the significance of patient education and compliance with dosing protocols (62).

Pyrogallol has the highest toxicity level of all the compounds in this study (Class 3). Class 1 (extremely hazardous), Class 2 (highly hazardous), and Class 3 (moderately hazardous) are typically inappropriate for drug development due to safety concerns, although exceptions may occur under rigorous conditions (63).

CONCLUSION

In summary, there were three compounds of *H. atra* that had high activity to inhibit Plasmepsin 2: rutin, chlorogenic acid, and catechin. All active compounds except rutin conform to Lipinski's rule. Coumaric acid exhibits superior bioavailability compared to chlorogenic acid, rutin, and catechin. Rutin has the potency to induce liver injury. Catechin exhibits the lowest toxicity level (6 level) in comparison to rutin (5 level) and chlorogenic acid (4 level).

The findings of this research represent a preliminary phase in the identification of a novel antimalarial treatment protocol. The subsequent steps, which constitute the primary prerequisites for the formulation of a novel drug regimen, encompass in vitro/in vivo activity, selectivity, ADMET profile, and potential for combination therapy. Validation via early-phase clinical trials is essential prior to commercialization.

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