EVALUATION OF THE ANTIPLASMODIAL POTENTIAL AND SAFETY OF COMBINED OF COMBINED ALCHORNEA CORDIFOLIA AND ENANTIA CHLORANTHA EXTRACTS AGAINST PLASMODIUM FALCIPARUM PLASMEPSIN II USING IN SILICO AND IN VIVO APPROACHES

BY

AYEDOGBA BAMIDELE

PHA1908471

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

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PHA1908471

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SUPERVISED BY:

VINCENT O. IMIEJE [B.Pharm; MSc; PhD]

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this work was done by **Ayedogba Bamidele** in, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, in partial fulfillment of the requirements of the award of the Doctor of Pharmacy Degree (Pharm D).

DEDICATION

This work is dedicated to the unfailing, infinite God, whose presence carried me through every challenge, every sleepless night, and every moment of doubt. His divine orchestration placed extraordinary people in my path, each one a beacon of support and encouragement.

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Six years ago, I stepped into the Faculty of Pharmacy at the University of Benin with a heart full of hope and a mind ready to be shaped. What followed was a journey marked by grit, growth, and grace, a journey I could never have completed alone.

First and foremost, I give all glory to Almighty God, whose presence carried me through every challenge, every sleepless night, and every moment of doubt. His divine orchestration placed extraordinary people in my path, each one a beacon of support and encouragement.

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To my non-relenting guardian and father, Mr. Pemi Igunikilegha, thank you for your enormous contributions to my life. Without you, I wouldn't have come this far. A special shoutout to Mr. P.A. Okunniga's family. Thank you so much for everything.

And to my mother, Kasimawo Sedara — my rock, my inspiration, my fiercest supporter. Your unwavering belief in me has been the heartbeat behind every step I've taken. This achievement is as much yours as it is mine.

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TABLE OF CONTENTS

ABSTRACT

Plasmodium falciparum malaria, contributing 26.8% of global malaria deaths in 2022, drives Nigeria's health burden, with artemisinin resistance necessitating novel natural product-derived treatments. This study bridges Nigeria's ethnobotanical heritage with modern pharmacognosy to evaluate the extracts of *Alchornea cordifolia* (Ogwu obi) and *Enantia chlorantha* (Awopa). This study aims to assess the antiplasmodial potential of a combined *A. cordifolia* and *E. chlorantha* extract via molecular docking against *P. falciparum* Plasmepsin II and evaluate its acute toxicity in mice. Leaves of *A. cordifolia* and stem bark of *E. chlorantha* were extracted with methanol, freeze-dried, and concentrated using a rotary evaporator at 40°C, respectively. Molecular docking targeted Plasmepsin II (PDB ID: 1LEE) using PyRx 0.9.8, with dihydroartemisinin (standard antimalarial drug) and R36 (co-crystallized ligand) as comparators. Compounds with binding affinities ≤ -6.5 kcal/mol were prioritised. Acute toxicity was assessed in Swiss mice using Lorke's method (10–5000 mg/kg). Drug-likeness was evaluated via SwissADME 2025.2 and ProTox-3.0. Binding affinity trends were analysed descriptively. Docking revealed 10 *A. cordifolia* compounds (e.g., CID:236432, -9.1 kcal/mol) and 15 *E. chlorantha* compounds (e.g., CID:91710667, -8.6 kcal/mol) with binding affinities ≤ -6.5 kcal/mol, comparable to dihydroartemisinin (-7.1 kcal/mol) and R36 (-10.0 kcal/mol), indicating strong Plasmepsin II inhibition. No toxicity was observed at a dose of 5000 mg/kg. Most compounds showed favourable drug-likeness.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Malaria remains a serious global health problem, particularly in tropical and subtropical regions where environmental and socio-economic conditions favour transmission. Despite decades of intervention, the disease continues to cause substantial morbidity and mortality, with Africa bearing the highest burden of cases and deaths. In 2021, the World Health Organization (2022) reported more than 247 million malaria cases worldwide, with children under five and pregnant women constituting the most vulnerable groups. Climate change, poor access to healthcare, and drug resistance further compound the problem, limiting the effectiveness of existing control measures (Abbasi, 2022; Leal Filho *et al*., 2023).

Among the causative agents, *Plasmodium falciparum* is the most virulent species and is responsible for the majority of malaria-related deaths globally (White, 2022). A major challenge to malaria elimination is the parasite's remarkable ability to develop resistance to conventional drugs. Chloroquine, sulfadoxine-pyrimethamine, and, more recently, artemisinin-based therapies have all faced emerging resistance, particularly in Africa and Southeast Asia (Ippolito *et al*., 2021; Li *et al*., 2024). This increasing resistance undermines gains in malaria control and underscores the urgent need for new drugs with novel mechanisms of action (Girgis *et al*., 2023).

One promising approach in antimalarial drug discovery involves targeting proteases essential to parasite survival. Plasmepsins, a family of aspartic proteases, play a vital role in the degradation of host haemoglobin within the parasite's digestive vacuole. Plasmepsin II, in particular, is indispensable in haemoglobin catabolism, making it a prime target for selective

inhibition (Adeoye & Lobb, 2025). Several computational and biochemical studies have identified both natural and synthetic compounds capable of inhibiting plasmepsin II, suggesting its therapeutic relevance (Manhas *et al*., 2022; En-Nahli *et al*., 2023; Syed *et al*., 2025).

Medicinal plants have historically provided critical leads in malaria therapy, as exemplified by the discovery of quinine and artemisinin (Kingston & Cassera, 2022). Their continued ethnomedicinal use across endemic regions underscores their accessibility and cultural acceptance (Nigussie & Wale, 2022; Napagoda &Wijesundara, 2022). Phytochemicals such as alkaloids, terpenoids, and flavonoids isolated from various plants have shown significant antiplasmodial activity and remain important scaffolds in modern drug discovery (Cheuka *et al*., 2024; Habibi *et al*., 2022; Hadi *et al*., 2025).

In this context, *Alchornea cordifolia* (Schumach. &Thonn.) Müll. Arg. and *Enantia chlorantha* Oliv. represent two ethnomedicinal plants with longstanding use in traditional medicine for treating malaria, fever, and other infectious diseases (Blaise *et al*., 2022; Evbuomwan *et al*., 2023). Both plants are rich in bioactive phytochemicals with demonstrated antimicrobial and antiparasitic activities. Studies have shown their potential antiplasmodial effects, both *in vitro* and *in vivo,* as well as promising inhibitory activity against parasite proteases (Imieje *et al*., 2021; Ibrahim *et al*., 2024; Evbuomwan *et al*., 2025). Importantly, combining their extracts may produce synergistic effects that enhance efficacy and mitigate toxicity, providing a scientific rationale for their investigation (Mahama *et al*., 2022).

Modern pharmacological research increasingly integrates *in silico* and *in vivo* approaches to accelerate drug discovery. Computational methods, such as molecular docking and ADMET prediction, facilitate rapid identification of potential inhibitors and the evaluation of their pharmacokinetic properties (Fernando *et al*., 2024; Sulyman *et al*., 2023). These predictive tools, when complemented with *in vivo* toxicity assessments, provide a holistic evaluation of

candidate compounds, improving the precision and efficiency of preclinical studies (Cheuka *et al*., 2024; Dkhil *et al*., 2021). Thus, the investigation of *A. cordifolia* and *E. chlorantha* extracts against *P. falciparum* plasmepsin II through *in silico* and *in vivo* techniques offers a robust approach to validate their therapeutic potential and safety profile.

1.2 History and Relevance of Medicinal Plants in Antimalarial Therapy

Historical Use of Plants in Traditional Medicine

The use of plants for disease treatment dates back to human civilization. Long before the development of synthetic pharmaceuticals, communities across Africa, Asia, and South America relied on medicinal plants as their primary source of healthcare (Napagoda & Wijesundara, 2022). This reliance was particularly critical in malaria-endemic regions, where traditional healers prescribed plant preparations for fevers, chills, and other malaria-like symptoms (Nigussie& Wale, 2022). In many cultures, herbal remedies were administered as decoctions, infusions, or powders, often derived from the leaves, roots, or bark of locally available species (Obonti *et al*., 2021).

Traditional medicine not only provided accessible treatment but also preserved valuable ethnopharmacological knowledge, much of which is still in use today. Modern scientific studies continue to validate these practices by identifying bioactive phytochemicals with antiplasmodial potential from plants historically used to treat malaria (Irungu *et al*., 2023). Thus, medicinal plants represent a bridge between ancient wisdom and modern pharmacology, demonstrating the continuity of their importance across generations.

Contributions of Plants to Modern Antimalarial Drugs

The most significant advances in malaria treatment have originated from plants. A historic milestone was the isolation of quinine from the bark of the Cinchona species in the 17th

century. This alkaloid became the first effective antimalarial and remained the frontline treatment for centuries, saving millions of lives globally (Kingston &Cassera, 2022). Later, in the 20th century, another ground-breaking discovery emerged from traditional Chinese medicine: artemisinin, derived from Artemisia annua. Artemisinin and its derivatives form the basis of artemisinin-based combination therapies (ACTs), which remain the cornerstone of malaria treatment (Duffy *et al*., 2024; Stanisic& Good, 2023).

These two examples illustrate how traditional medicinal knowledge directly contributed to transformative breakthroughs in modern antimalarial therapy. Moreover, they highlight the ability of plant-derived compounds not only to serve as direct therapeutic agents but also to inspire the development of synthetic analogs with improved pharmacological profiles (Habibi *et al*., 2022). Current research continues to explore plants as reservoirs of secondary metabolites, alkaloids, terpenoids, flavonoids, and phenolics, many of which show significant *in vitro* and *in vivo* antiplasmodial activity (Cheuka *et al*., 2024; Hadi *et al*., 2025). This enduring relevance underscores the importance of medicinal plants as both historical and future contributors to malaria control.

Ethnobotanical Importance of *Alchornea cordifolia* and *Enantiachlorantha*

Within the African context, *Alchornea cordifolia* (Schumach. &Thonn.) Müll. Arg. and *Enantia chlorantha* Oliv. stand out as ethnomedicinal plants of remarkable antimalarial relevance. *Alchornea cordifolia*, commonly known as Christmas bush, is widely distributed across West and Central Africa and is traditionally used to treat malaria, fever, diarrhea, and microbial infections (Blaise *et al*., 2022). Phytochemical investigations reveal that the plant is rich in flavonoids, tannins, alkaloids, and terpenoids, compounds known to exert antiparasitic, antioxidant, and antimicrobial effects (Imieje *et al*., 2021). Animal model studies further support its antiplasmodial efficacy, validating its ethnobotanical use (Mahama *et al*., 2022).

Similarly, *Enantia chlorantha*, commonly known as African yellow wood, has been traditionally employed for malaria, jaundice, and various infectious diseases. Its stem bark is particularly rich in protoberberine alkaloids such as berberine, palmatine, and jatrorrhizine, which possess potent antimicrobial and antiplasmodial activities (Aladi, 2022; Imieje *et al*., 2024). Recent pharmacological studies confirm that aqueous and ethanolic extracts of *E. chlorantha* not only exhibit strong antimalarial activity but also improve redox balance and biochemical alterations in infected animal models (Evbuomwan *et al*., 2025).

Ethnobotanical surveys show that both plants are frequently used in polyherbal combinations for malaria treatment, where their synergistic effects are believed to enhance efficacy and reduce toxicity (Evbuomwan *et al*., 2023). Their continued use in African traditional medicine, supported by emerging scientific validation, positions them as promising candidates for modern antimalarial drug discovery.

Figure 1.1: *Alchornea cordifolia* leaf in its natural habitat.

Figure 1.2: *Enantia chlorantha* in its natural habitat

1.3 Malaria and Its Global Impact

1.3.1 Overview of Malaria

Malaria is one of the most significant parasitic diseases affecting humans, causing high morbidity and mortality across tropical and subtropical regions. It is caused by protozoan parasites of the genus Plasmodium, transmitted through the bite of infected female Anopheles mosquitoes (World Health Organization [WHO], 2022). Among the five species known to infect humans *Plasmodium falciparum*, *P. vivax, P. malariae, P. ovale*, and *P. knowlesi P. falciparum* is the most lethal and responsible for the majority of malaria-related deaths, particularly in sub-Saharan Africa (White, 2022; Stanisic & Good, 2023).

The life cycle of Plasmodium parasites involves both a human host and a mosquito vector. Transmission begins when an infected mosquito injects sporozoites into the human bloodstream during a blood meal. These sporozoites travel to the liver, where they invade hepatocytes and undergo asexual replication to form merozoites (Girgis *et al*., 2023). The

merozoites are subsequently released into the bloodstream, invade red blood cells (RBCs), and initiate cycles of erythrocytic schizogony. Within RBCs, the parasites mature into trophozoites, multiply, and rupture the cells, releasing more merozoites and causing the characteristic periodic fevers of malaria (Dkhil *et al*., 2021). Some parasites differentiate into sexual forms known as gametocytes, which are taken up by mosquitoes during feeding. Within the mosquito gut, gametocytes fuse to form zygotes, which develop into ookinetes, oocysts, and eventually sporozoites that migrate to the salivary glands completing the transmission cycle (Adeoye & Lobb, 2025).

Transmission dynamics are influenced by a complex interplay of environmental, vector-related, and socio-economic factors. Warm temperatures and high humidity provide optimal breeding conditions for Anopheles mosquitoes, which explains the endemicity of malaria in tropical regions (Filho *et al*., 2023). Population mobility, inadequate healthcare access, and poor housing also contribute to sustained transmission (Abbasi, 2022). Furthermore, the emergence of insecticide-resistant mosquitoes and drug-resistant Plasmodium strains has complicated malaria control and hindered global elimination efforts (Li *et al*., 2024; Ippolito *et al*., 2021).

1.3.2 Symptoms of Malaria

The clinical manifestations of malaria vary depending on the infecting Plasmodium species, the host's immune status, and the severity of infection. Malaria symptoms are generally classified into uncomplicated malaria and severe malaria (WHO, 2022).

Uncomplicated malaria typically presents as fever, chills, sweating, headache, nausea, vomiting, fatigue, and muscle pains. These symptoms are nonspecific and can resemble other febrile illnesses, making early diagnosis challenging (Obonti *et al*., 2021). In *P. falciparum* infections, fever often exhibits a tertian periodicity (every 48 hours), corresponding to the

synchronous rupture of infected erythrocytes, though irregular patterns are also common in endemic areas (Stanisic & Good, 2023).

Severe malaria, most commonly caused by *P. falciparum*, is associated with life-threatening complications resulting from high parasitemia and microvascular sequestration of infected red blood cells. Severe clinical features include cerebral malaria (manifesting as seizures, altered consciousness, or coma), severe anemia, jaundice, metabolic acidosis, renal failure, respiratory distress, and hypoglycemia (White, 2022; Dkhil *et al*., 2021). Children under five years of age and pregnant women are disproportionately affected due to their relatively weaker immunity (WHO, 2022). Without prompt diagnosis and treatment, severe malaria often results in death.

1.3.3 The Burden of Malaria

Global Prevalence and Mortality

Malaria remains one of the leading global health challenges, disproportionately affecting low- and middle-income countries. According to the World Health Organization (2022), there were approximately 247 million malaria cases and 619,000 deaths worldwide in 2021, with Africa accounting for about 95% of cases and deaths. Children under five years of age represented nearly 80% of malaria-related mortality, highlighting their vulnerability to the disease (Stanisic& Good, 2023). *Plasmodium falciparum* remain the dominant species in sub-Saharan Africa and is responsible for the highest rates of morbidity and mortality (White, 2022).

Despite progress achieved through vector control interventions, diagnostic improvements, and artemisinin-based combination therapies (ACTs), malaria incidence remains stubbornly high. Resistance to both insecticides and frontline drugs has emerged as a critical obstacle to sustained global control efforts (Ippolito *et al*., 2021; Li *et al*., 2024). Moreover, climate change, urbanization, and population displacement due to conflict and migration have further

complicated elimination strategies by expanding transmission zones into areas previously free of malaria (Abbasi, 2022; Filho *et al*., 2023).

Socioeconomic Impact

The burden of malaria extends beyond morbidity and mortality to significant socioeconomic consequences. Malaria is a leading cause of school and work absenteeism, reducing productivity and perpetuating cycles of poverty in endemic regions (Girgis *et al*., 2023). According to WHO (2022), the disease costs Africa an estimated USD 12 billion annually in lost productivity, treatment, and prevention expenses. The impact is especially severe in rural communities where access to healthcare facilities is limited and out-of-pocket treatment cost are high (Obonti *et al*., 2021).

Furthermore, malaria imposes a strain on healthcare systems through recurrent hospital admissions, blood transfusions for severe anaemia, and intensive care for cerebral malaria (Dkhil *et al*., 2021). The indirect costs, such as impaired cognitive development in children and reduced agricultural labour, exacerbate long-term socioeconomic instability (Cheuka *et al*., 2024). Thus, malaria is not only a public health issue but also a major barrier to sustainable economic development in endemic countries.

1.3.4 Prevalence of Malaria in Nigeria

Epidemiological Data

Nigeria bears the highest global malaria burden, accounting for approximately 27% of all malaria cases and 31% of global malaria deaths in 2021 (WHO, 2022). The disease remains one of the top causes of outpatient visits, hospitalizations, and mortality, particularly among children and pregnant women (White, 2022). Despite extensive efforts such as long-lasting

insecticidal nets (LLINs), indoor residual spraying, and ACT distribution, malaria prevalence in Nigeria has declined only modestly in the last decade (Evbuomwan *et al*., 2023).

National surveys indicate variations in prevalence across geopolitical zones, with the northern regions experiencing higher transmission intensity compared to the southern states. This disparity is linked to climatic factors, population density, and socio-economic determinants of health (Abbasi, 2022; Filho *et al*., 2023). *P. falciparum* accounts for over 95% of infections in Nigeria, making it the most significant species of concern (Stanisic& Good, 2023).

Endemicity and Transmission Patterns

Nigeria is holoendemic for malaria, meaning transmission occurs year-round, with seasonal peaks during the rainy season when vector breeding is intensified (Obonti *et al*., 2021). The major vectors are members of the *Anopheles gambiae* complex, which thrive in the warm and humid climate prevalent across much of the country (White, 2022). Transmission is further sustained by poor housing, limited use of preventive measures, and widespread insecticide resistance among mosquito populations (Ippolito *et al*., 2021).

Endemicity also varies between rural and urban areas. Rural communities, where healthcare access is limited, bear the heaviest burden of transmission, while urban areas experience more sporadic outbreaks often associated with poor sanitation and drainage (Girgis *et al*., 2023). Despite ongoing malaria control initiatives, challenges such as drug resistance, limited healthcare infrastructure, and socio-economic inequalities hinder elimination efforts. Nigeria's disproportionate malaria burden emphasizes the urgency for novel therapeutic interventions, including those derived from medicinal plants with validated efficacy and safety profiles (Cheuka *et al*., 2024; Evbuomwan *et al*., 2025).

1.4 Management of Malaria

1.4.1 Antimalarial Drugs and Treatment Approaches

Classification of Antimalarials

Antimalarial drugs form the cornerstone of malaria treatment and control strategies. These agents are classified based on their chemical structure, mechanism of action, or stage of parasite development they target. The major classes include quinoline derivatives, antifolates, artemisinin and its derivatives, antibiotics, and endoperoxides (White, 2022; Ippolito *et al*., 2021).

- **Quinolines**: This group includes chloroquine, quinine, mefloquine, amodiaquine, and primaquine. Quinine, one of the earliest antimalarial agents, was derived from the bark of the *Cinchona* tree and remains clinically relevant, particularly for severe malaria (Cheuka *et al*., 2024). Chloroquine, once the first-line treatment for *P. falciparum* malaria, has lost efficacy in most endemic regions due to widespread resistance (White, 2022). Primaquine, a unique 8-aminoquinoline, is effective against dormant liver hypnozoites of *P. vivax* and *P. ovale*, making it essential for radical cure (Girgis *et al*., 2023).
- **Antifolates**: Drugs such as sulfadoxine-pyrimethamine (SP) inhibit folate biosynthesis, thereby blocking DNA and protein synthesis in the parasite (Li *et al*., 2024). SP has been widely used for intermittent preventive treatment in pregnancy (IPTp), though resistance has limited its effectiveness in some regions (Obonti *et al*., 2021).
- **Artemisinin Derivatives**: Artemisinin, isolated from *Artemisia annua*, and its semisynthetic derivatives (artesunate, artemether, dihydroartemisinin) are the most effective and fast-acting antimalarials available today (Stanisic& Good, 2023). They form the basis of artemisinin-based combination therapies (ACTs), which pair

artemisinin derivatives with longer-acting partner drugs to enhance efficacy and reduce the risk of resistance (WHO, 2022).

- **Other Agents**: Antibiotics such as doxycycline and clindamycin have also been employed as adjunctive therapies, especially in non-endemic travellers or cases of drug resistance (Abbasi, 2022).

Mechanisms of Action

Each class of antimalarial drugs targets specific pathways in the parasite:

- **Quinolines** act by interfering with heme detoxification in the parasite's digestive vacuole. Normally, *Plasmodium* parasites digest haemoglobin and release toxic heme, which they detoxify by polymerizing into hemozoin. Quinolines disrupt this process, leading to the accumulation of toxic heme and parasite death (White, 2022).
- **Antifolates** inhibit enzymes such as dihydropteroate synthase and dihydrofolate reductase, disrupting folate metabolism and nucleic acid synthesis (Li *et al*., 2024).
- **Artemisinin and its derivatives** generate free radicals upon activation by heme iron within the parasite, causing widespread damage to parasite proteins and membranes (Stanisic& Good, 2023).
- **Primaquine** is believed to interfere with mitochondrial function and electron transport in parasites, making it effective against liver stages and gametocytes (Girgis *et al*., 2023).

Limitations of Antimalarial Drugs

Despite advances in chemotherapy, significant challenges hinder malaria management. The most critical limitation is the emergence of drug resistance. Chloroquine resistance emerged in the 1950s and spread globally, leading to its replacement by ACTs (White, 2022). Resistance to sulfadoxine-pyrimethamine is widespread, and there is growing concern over partial

resistance to artemisinin, particularly in Southeast Asia and, more recently, Africa (Ippolito *et al*., 2021; Li *et al*., 2024). This threatens the efficacy of ACTs, the cornerstone of malaria treatment.

Other limitations include adverse effects and tolerability issues. For instance, quinine is associated with cinchonism (tinnitus, dizziness, nausea), while mefloquine can cause neuropsychiatric effects (Cheuka *et al*., 2024). Primaquine, although vital for radical cure, can cause haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Girgis *et al*., 2023). Furthermore, the short half-life of artemisinin necessitates combination therapies, increasing costs and accessibility concerns in resource-limited settings (WHO, 2022).

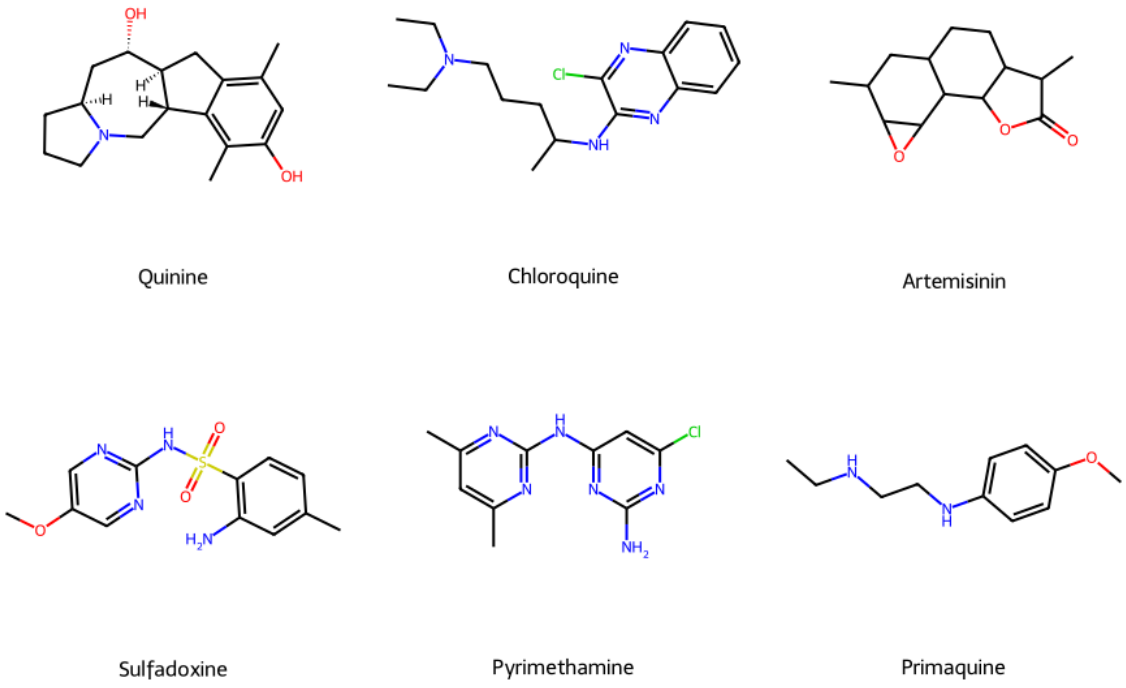

Figure 1.3: Antimalarial drugs

1.4.2 Prevention Strategies

Malaria prevention remains a cornerstone of control and elimination programs, particularly in endemic regions where the burden of disease remains high. Strategies for prevention are multifaceted and include vector control**,** chemoprevention, and vaccination efforts. These interventions have proven effective in reducing the incidence, morbidity, and mortality associated with *Plasmodium falciparum* and *Plasmodium vivax* infections.

1. **Vector Control (ITNs, IRS)**

Vector control interventions are considered the most effective preventive strategies against malaria. The use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) has contributed substantially to reducing malaria transmission in sub-Saharan Africa and other endemic regions (World Health Organization [WHO], 2022; Collins & Duffy, 2022). ITNs provide a protective barrier against mosquito bites, and large-scale distribution campaigns have demonstrated significant reductions in morbidity and mortality, particularly among children under five and pregnant women (Dasgupta *et al*., 2022). IRS, on the other hand, reduces mosquito density and longevity by applying insecticides to interior walls where vectors commonly rest (Hamilton *et al*., 2023). Despite their effectiveness, challenges such as insecticide resistance and sustainability of distribution programs continue to hinder optimal outcomes (Ippolito *et al*., 2021).

2. **Chemoprevention**

Chemo-preventive approaches remain critical for high-risk populations. Intermittent preventive treatment in pregnancy (IPTp) and seasonal malaria chemoprevention (SMC) for children are widely adopted strategies (de Cola *et al*., 2022; Isiko *et al*., 2024). IPTp with sulfadoxine-pyrimethamine has been shown to reduce the risk of maternal anaemia, low birth weight, and neonatal mortality (Adebusuyi *et al*., 2024). SMC, which involves monthly

administration of antimalarial drugs during peak transmission seasons, has significantly reduced malaria prevalence in West African countries (de Cola *et al*., 2022). Furthermore, chemoprevention remains an important tool to complement vector control in settings of high transmission and drug resistance (Daily *et al*., 2022).

3. **Vaccination Efforts**

Vaccination represents a promising advancement in malaria prevention. The RTS, S/AS01 vaccine, which targets the *Plasmodium falciparum* circumsporozoite protein, has shown modest efficacy in reducing clinical malaria among children (Stanisic& Good, 2023; Duffy *et al*., 2024). Ongoing developments, including the R21/Matrix-M vaccine, have demonstrated higher efficacy rates and are currently under wider evaluation (Hamilton *et al*., 2023). Mathematical modelling indicates that malaria vaccines, when combined with ITNs and SMC, could significantly reduce the disease burden and mitigate the risk of drug resistance in Africa (Hamilton *et al*., 2023). Nevertheless, challenges remain regarding long-term immunity, cost, and large-scale implementation in resource-limited settings (Li *et al*., 2024; Venkatesan, 2025).

1.5 Plasmepsin II as a Drug Target in *Plasmodium falciparum*

Role in Haemoglobin Degradation

Plasmepsin II is an aspartic protease localized in the acidic food vacuole of *Plasmodium falciparum,* where it plays a pivotal role in the initial stages of haemoglobin degradation. The parasite relies on host haemoglobin as a major nutrient source, breaking it down into peptides and amino acids necessary for its growth and replication (Adeoye & Lobb, 2025; Faloye *et al*., 2025). Specifically, plasmepsin II catalyses the cleavage of haemoglobin into smaller fragments, which are further processed by falcipains (cysteine proteases) and metalloproteases (Ji *et al*., 2022; Ishola *et al*., 2023). Inhibition of plasmepsin II disrupts this catabolic process,

leading to parasite starvation and the accumulation of toxic heme by products, ultimately resulting in parasite death (Syed *et al*., 2025; Manhas *et al*., 2022). Thus, the essential role of plasmepsin II in haemoglobin catabolism establishes it as a crucial metabolic enzyme for parasite survival.

Validation as a Drug Target

The essentiality of plasmepsin II for parasite survival has validated it as a potential drug target. Structural biology and molecular docking studies have provided evidence that inhibiting plasmepsin II can significantly impair parasite development (En-Nahli *et al*., 2023; Faloye *et al*., 2025). Several computational approaches, including high-throughput virtual screening and molecular dynamics simulations, have identified natural compounds and synthetic analogs with strong binding affinities to plasmepsin II (Manhas *et al*., 2022; Fernando *et al*., 2024). Furthermore, comparative studies indicate that targeting plasmepsin II could overcome some of the limitations of current antimalarial therapies, particularly in the face of increasing resistance to artemisinin and partner drugs (Adeoye & Lobb, 2025; Cheuka *et al*., 2024). The convergence of biochemical assays, *in silico* modelling, and parasite culture studies continues to validate plasmepsin II as a promising therapeutic target for next-generation antimalarial agents.

Potential for Inhibitor Design

The potential for designing effective plasmepsin II inhibitors lies in exploiting its well-characterized crystal structure and active site specificity. Advances in molecular docking and structure–activity relationship studies have facilitated the identification of plant-derived phytochemicals, transition metal complexes, and synthetic analogs with inhibitory potential (Hamza *et al*., 2024; Ibrahim *et al*., 2024). Natural compounds from medicinal plants such as *Enantiachlorantha* and *Alchornea cordifolia* have also shown promising interactions with

plasmepsin II, reinforcing the therapeutic potential of ethnomedicinal resources (Evbuomwan *et al*., 2025; Imieje *et al*., 2021). *In silico* screening and computational modelling have revealed strong binding affinities for flavonoids, alkaloids, and terpenoids, suggesting their suitability for rational drug design (Faloye *et al*., 2025; Ishola *et al*., 2023). Additionally, novel approaches combining plasmepsin II inhibitors with other protease inhibitors or standard antimalarial drugs could enhance efficacy and reduce the likelihood of resistance development (Syed *et al*., 2025). Collectively, the integration of computational chemistry, medicinal plant bioprospecting, and rational drug design provides a strong foundation for the development of novel plasmepsin II inhibitors as potent antimalarial candidates.

1.6 Computer-Aided Drug Design (In Silico Screening)

1.6.1 Overview of CADD

Computer-aided drug design (CADD) has become a fundamental strategy in modern pharmaceutical research, particularly in the era of rising antimicrobial resistance. Malaria, caused primarily by *Plasmodium falciparum*, continues to pose a major public health threat, and the rapid development of resistance to frontline drugs such as chloroquine and artemisinin highlights the urgent need for innovative drug discovery methods (Ippolito *et al*., 2021; Adeoye & Lobb, 2025). CADD provides a platform to accelerate this process by leveraging computational tools to analyse interactions between potential drug molecules and biological targets.

The significance of CADD in drug discovery lies in its ability to bridge the gap between theoretical predictions and experimental validations. It enables the identification of novel lead compounds from vast chemical libraries, including synthetic molecules and phytochemicals, that may not be immediately accessible through traditional laboratory screening (Cheuka *et al*., 2024; En-Nahli *et al*., 2023). This is particularly valuable in antimalarial research where

medicinal plants such as *Enantiachlorantha* and *Alchornea cordifolia* have historically provided important therapeutic leads (Evbuomwan *et al*., 2025; Imieje *et al*., 2021).

CADD also offers several advantages compared to conventional drug discovery methods. It is cost-effective because it reduces reliance on high-cost wet-laboratory experiments in the early stages by filtering out compounds with poor pharmacokinetic or toxicological properties (Fernando *et al*., 2024). It is also time-saving, as virtual screening of thousands of compounds can be conducted in days, compared to months or years with conventional assays (Ji *et al*., 2022; Manhas *et al*., 2022). Additionally, it allows high-throughput analyses, providing simultaneous evaluation of structural binding, energetics, and predicted biological activity across diverse compound classes (Faloye *et al*., 2025). As such, CADD has evolved into an indispensable tool for modern antimalarial drug discovery programs.

1.6.2 Structure-Based Drug Design (Molecular Docking)

Structure-based drug design (SBDD), with molecular docking as its central technique, has revolutionized malaria drug discovery. Docking involves simulating how small molecules (ligands) interact with the three-dimensional structure of target proteins, predicting both the binding orientation and the interaction affinity (Ji *et al*., 2022). The process typically begins with preparing the protein target (e.g., plasmepsin II, falcipain-2, or dihydrofolate reductase), followed by optimizing potential ligands, and finally using scoring functions to estimate the strength of ligand–protein binding (Fernando *et al*., 2024).

In malaria research, molecular docking has been extensively applied to validate proteases as drug targets. For example, plasmepsin II, an aspartic protease critical in haemoglobin degradation, has been shown through docking studies to bind strongly with plant-derived alkaloids, flavonoids, and synthetic inhibitors, supporting its role as a druggable enzyme (Faloye *et al*., 2025; Syed *et al*., 2025). Docking has also been employed to identify lead

phytochemicals from African medicinal plants, including compounds isolated from *Enantia chlorantha* and *Alchornea cordifolia*, which demonstrated significant binding potential for plasmepsin II and related enzymes (Evbuomwan *et al*., 2025; Imieje *et al*., 2021).

Furthermore, docking applications extend to drug repurposing, in which existing drugs are screened against malaria targets to identify new uses. For example, fluoroquinolones have been computationally validated as plasmepsin II inhibitors, indicating their potential as scaffolds for antimalarial drug development (Syed *et al*., 2025). Docking has also facilitated lead optimization, where structural modifications to phytochemicals or synthetic scaffolds improve binding affinity, selectivity, and stability (Manhas *et al*., 2022; En-Nahli *et al*., 2023). These advances illustrate how molecular docking not only aids in drug discovery but also accelerates the translation of natural compounds into clinically viable candidates.

1.6.3 ADMET and Drug-Likeness Predictions

While strong binding affinity is important, drug candidates must also exhibit favourable pharmacological and safety properties to progress successfully through the drug pipeline. Computational modelling of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) parameters has therefore become a vital component of CADD workflows (Hamza *et al*., 2024; Ibrahim *et al*., 2024).

A key concept in drug-likeness prediction is Lipinski's Rule of Five, which stipulates that compounds are more likely to be orally bioavailable if they have: molecular weight below 5000 Da, no more than five hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a logP value (lipophilicity) below five (Ain *et al*., 2024). Many phytochemicals identified from antimalarial plants have been evaluated against this criterion, and those that meet the criteria are prioritized for further development (Faloye *et al*., 2025).

ADMET predictions also extend to toxicity assessment, including hepatotoxicity, mutagenicity, and cardiotoxicity risks, which are among the leading causes of late-stage drug development failures (Alum, 2024; Sulyman *et al*., 2023). For instance, transition-metal complexes investigated as plasmepsin inhibitors have undergone *in silico* ADMET profiling, which demonstrated acceptable safety margins before *in vivo* validation (Hamza *et al*., 2024). Similarly, computational pharmacokinetic models have been used to evaluate the bioavailability and distribution of phytochemicals from *Enantia chlorantha* and *Alchornea cordifolia*, ensuring that promising compounds also meet safety thresholds before preclinical testing (Ibrahim *et al*., 2024).

By integrating drug-likeness rules with ADMET modelling, researchers can filter out unsuitable candidates early, thereby reducing the likelihood of costly failures in experimental studies. This makes ADMET and drug-likeness predictions an indispensable complement to molecular docking in the rational design of novel antimalarial drugs.

1.7 In Vivo Toxicity Assessment

Importance of Safety Evaluation in Drug Discovery

The process of drug discovery not only focuses on efficacy but also prioritizes safety evaluation to ensure that promising therapeutic candidates do not pose unacceptable risks to humans. Safety assessment is particularly critical in antimalarial drug development, where compounds are often administered in endemic regions with vulnerable populations such as children and pregnant women (Daily *et al*., 2022; White, 2022). Despite promising *in silico* and *in vitro* outcomes, compounds may still exhibit harmful side effects in whole organisms due to complex pharmacokinetic and metabolic interactions (Adeoye & Lobb, 2025; Cheuka *et al*., 2024). Therefore, *in vivo* toxicity studies remain an indispensable step in bridging preclinical findings

with clinical application, reducing the likelihood of drug attrition during later development stages.

Acute Toxicity Studies in Laboratory Animals

Acute toxicity testing is one of the earliest steps in evaluating the safety of novel compounds. It involves administration a single or repeated dose of a substance to laboratory animals, typically rodents, to determine immediate harmful effects is establish the lethal dose 50 (LD50) value (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). This approach provides insights into potential systemic toxicity, behavioural alterations, and biochemical imbalances that may arise from exposure. For example, the aqueous extract of *Enantia chlorantha* has been shown to exhibit antimalarial activity with a favourable safety profile in mice, demonstrating its potential as a safe herbal remedy (Evbuomwan *et al*., 2025). Similarly, combined plant extracts such as *Alchornea cordifolia* have undergone murine toxicity testing, with findings confirming low toxicity at therapeutic doses (Mahama *et al*., 2022). These studies highlight the importance of acute toxicity assessments for validating both synthetic and plant-derived compounds before clinical use.

OECD Guidelines for Toxicity Testing

To standardize safety evaluations globally, the Organisation for Economic Co-operation and Development (OECD) provides internationally accepted guidelines for toxicity testing. OECD protocols outline standardized methodologies for assessing acute oral toxicity, repeated-dose toxicity, and other safety endpoints in animal models (Babandi, 2025; Andrade *et al*., 2022). For acute toxicity, OECD Test Guidelines 423 and 425 are commonly employed, which recommend stepwise dose administration and animal observation to determine safety thresholds. The use of these guidelines ensures reproducibility, reliability, and ethical compliance by minimizing the number of animals used while maximizing the quality and safety

of the data (Dasgupta *et al*., 2022; Dkhil *et al*., 2021). In malaria research, adherence to OECD standards strengthens the credibility of toxicity assessments, facilitating regulatory approval and global acceptance of new drug candidates.

Relevance to Herbal Medicine Validation

The importance of *in vivo* toxicity assessment is particularly pronounced in validating herbal medicines, which are widely used for malaria treatment across Africa and Asia. Despite their traditional use, many herbal formulations lack rigorous scientific safety data, raising concerns about potential toxicity, drug–herb interactions, and dosage variability (Nigussie& Wale, 2022; Irungu *et al*., 2023). Acute toxicity studies provide the first scientific evidence to support or refute the safety claims of ethnomedicinal plants. For instance, extracts of *Alchornea cordifolia* and *Enantia chlorantha* have been traditionally used in , and their validation through OECD-guided toxicity studies enhances their credibility as potential phytotherapeutic agents (Blaise *et al*., 2022; Evbuomwan *et al*., 2023).

Moreover, toxicity testing helps establish therapeutic windows, distinguishing safe dosage levels from harmful concentrations. This is crucial in herbal drug development, where plant extracts often contain multiple bioactive compounds with synergistic or antagonistic effects (Habibi *et al*., 2022). By providing evidence-based safety profiles, *in vivo* toxicity assessment ensures that traditional remedies can be translated into standardized, clinically acceptable medicines, thereby bridging ethnopharmacology with modern drug discovery.

Locke's Procedure in In Vivo Toxicity Assessment

In the evaluation of acute and subacute toxicity of plant extracts or synthetic compounds, standardized procedures are critical to ensuring reproducibility and reliability of results. One such recognized approach is **Locke's method**, which is often referenced in pharmacological and toxicological studies involving laboratory animals. Locke's procedure provides a

systematic framework for administering test substances, monitoring animals, and recording physiological and behavioural responses to establish safety margins and determine the median lethal dose (LD_{50}) (Mahama *et al*., 2022; Evbuomwan *et al*., 2025).

Principles of Locke's Procedure

Locke's method is based on the premise that the acute administration of a test compound at graded doses allows researchers to identify immediate signs of systemic toxicity and mortality patterns. It incorporates close observation of animals following oral or intraperitoneal administration of the extract under study, with emphasis on:

Clinical signs such as changes in locomotion, feeding behavior, respiration, salivation, tremors, or convulsions.

Physiological markers include body weight, grooming, and eye/nasal discharge.

Mortality tracking across a 24–72-hour period, extended to 14 days where necessary to capture delayed toxicity manifestations (Dkhil *et al*., 2021).

This framework enables researchers to establish the LD_{50} value, which remains a key index of acute toxicity.

Relevance to Herbal Medicine Evaluation

Locke's procedure has been widely applied in validating the safety of ethnomedicinal plants such as *Alchornea cordifolia* and *Enantia chlorantha*. For instance, aqueous and ethanolic extracts of these plants have been tested in rodents using Locke's framework, with findings demonstrating **low toxicity at therapeutic doses** and providing preliminary evidence of their safety for use in traditional medicine (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). This aligns with OECD guidelines for toxicity testing, reinforcing that acute toxicity evaluation

remains indispensable before advancing plant extracts into more complex sub-chronic or chronic studies (Andrade *et al*., 2022).

Advantages of Locke's Procedure

1. **Standardization** – Locke's method provides a replicable structure for comparing toxicological profiles of different compounds or extracts.
2. **Ethical efficiency** – It minimizes the number of animals used while maximizing data quality, complementing OECD principles of humane experimentation.
3. **Translational value** – By establishing safety thresholds early, the procedure supports the rational development of herbal therapies into standardized phytomedicines.

Limitations

Despite its usefulness, Locke's procedure has limitations. It primarily addresses **acute toxicity**, leaving gaps in chronic, reproductive, and genotoxicity evaluations, which are critical for clinical translation (Ippolito *et al*., 2021). Furthermore, plant extracts often contain multiple bioactive constituents with complex interactions, making it difficult to attribute observed toxic effects to specific compounds (Nigussie & Wale, 2022).

1.8 Literature Review

Studies on *Alchornea cordifolia:* Phytochemistry, Pharmacological Activities, and Traditional Uses

Alchornea cordifolia (commonly known as "Christmas bush") is a medicinal plant widely distributed in tropical Africa and used in various ethnomedicinal systems. Over the decades, this plant has attracted significant attention for its phytochemical richness, diverse pharmacological activities, and a long history of traditional applications for managing infectious and non-infectious diseases.

Phytochemistry

Phytochemical investigations of *A. cordifolia* have revealed a wide range of bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds (Irungu *et al*., 2023; Blaise *et al*., 2022). These metabolites are believed to contribute to its antimalarial, antimicrobial, and anti-inflammatory properties. For example, anthocyanins and ellagic acid derivatives isolated from the plant have demonstrated strong antioxidant activity, supporting its therapeutic role in oxidative stress-related conditions (Habibi *et al*., 2022). The presence of such diverse secondary metabolites underscores the plant's potential as a source of novel drug candidates, particularly for the treatment of parasitic infections such as malaria (Evbuomwan *et al*., 2023).

Phytochemicals from *Alchornea cordifolia*

1. Ellagic acid (polyphenolic antioxidant, widely reported in *A. cordifolia*) IUPAC: 2,3,7,8-Tetrahydroxy [1] benzopyrano[5,4,3-cde][1] benzopyran-5,10-dione
2. Gallic acid (hydrolysable tannin precursor) IUPAC: 3,4,5-Trihydroxybenzoic acid
3. Quercetin (flavonoid with strong antioxidant/antimalarial potential) IUPAC: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
4. β -sitosterol (triterpenoid with anti-inflammatory & immunomodulatory effects) IUPAC: (3β)-stigmast-5-en-3-ol

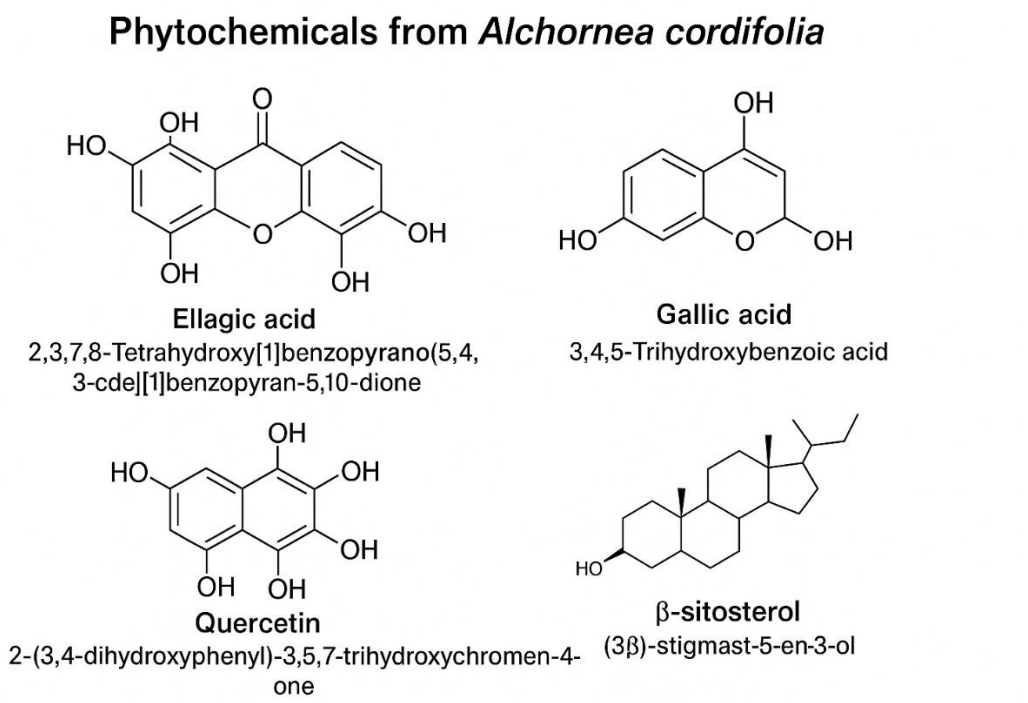

Figure 1.4: Chemical structures of major phytochemical constituents from *Alchornea cordifolia*

Pharmacological Activities

Several pharmacological studies have validated the therapeutic potential of *A. cordifolia*. Extracts from the leaves, stem bark, and roots have demonstrated antiplasmodial activity, both *in vitro* and *in vivo*, suggesting their potential as a complementary therapy in malaria management (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). Beyond its antimalarial effects, the plant also exhibits significant antimicrobial properties, effective against bacterial and fungal pathogens, thereby supporting its ethnopharmacological use in treating infections (Nigussie& Wale, 2022).

Toxicological studies further indicate that *A. cordifolia* extracts are relatively safe at therapeutic doses, with acute toxicity studies in rodents showing no severe adverse effects at moderate concentrations (Mahama *et al*., 2022). Additionally, its anti-inflammatory and wound-healing properties have been reported, attributed mainly to its tannin and flavonoid content (Blaise *et*

al., 2022). These findings underscore the plant's broad spectrum of bioactivity and its pharmacological relevance in modern drug discovery.

Traditional Uses

Traditionally, *Alchornea cordifolia* has been extensively employed in African ethnomedicine. Decoctions of its leaves and bark are commonly used to treat malaria, fever, diarrhea, respiratory infections, and wounds (Irungu *et al*., 2023). Its role in malaria treatment is particularly noteworthy, as local healers often combine it with other herbs such as *Enantia chlorantha* to enhance therapeutic efficacy (Evbuomwan *et al*., 2025). In addition to infectious diseases, it is used to manage inflammatory disorders, gastrointestinal complaints, and skin ailments, reflecting its broad traditional pharmacopoeia (Nigussie & Wale, 2022).

The persistence of its use across generations and regions demonstrates strong cultural trust in its medicinal properties. However, the growing body of scientific validation not only supports these traditional claims but also provides a foundation for integrating *A. cordifolia* into modern therapeutic strategies against malaria and other diseases.

Studies on *Enantia chlorantha*: Phytochemistry, Pharmacological Activities, and Traditional Uses

Phytochemistry

Enantia chlorantha (African yellow wood) is chemically rich, with studies consistently reporting abundant alkaloids alongside phenolics, flavonoids, and terpenoids (Aladi, 2022; Imieje *et al*., 2024; Ibrahim *et al*., 2024). The stem and root bark are particularly rich in alkaloids and have yielded multiple bioactive constituents that underpin its broad pharmacology (Imieje *et al*., 2024; Ibrahim *et al*., 2024). *In silico* profiling of root-bark metabolites highlights structural features compatible with antimalarial target engagement, including H-bonding and π–π interactions at protease active sites (Ibrahim *et al*., 2024).

Complementary computational chemistry (e.g., DFT) on isolated alkaloids from the stem bark supports their electronic readiness for biological activity and correlates with observed anti-infective effects (Imieje *et al*., 2024). Beyond alkaloids, enzyme-modulating phytochemicals that inhibit carbohydrate-digestive enzymes (α-amylase/α-glucosidase) were reported in the stem bark, suggesting metabolic benefits that may support host resilience during infection (Aladi, 2022). Collectively, this phytochemical breadth provides a rational basis for the plant's multi-target therapeutic profile and its recurrent appearance in antimalarial ethnomedicine (Ceravolo *et al*., 2021; Kingston &Cassera, 2022).

Phytochemicals from *Enantia chlorantha*

1. Berberine (protoberberine alkaloid, yellow pigment, strong antimicrobial/antiplasmodial activity) IUPAC: 2,3-Methylenedioxy-9,10-dimethoxyprotoberberinium
2. Palmatine (isoquinoline alkaloid, structurally related to berberine) IUPAC: 2,3-Methylenedioxy-9,10-dimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium
3. Jatrorrhizine (isoquinoline alkaloid, antimicrobial and antioxidant) IUPAC: 2,3-dihydroxy-9,10-dimethoxyprotoberberinium
4. Magnoflorine (aporphine alkaloid, often isolated from *E. chlorantha* bark) IUPAC: (S)-1,2,3,10-tetramethoxyaporphin-7-ium

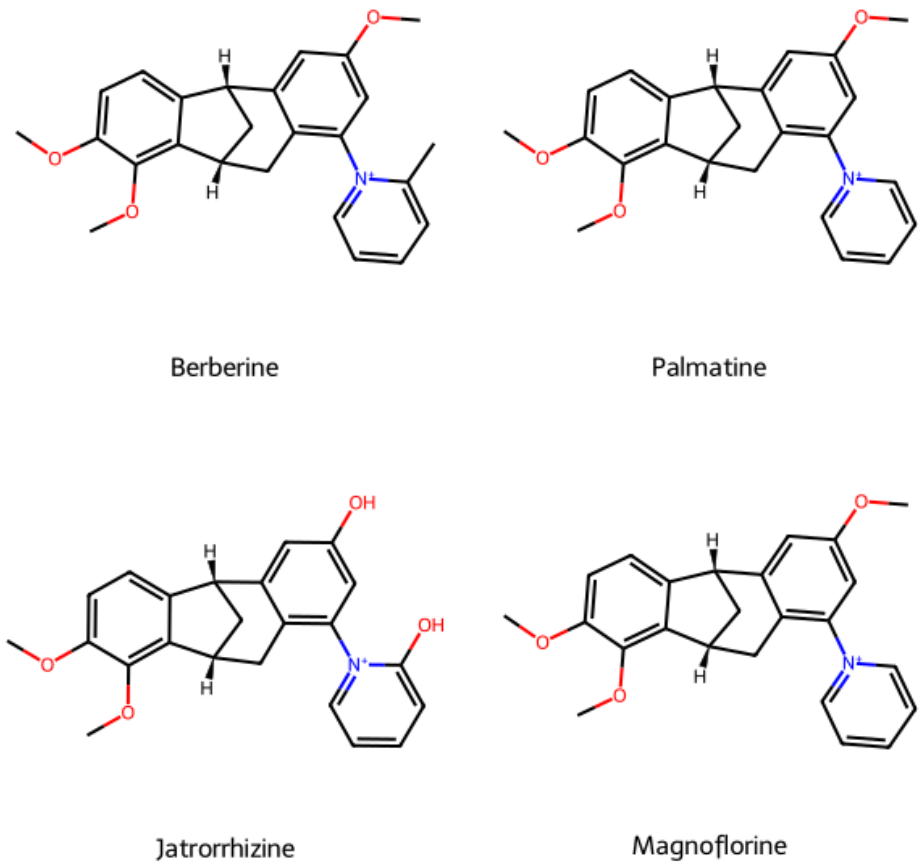

Figure 1.5: Chemical structures of major phytochemical constituents from *Enantia chlorantha*

Pharmacological Activities

Antimalarial activity. Multiple lines of evidence support antiplasmodial potential. An aqueous stem-bark extract produced significant antimalarial effects in mice, while also improving redox balance and normalizing biochemical perturbations, indicating both efficacy and a favorable safety signal *in vivo* (Evbuomwan *et al*., 2025). Ethnobotanical mapping in Nigeria independently documents frequent use of *E. chlorantha* in community malaria remedies, aligning traditional practice with laboratory signals (Evbuomwan *et al*., 2023). At the mechanistic level, *in silico* studies on root-bark constituents predict strong binding to key *P. falciparum* targets, including haemoglobin-degrading proteases supporting a plausible

molecular basis for the observed activity (Ibrahim *et al*., 2024). More broadly, computational campaigns against malarial proteases and other validated targets position *E. chlorantha* metabolites as promising scaffolds within current antimalarial discovery pipelines (Cheuka *et al*., 2024; Ji *et al*., 2022).

Anti-infective and broad antimicrobial effects. Isolated stem-bark alkaloids exhibited anti-infective activity in biological assays, complemented by mechanistic insights from DFT that link electronic properties to the pro-apoptotic impacts in target organisms (Imieje *et al*., 2024). Reviews of medicinal plants used against malaria also cite *E. chlorantha* for antibacterial and antifunga**l** activities, properties that may reduce malaria-related co-infections and secondary complications (Ceravolo *et al*., 2021; Obonti *et al*., 2021).

Antioxidant and host-protective actions. Oxidative stress accompanies severe malaria; extracts of *E. chlorantha* mitigated redox imbalance and biochemical alterations in infected mice, suggesting antioxidant and cytoprotective benefits that complement parasite suppression (Evbuomwan *et al*., 2025).

Metabolic enzyme modulation. Inhibition of α-amylase/α-glucosidase by stem-bark constituents indicates potential glycaemic control benefits (Aladi, 2022). While not antimalarial per se, improved metabolic homeostasis may enhance recovery trajectories during or after infection.

Safety/Toxicology. The murine study reporting antimalarial efficacy also observed favourable safety indicators, supporting a workable therapeutic window (Evbuomwan *et al*., 2025). This aligns with broader guidance that plant-based leads should proceed with standardized OECD-style toxicity assessments in parallel with efficacy testing (Dkhil *et al*., 2021; Andrade *et al*., 2022).

Traditional Uses

Across West and Central Africa, *E. chlorantha* features prominently in traditional malaria treatments, either alone or in polyherbal combinations often paired with other antimalarial plants in decoctions and macerates (Evbuomwan *et al*., 2023; Akhtar *et al*., 2024). Beyond malaria, communities use the stem and root bark for fevers, gastrointestinal complaints, respiratory infections, wound care, and general "blood cleansing," reflecting its broad anti-infective reputation (Obonti *et al*., 2021; Ceravolo *et al*., 2021). Ethnobotanical prevalence, together with convergent pharmacology (antiplasmodial, antimicrobial, antioxidant), explains its continued prominence in primary healthcare and its prioritization in modern bioprospecting efforts (Kingston &Cassera, 2022; Irungu *et al*., 2023). Recent Nigerian field studies documenting sustained community reliance on *E. chlorantha* underscore the cultural continuity and practical accessibility that often drive real-world utilization (Evbuomwan *et al*., 2023).

Synthesis and Implications

Bringing together phytochemistry (alkaloid-rich profiles with drug-like properties), experimental pharmacology (murine antimalarial efficacy and anti-infective activity), and traditional evidence (widespread use in malaria remedies), *E. chlorantha* stands out as a credible lead source for antimalarial discovery. The alignment between ethnomedical prominence and modern *in silico*/*in vivo* signals strengthens its candidacy for structure-guided optimization (e.g., plasmepsin-focused inhibitor design) and for standardized safety validation to support future clinical translation (Ibrahim *et al*., 2024; Cheuka *et al*., 2024; Evbuomwan *et al*., 2025).

Previous reports on antiplasmodial activities of the plants (individual or combined)

Individual plant studies

***Alchornea cordifolia*.** Multiple studies document the antiplasmodial potential of *A. cordifolia* across *in vitro* and *in vivo* models. Crude extracts from leaves and other plant parts have shown inhibitory activity against *Plasmodium* spp. in cell-based assays, and fractionation studies have identified bioactive fractions with enhanced potency (Imieje *et al*., 2021; Mahama *et al*., 2022). Ethnopharmacological surveys and mini-reviews summarize that the plant contains alkaloids, flavonoids, tannins, and other secondary metabolites plausibly responsible for antiplasmodial effects (Blaise *et al*., 2022; Ceravolo *et al*., 2021). In murine models, chloroform fractions and other preparations reduced parasitemia with acceptable acute safety signals at therapeutic doses (Mahama *et al*., 2022). These experimental and ethnobotanical data together underpin *A. cordifolia* as a validated source of antiplasmodial leads (Imieje *et al*., 2021; Blaise *et al*., 2022).

***Enantia chlorantha*.** Similarly, *E. chlorantha* has a substantive experimental record supporting antimalarial activity. *In vivo* studies with aqueous stem-bark extracts demonstrated antimalarial efficacy in mice, coupled with improvements in redox status and suggesting correction of these abnormalities and both antiparasitic and host-protective effects (Evbuomwan *et al*., 2025). Phytochemical and computational characterizations report an alkaloid-rich profile and predict strong interactions of isolated constituents with parasite targets (Ibrahim *et al*., 2024; Imieje *et al*., 2024). Ethnobotanical work confirms widespread traditional use of *E. chlorantha* for fever and malaria, lending concordant cultural support to laboratory findings (Evbuomwan *et al*., 2023; Ceravolo *et al*., 2021).

Reports on combinations and polyherbal use

Ethnobotanical co-use and formulation. Traditional medicine frequently employs *A. cordifolia* and *E. chlorantha* either singly or in combinations with other antimalarial plants.

Ethnobotanical surveys document polyherbal decoctions in which these species are combined to potentiate activity or broaden the therapeutic spectrum (Evbuomwan *et al*., 2023; Kingston & Cassera, 2022). Such combined use is every day in community practice and motivates formal study of synergistic interactions.

Although ethnomedicinal practices consistently highlight the co-use of *Alchornea cordifolia* and *Enantia chlorantha*, direct experimental studies on their defined combinations remain limited. Available evidence largely demonstrates that each plant, when tested individually, exhibits significant antiplasmodial activity and acceptable acute safety in rodent models (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). Ethnobotanical surveys also report frequent co-use of these plants in polyherbal formulations for malaria management, supporting their cultural and therapeutic relevance (Evbuomwan *et al*., 2023). However, systematic evaluations of their combined extracts specifically study that assess synergy, additivity, or antagonism through rigorous *in vitro* and *in vivo* approache sare sparse. Few reports employ standardized methodologies such as dose–response analysis, isobologram assessment, or interaction indices to quantify pharmacological interactions (Ceravolo *et al*., 2021; Kingston &Cassera, 2022). Consequently, while traditional practice and individual pharmacological findings provide a strong rationale for investigating *A. cordifolia* and *E. chlorantha* in combination, robust experimental validation of their interactive effects is still lacking (Imieje *et al*., 2021; Evbuomwan *et al*., 2023).

Computational Leads Relevant to Each Plant

In silico studies have highlighted the potential of African medicinal plants as sources of protease inhibitors, including compounds structurally similar to those present in *Alchornea cordifolia* and *Enantia chlorantha*. Molecular docking and virtual screening campaigns have identified several phytochemicals with promising inhibitory activity against plasmepsins and related parasite proteases (Manhas *et al*., 2022; En-Nahli *et al*., 2023; Faloye *et al*., 2025). For

E. chlorantha, specific evaluations of root-bark alkaloids indicate strong binding affinities to parasite proteases, alongside favourable drug-likeness profiles (Ibrahim *et al*., 2024). Comparable computational studies on *A. cordifolia* constituents are available in the broader antimalarial phytochemical literature, yet direct comparative analyses of the metabolomes of both species remain scarce (Imieje *et al*., 2021; Faloye *et al*., 2025).

Knowledge gaps in *in silico* and *in vivo* validation

Despite encouraging ethnobotanical and preliminary experimental evidence, several important knowledge gaps limit the translational potential of *A. cordifolia* and *E. chlorantha* as standardized antimalarial therapies. These gaps fall into methodological, mechanistic, and regulatory categories.

Gaps in in silico validation

- **Limited integration of docking with molecular dynamics (MD).** Many virtual screening reports rely on docking scores alone to rank ligands (En-Nahli *et al*., 2023; Manhas *et al*., 2022). Docking provides useful hypotheses of binding mode and affinity, but does not fully capture the dynamic behaviour of ligand–protein complexes. There is a relative scarcity of follow-up MD simulations or free-energy calculations for phytochemicals from *A. cordifolia* and *E. chlorantha* that would provide more robust estimates of stability and binding energy (Faloye *et al*., 2025; Syed *et al*., 2025).
- **Insufficient target breadth and multi-target profiling.** Most *in silico* efforts tend to focus on single validated targets (e.g., plasmepsin II). Given the complex, multi-enzyme biology of *P. falciparum*, multi-target screening (polypharmacology) and network-based approaches are underutilized for these plant metabolites (Cheuka *et al*., 2024; Ji *et al*., 2022). Such approaches could identify synergistic mechanisms whereby multiple low-potency interactions produce strong phenotypic effects.

- **Sparse ADMET and off-target prediction for phytochemicals.** Although drug-likeness (e.g., Lipinski filters) is sometimes applied, comprehensive ADMET modeling, including metabolic stability, CYP interactions, hERG liability, and predicted toxicities, is not consistently carried out for many phytoconstituents (Hamza *et al*., 2024; Ibrahim *et al*., 2024). This leaves a gap between predicted binding and realistic drug viability.
- **Lack of standardized chemical libraries and dereplication.** Diverse naming, lack of stereochemical detail, and incomplete structural annotation of plant metabolites hinder the reproducibility of virtual screens and accurate comparison across studies (Kingston & Cassera, 2022; Ceravolo *et al*., 2021).

Gaps in in vivo validation

- **Few standardized combination studies.** Although traditional practice frequently combines *A. cordifolia* and *E. chlorantha*, rigorous animal studies evaluating defined extract combinations (with controlled ratios, standardized phytochemical content, and formal interaction analyses) are scant (Evbuomwan *et al*., 2023; Mahama *et al*., 2022). Without these data, claims of synergy remain largely anecdotal.
- **Limited chronic and sub-chronic toxicity data.** Most toxicity work reported is acute or limited to single-dose studies (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). Long-term, reproductive, and genotoxicity studies following OECD protocols are under-reported for both species, yet are essential for advancing to clinical evaluation (Andrade *et al*., 2022; Dkhil *et al*., 2021).
- **Inadequate phytochemical standardization in biological assays.** Many *in vivo* reports use crude extracts without robust phytochemical profiling or batch standardization, making interstudy comparisons and dose-response interpretation difficult (Blaise *et al*., 2022; Imieje *et al*., 2021). Standardization (e.g., marker

compound quantification) is necessary to relate observed effects to specific constituents and to permit reproducible efficacy and toxicity assessments.

- **Sparse pharmacokinetic (PK) and exposure data.** There is a paucity of PK studies evaluating the absorption, bioavailability, plasma exposure, tissue distribution, or metabolism of the major phytoconstituents from these plants. Without PK, linking *in vitro* potency to observed *in vivo* efficacy (or toxicity) is conjectural (Ibrahim *et al*., 2024; Hamza *et al*., 2024).
- **Limited evaluation against resistant parasite strains and clinical isolates.** Much preclinical work uses standard laboratory strains or murine models; limited evidence exists on activity against clinically relevant, drug-resistant *P. falciparum* isolates, which is critical given the clinical challenge of resistance (Ippolito *et al*., 2021; Girgis *et al*., 2023).
- **Regulatory and quality-control gaps in herbal formulations.** Even when efficacy and acute safety are reported, steps needed for clinical translation, including Good Manufacturing Practice (GMP) extract production, stability studies, and regulatory toxicology packages, are seldom addressed in the published experimental literature (Ceravolo *et al*., 2021; Babandi, 2025).

Practical and conceptual implications of these gaps

- **Translational risk.** The combination of promising *in silico* docking hits and isolated *in vivo* efficacy, without integrated ADMET/Pk and rigorous combination studies, increases the risk of late-stage failures or safety surprises when moving toward human studies (Hamza *et al*., 2024; Faloye *et al*., 2025).
- **Research priorities.** High-priority steps include (a) standardizing extracts and characterizing major constituents, (b) performing integrated CADD pipelines that include MD and ADMET prediction, (c) executing well-designed combination *in vitro*

synergy assays and confirmatory *in vivo* combination studies with formal interaction analysis, and (d) expanding toxicity testing to subchronic and regulatory guideline–compliant studies (Andrade *et al*., 2022; En-Nahli *et al*., 2023; Mahama *et al*., 2022).

- **Opportunity for multidisciplinary work.** Coordinated efforts linking ethnobotany, phytochemistry, computational chemistry, pharmacology, and regulatory toxicology are required to advance these promising plant leads (individually and in combination) toward validated antimalarial candidates (Kingston & Cassera, 2022; Ceravolo *et al*., 2021).

1.9 Justification of the Study

Malaria remains one of the most serious global health challenges, particularly in sub-Saharan Africa, where *Plasmodium falciparum* is responsible for the majority of morbidity and mortality (WHO, 2024; Ippolito *et al*., 2021). However, artemisinin-based combination therapies (ACTs) have been the cornerstone of malaria treatment, emerging resistance in Southeast Asia and parts of Africa threatens their long-term efficacy (Girgis *et al*., 2023; Ippolito *et al*., 2021). Resistance mechanisms, including mutations in kelch13 and alterations in parasite proteases, compromise therapeutic success and necessitate urgent discovery of novel antimalarial agents (Cheuka *et al*., 2024; Faloye *et al*., 2025). This resistance crisis justifies the exploration of alternative chemotypes and bioactive natural products that may overcome or bypass known resistance mechanisms (Kingston & Cassera, 2022; Syed *et al*., 2025).

Plants have historically provided crucial leads in antimalarial drug development, including quinine from Cinchona and artemisinin from Artemisia annua (Kingston & Cassera, 2022). African medicinal flora, such as *Alchornea cordifolia* and *Enantia chlorantha*, are rich in alkaloids, flavonoids, and tannins with demonstrated bioactivity against Plasmodium species (Imieje *et al*., 2021; Blaise *et al*., 2022; Ibrahim *et al*., 2024). Moreover, preclinical studies

confirm that extracts of both plants reduce parasitemia in rodent malaria models, often with favorable acute safety margins (Mahama *et al*., 2022; Evbuomwan *et al*., 2025). The relative safety of these plants in ethnomedicine, coupled with the growing push for natural, low-cost therapies, underscores their potential as sources of novel drug scaffolds. Unlike synthetic high-throughput screens, phytochemicals are often "privileged structures" that have been optimized through evolution for bioactivity, making them promising candidates for drug discovery (Ceravolo *et al*., 2021; Manhas *et al*., 2022).

Traditional medicine in West and Central Africa frequently employs *A. cordifolia* and *E. chlorantha* either singly or in polyherbal formulations for the treatment of malaria and febrile illnesses (Evbuomwan *et al*., 2023; Ceravolo *et al*., 2021). Ethnopharmacological surveys confirm that healers often combine multiple species to enhance therapeutic efficacy and reduce side effects (Kingston &Cassera, 2022). Both plants contain distinct classes of secondary metabolites: *A. cordifolia* is rich in flavonoids, tannins, and triterpenoids, while *E. chlorantha* primarily contains protoberberine alkaloids such as berberine and palmatine (Imieje *et al*., 2021; Ibrahim *et al*., 2024). Combining these phytochemical profiles may yield synergistic interactions at multiple parasite targets, such as plasmepsins, falcipains, and redox enzymes, thereby enhancing potency while mitigating resistance (Faloye *et al*., 2025; Manhas *et al*., 2022). However, despite this strong ethnobotanical basis, systematic studies that validate their combination through *in silico* molecular docking, ADMET predictions, and *in vivo* toxicity testing are scarce (Evbuomwan *et al*., 2023; Mahama *et al*., 2022). This gap provides a compelling rationale for scientifically validating their co-use.

Traditional medicine remains a cornerstone of healthcare in malaria-endemic regions, with more than 80% of the African population relying on plant-based remedies for primary health care (Ceravolo *et al*., 2021; Evbuomwan *et al*., 2023). While empirical use supports their relevance, scientific validation through computational and experimental approaches is critical

to ensure safety, efficacy, and acceptance within modern drug discovery pipelines (Andrade *et al*., 2022; Hamza *et al*., 2024). Computer-aided drug design (CADD) now enables high-throughput screening of phytochemicals against validated parasite targets such as plasmepsin II, thereby narrowing down active constituents for further testing (Manhas *et al*., 2022; En-Nahli *et al*., 2023). Parallel *in vivo* toxicity studies aligned with OECD guidelines are essential to guarantee safety (Andrade *et al*., 2022; Dkhil *et al*., 2021). By combining traditional knowledge with cutting-edge computational chemistry and pharmacological testing, this study aims to transform ethnobotanical wisdom into evidence-based therapeutic leads that address both public health and scientific priorities.

1.10 Aim and Objectives of the Study

General Aim

To evaluate the antiplasmodial potential and safety of combined *Alchornea cordifolia* and *Enantia chlorantha* extracts using *in silico* and *in vivo* approaches.

Objectives: This study aims to assess the antiplasmodial potential of a combined *A. cordifolia* and *E. chlorantha* extract via molecular docking against *P. falciparum* Plasmepsin II and evaluate its acute toxicity in mice.

Specific Objectives

1. To identify and screen phytoconstituents of *A. cordifolia* and *E. chlorantha* against *P. falciparum* plasmepsin II using molecular docking.
2. To assess the drug-likeness and ADMET properties of identified phytoconstituents.
3. To evaluate the acute toxicity profile of the combined plant extracts in animal models.
4. To compare the binding affinities and safety profile with standard antimalarial drugs.

CHAPTER TWO

MATERIALS AND METHODS

2.0 Materials

2.0.1 Device Used

This study was conducted on a Dell Latitude 7410 x360 laptop equipped with a 512 GB Solid State Drive (SSD), an Intel Core i7 vPRO processor (2.30 GHz), 16 GB RAM, and a 64-bit Windows 11 Pro operating system. A high-precision mouse was employed to ensure seamless computational operations.

2.0.2 RCSB Protein Data Bank (https://www.rcsb.org)

The 3D structure of *Plasmodium falciparum* Plasmepsin II (PDB ID: 1LEE) was retrieved from the RCSB Protein Data Bank. This protein is a validated molecular target in antimalarial drug discovery due to its role in hemoglobin degradation (Adeoye & Lobb, 2025; Cheuka *et al*., 2024).

2.0.3 PubChem (https://pubchem.ncbi.nlm.nih.gov)

Bioactive compounds from *Alchornea cordifolia* and *Enantia chlorantha* were retrieved in SDF format from the PubChem and Google Scholar databases. PubChem provides structural, chemical, and bioactivity data critical to computational drug discovery (Ibrahim *et al*., 2024; Imieje *et al*., 2021).

2.0.4 PyRx 0.9.8 (SourceForge, San Diego, USA) (https://pyrx.sourceforge.io/)

PyRx integrates AutoDock Vina for virtual screening and docking simulations. It was used for ligand preparation, energy minimization, and docking against Plasmepsin II (Faloye *et al*., 2025; Hamza *et al*., 2024).

2.0.5 PyMOL 3.1 (Schrödinger LLC, New York, USA)(https://www.pymol.org/)

PyMOL was used for visualization and identification of the active site residues of Plasmepsin II, based on its co-crystallized ligand (R36). This ensured accurate docking studies (En-Nahli *et al*., 2023; Manhas *et al*., 2022).

2.0.6 Biovia Discovery Studio 2025 (Dassault Systèmes, Paris, France)

Biovia Discovery Studio was employed to refine protein structures, remove water molecules, add hydrogen atoms, and visualize ligand-protein interactions. It allowed for both 2D and 3D post-docking interaction analyses (Syed *et al*., 2025; Ishola *et al*., 2023)., Ensuring cutting-edge molecular docking and visualization aligned with Nigeria's ethnobotanical quest to combat *Plasmodium falciparum* malaria.

2.0.7 SwissADME (https://www.swissadme.ch)

SwissADME was used to predict pharmacokinetic parameters, drug-likeness, and medicinal chemistry properties of the docked ligands. It ensured that only compounds with favourable absorption and metabolism profiles were advanced (Sulyman *et al*., 2023; Ain *et al*., 2024).

2.0.8 ProTox-III (https://comptox-charite.de/protox3)

The ProTox-III webserver was used to predict toxicity endpoints, including hepatotoxicity, mutagenicity, and carcinogenicity, for selected ligands (Mahama *et al*., 2022; Hadi *et al*., 2025).

2.1 Methods

2.1.1 Preparation of Protein Target

The structure of Plasmepsin II (PDB ID: 1LEE) was retrieved from RCSB PDB and prepared using Biovia Discovery Studio. Non-standard residues, extraneous chains, and water molecules were removed, followed by energy minimization using 200 steepest descent iterations. Hydrogen atoms and charges were added, and the refined protein was saved in PDB format for docking (Adeoye & Lobb, 2025; Faloye *et al*., 2025).

2.1.2 Identification and Preparation of Ligands

Phytochemicals from *A. cordifolia* and *E. chlorantha*, identified in ethnobotanical and phytochemical studies (Evbuomwan *et al*., 2023; Blaise *et al*., 2022), were downloaded from PubChem as SDF files. Using PyRx's Open Babel plug-in, ligands were energy minimized and converted to pdbqt format for docking (Imieje *et al*., 2024; Aladi, 2022).

2.1.3 Identification of Binding Site

The active site of Plasmepsin II was identified using the co-crystallized ligand (R36). PyMOL was used to visualize and map the catalytic residues within the enzyme-binding pocket, enabling accurate placement of the docking grid for docking simulations (En-Nahli *et al*., 2023; Manhas *et al*., 2022).

2.1.4 Molecular Docking

Molecular docking was performed in PyRx 0.9.8 using the AutoDock Vina engine. The grid box was centered on the identified active site residues, and docking was run with an

exhaustiveness of 8. Binding affinities were expressed in kcal/mol, where more negative values indicated stronger binding interactions (Faloye *et al*., 2025; Hamza *et al*., 2024).

2.1.5 Post-Docking Analysis

Docked ligand conformations were analysed in Biovia Discovery Studio. Interactions such as hydrogen bonding, hydrophobic contacts, and π-π stacking were examined in both 2D and 3D. This enabled the identification of compounds with favourable orientations and strong inhibitory potential (Syed *et al*., 2025; Ishola *et al*., 2023).

2.1.6 ADMET Profiling

Ligands with binding affinities ≤ –6.5 kcal/mol were evaluated for pharmacokinetics and toxicity. ADME properties were predicted using SwissADME, with compounds passing Lipinski's Rule of Five considered drug-like. Toxicity endpoints were predicted using ProTox-III, ensuring safety in potential drug development (Sulyman *et al*., 2023; Mahama *et al*., 2022).

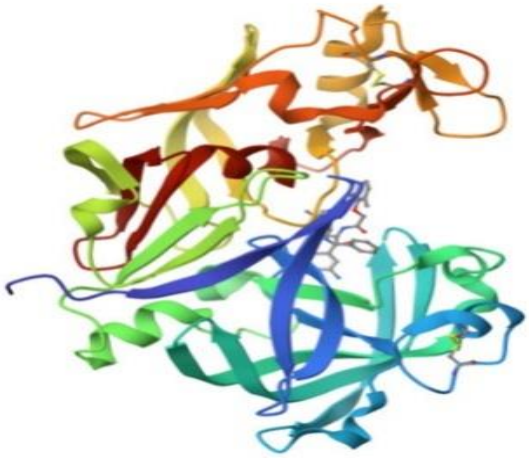

Figure 1: Crystal Structure of Plasmepsin II from *Plasmodium Falciparum* in Complex with Inhibitor R36

2.2 *In Vivo* Toxicity Assessment

To confirm the safety of the combined *A. cordifolia* and *E. chlorantha* extracts, an acute toxicity study was conducted in Wistar mice (17g-26g) using Lorke's method, a two-stage protocol (Lorke, 1983). Stage 1 involved nine mice, divided into three groups of three, each marked with single, double, or triple strokes of indelible ink in distinct colours for clear identification. After overnight fasting to ensure accurate dosing, mice were weighed, and doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the combined extract were administered orally via an orogastric tube. The extract was prepared by weighing 500 mg each of *A. cordifolia* and *E. chlorantha* extracts, and blending them with 250 mg of acacia gum powder to aid dissolution. This mixture was vigorously triturated in a mortar with 4 mL of distilled water, then carefully transferred to a calibrated universal bottle, topped up to 10 mL with distilled water to yield a 100 mg/mL solution. Serial dilutions were performed: 1 mL of this solution was withdrawn using a 10mL syringe, mixed with 9 mL of distilled water in another universal bottle to produce a 10 mg/mL solution, and repeated to obtain a 1 mg/mL solution. Each bottle was securely sealed and clearly labelled. The volume administered to each mouse was calculated based on its body weight, ensuring precision. Stage 2 utilized three mice, one per group, each marked with a unique colour of indelible ink for easy differentiation. Solutions of 100 mg/mL (for Groups 1 and 2) and 200 mg/mL (for Group 3) were prepared following the same procedure as Stage 1, with 500 mg of each extract and 250 mg of acacia gum triturated in 4 mL of distilled water, then adjusted to 10 mL or 5 mL for the respective concentrations. Doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg were administered orally via orogastric tube after weighing each mouse and calculating the precise volume based on body weight. All bottles were tightly sealed and labelled, ensuring meticulous care. This rigorous approach honoured Nigeria's healing traditions, validating the safety of Ogwu obi and Awopa for antimalarial drug development.

CHAPTER THREE

RESULTS

3.1 Binding Affinities

The results of the binding site amino acids identified with the aid of Pymol are shown in Table 3.1

The binding affinities of all the compounds from *Achornea cordifolia* and *Enantia chlorantha* with the protein target (1LEE, Plasmepsin II) are shown in Tables 3.2 and 3.3.

The chemical structures of compounds of *Achornea cordifolia* and *Enantia chlorantha* are shown in Figure 3.1 and Figure 3.2, respectively.

Table 3.1: Showing the binding site amino acids identified with Pymol

S/N	**Binding site amino acids**
1	GLY 36
2	ASN 76
3	TYR 77
4	VAL 78
5	SER 79
6	PHE 111
7	ILE 123
8	LEU 131
9	TYR 192
10	ASP 214
11	GLY 216

12	THR 217
13	THR 221
14	ILE 290
15	PHE 294

Table 3.2 Binding affinities of *Alchornea cordifolia* compounds with 1LEE

S/N	Compounds	Pubchem ID	Binding Affinity	Molecular Formula	Molecular weight g/mol
1	Acetyl aleuritolic acid	161616	**-7.1**	$C_{32}H_{50}O_4$	498.7
2	B-sitosterol	222284	**-8.1**	$C_{29}H_{50}O$	414.71
3	Daucosterol	5742590	**-7.2**	$C_{35}H_{60}O_6$	576.85
4	Ellagic acid	5281855	**-7.3**	$C_{14}H_6O_8$	302.19
5	Friedelin	91472	**-8.3**	$C_{30}H_{50}O$	426.72
6	Kaempferol-3-O-galactoside	5282149	**-7.8**	$C_{21}H_{20}O_{11}$	448.38
7	Lup-20(29)-en-3c-ol (Lupenol)	236432	**-9.1**	$C_{30}H_{50}O$	426.72
8	Quercetin	5280343	**-7.3**	$C_{15}H_{10}O_7$	302.24
9	Quercetin-3-O-alpha-D-arabinofuranoside	11968848	**-7.4**	$C_{20}H_{18}O_{11}$	434.35
10	Quercetin-3-O-alpha-D-arabinofuranoside	12309865	**-8**	$C_{20}H_{18}O_{11}$	434.35
11	*4-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide	R36	**-10**	$C_{32}H_{41}N_3O_4$	531.69
12	**Dihydroartemisinin	6918483	**-7.1**	$C_{15}H_{24}O_5$	284.35

*The native ligand (R36) **the positive control ligand (Dihydroartemisinin)

Table 3.3: Binding affinities of *Enantia chlorantha* compounds with 1LEE

S/N	Compounds	Pubchem ID	Binding Affinity	Molecular Formula	Molecular weight
1	Diazoprogesterone	104633	**-8.5**	$C_{21}H_{28}N_2O_2$	340.46
2	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one	13760785	**-6.9**	$C_{16}H_{28}O$	236.39
3	Pseudocolumbamine	182406	**-7**	$C_{20}H_{20}NO_4^+$	338.38
4	Palmatine	19009	**-7.3**	$C_{21}H_{22}NO_4^+$	352.4
5	β -Sitosterol	222284	**-8.2**	$C_{29}H_{50}O$	414.71
6	Berberine	2353	**-7.9**	$C_{20}H_{18}NO_4^+$	336.36
7	7,8-Dihydro-8-hydroxypalmatine	493569	**-7.4**	$C_{21}H_{23}NO_5$	369.41
8	Stigmasterol	5280794	**-8.4**	$C_{29}H_{48}O$	412.69
9	Isoshyobunone	5318673	**-6.6**	$C_{15}H_{24}O$	220.35
10	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	5363697	**-7.1**	$C_{18}H_{26}O$	258.4
11	4-Chloro-2-isopropyl-5-methylphenyl bicyclo[4.1.0]heptane-7-carboxylate	579217	**-7.7**	$C_{18}H_{23}ClO_2$	306.83
12	Columbamine	72310	**-7.5**	$C_{20}H_{20}NO_4^+$	338.38
13	Jatrorrhizine	72323	**-7.5**	$C_{20}H_{20}NO_4^+$	338.38
14	Magnoflorine	73337	**-7.4**	$C_{20}H_{24}NO_4^+$	342.41
15	1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)	91710667	**-8.6**	$C_{25}H_{24}O_4$	388.46
16	*4-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide	R36	**-10**	$C_{32}H_{41}N_3O_4$	531.69
17	**Dihydroartemisinin	6918483	**-7.1**	$C_{15}H_{24}O_5$	284.35

*The native ligand (R36) **the positive control ligand (Dihydroartemisinin)

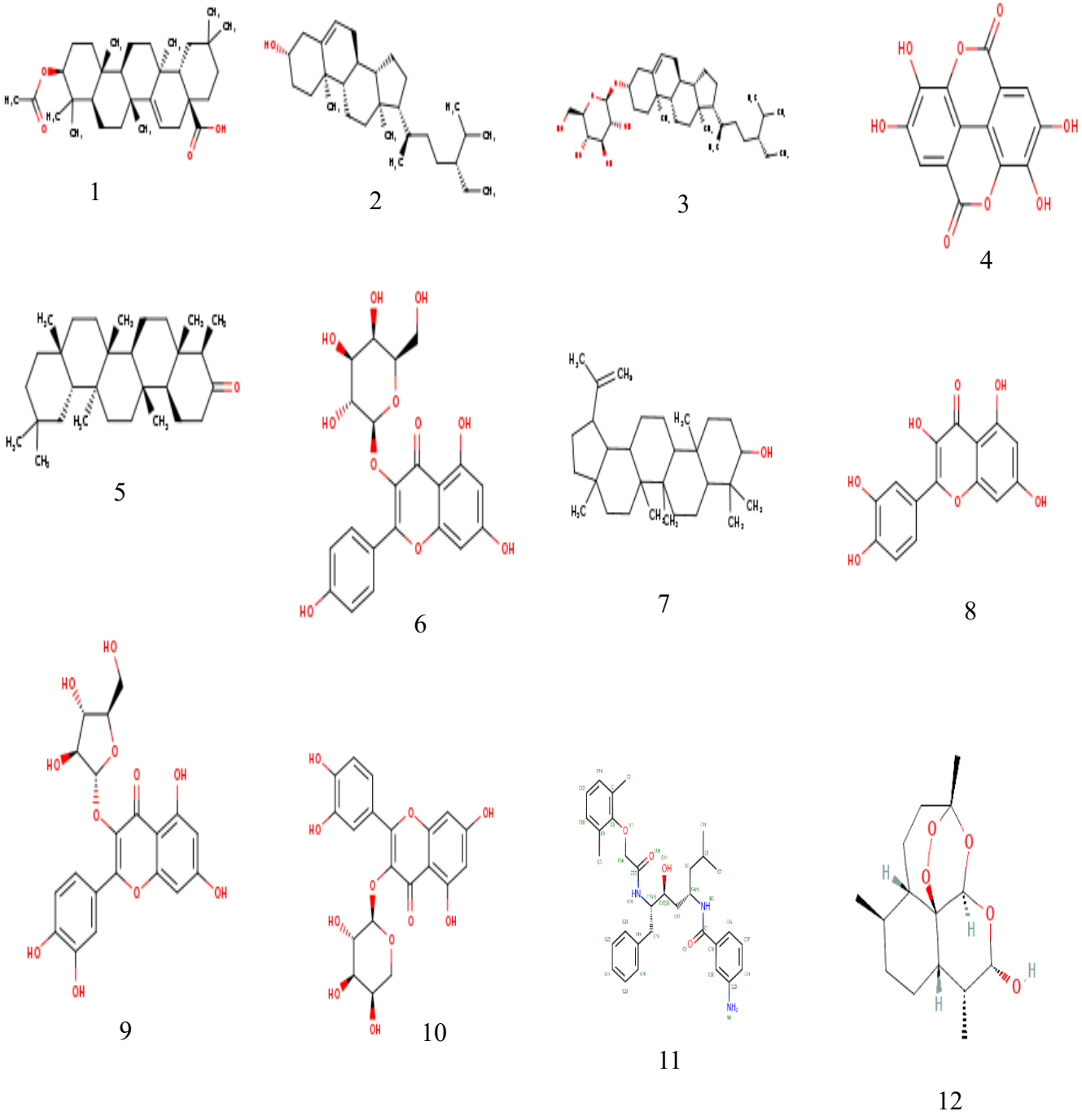

Figure 3.1 Showing the chemical structures of compounds **1-10** from *Alcornea cordifolia* and **11-12** for the co-crystallized ligand and Dihydroartemisinin, respectively (Table 3.2).

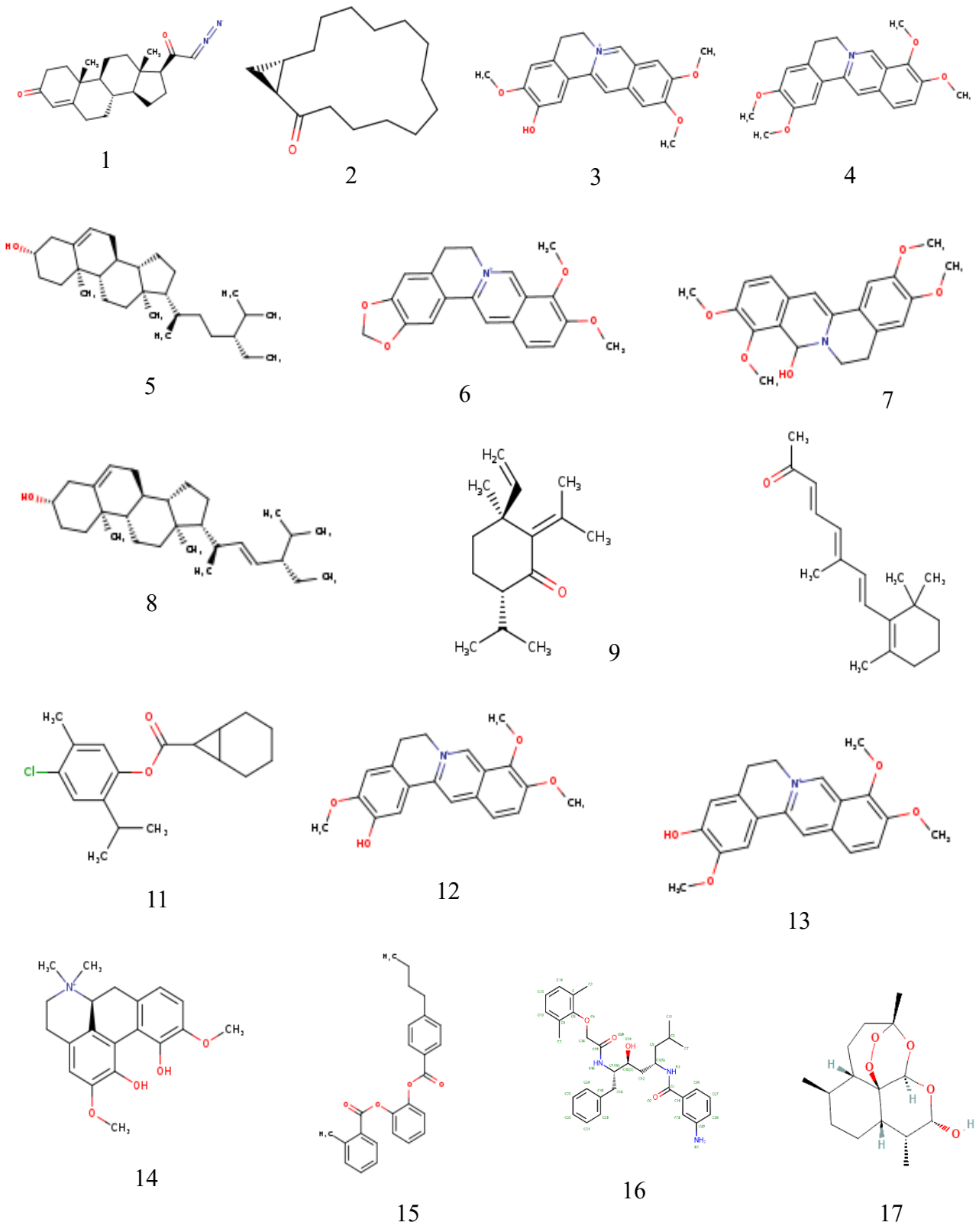

Figure 3.2: Showing the chemical structures of compounds **1-15** from *Enantia chlorantha* and **16-17** for the co-crystallized ligand and Dihydroartemisinin, respectively (Table 3.2).

3.2 The 2D, 3D, and H-bonds binding interactions of the phytoconstituents of *Alchornea cordifolia and Enantia chlorantha* with 1LEE.

The images below show the interactions of the ligands with a binding affinity score of -7 or lower with the amino acids of the target protein (Figures 3.3 –3.12). The interaction of the co-crystallised ligand and the positive control antimalarial agent, artesunate, with 1LF3 is also shown.

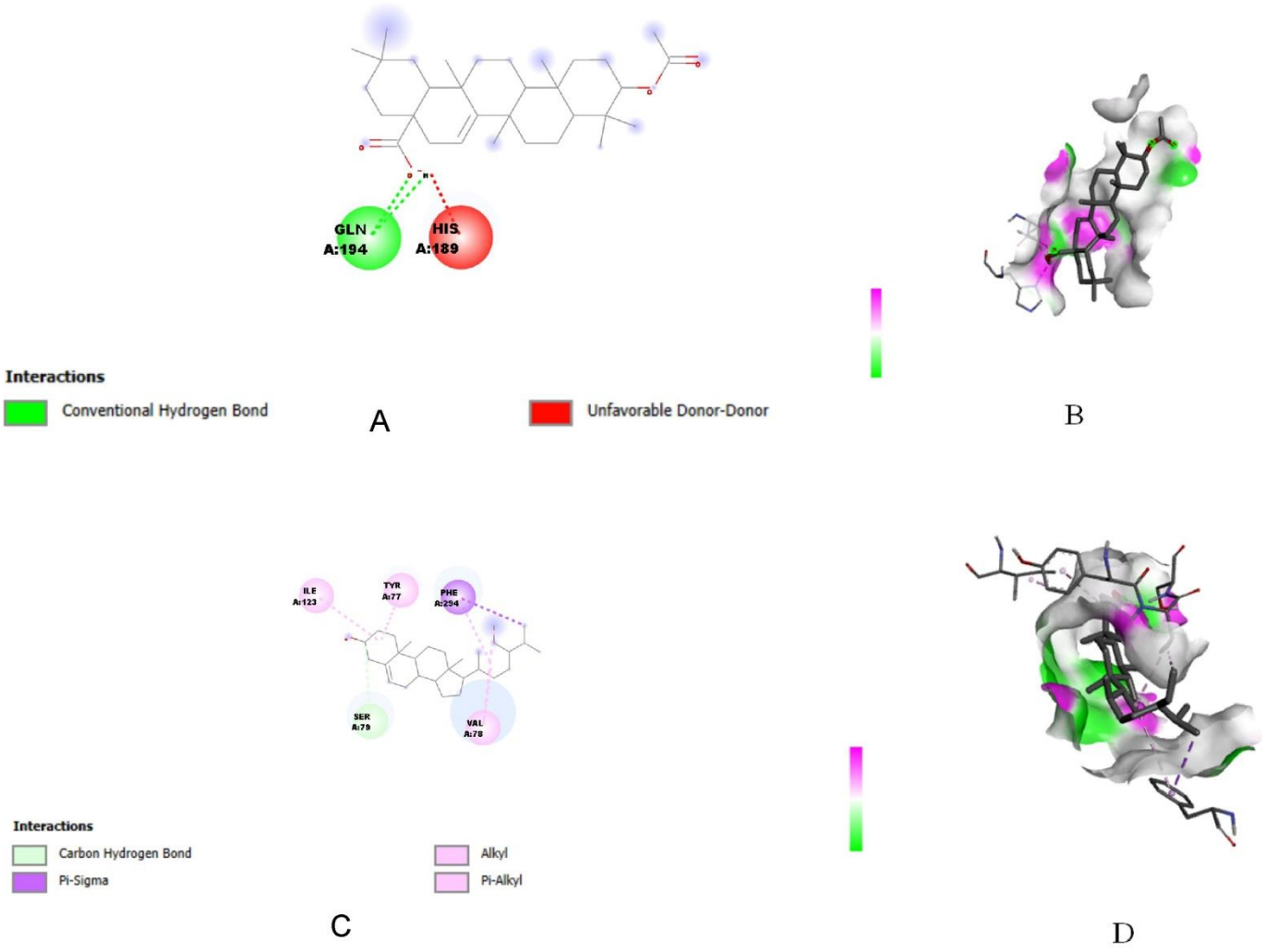

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 1 and 2 from *Alchornea cordifolia* (Table 3.2)with the amino-acid residues of Plasmepsin II receptor.

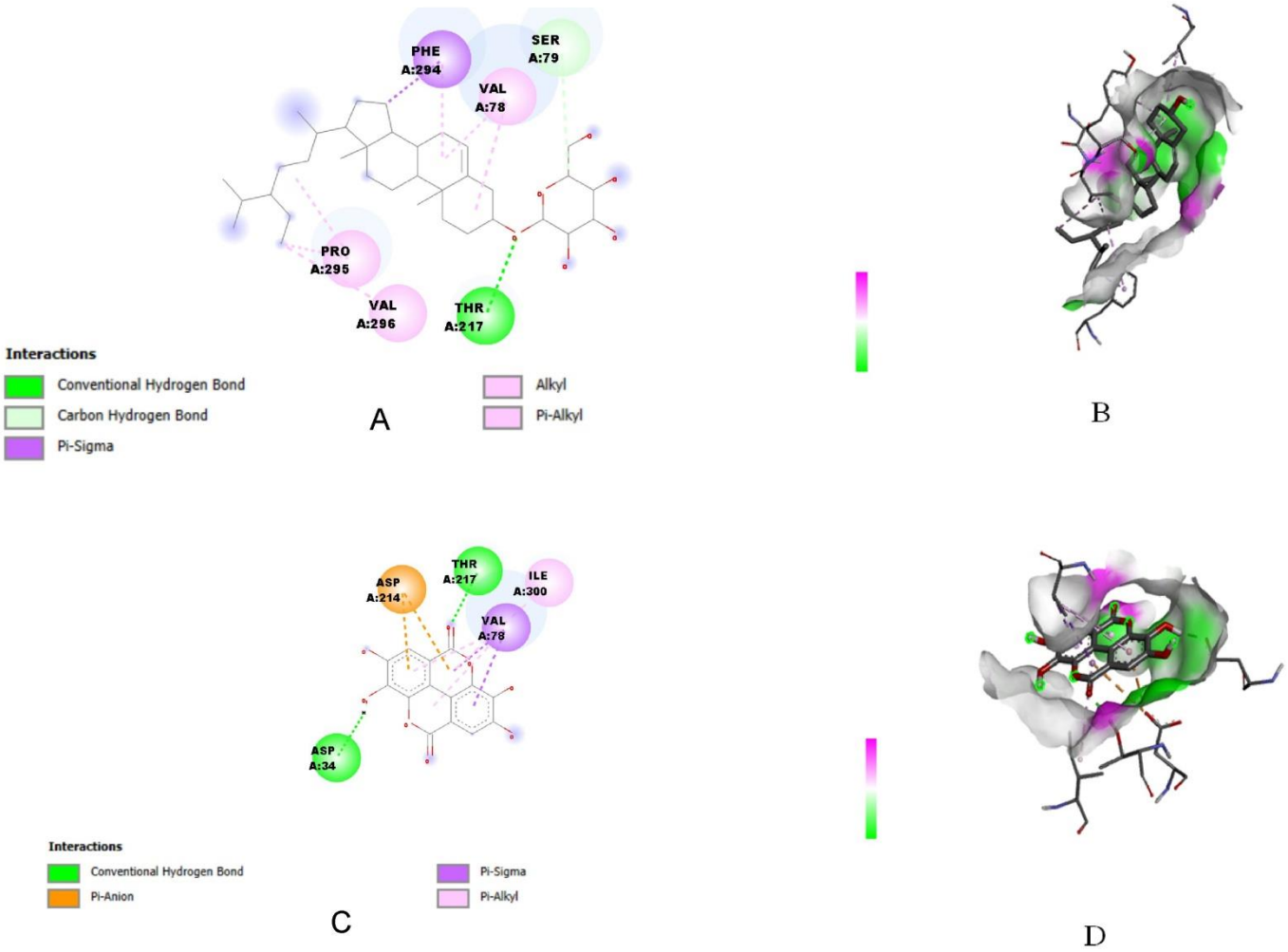

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 3 and 4 from *Alchornea cordifolia* (Table 3.2) with the amino-acid residues of Plasmepsin II receptor.

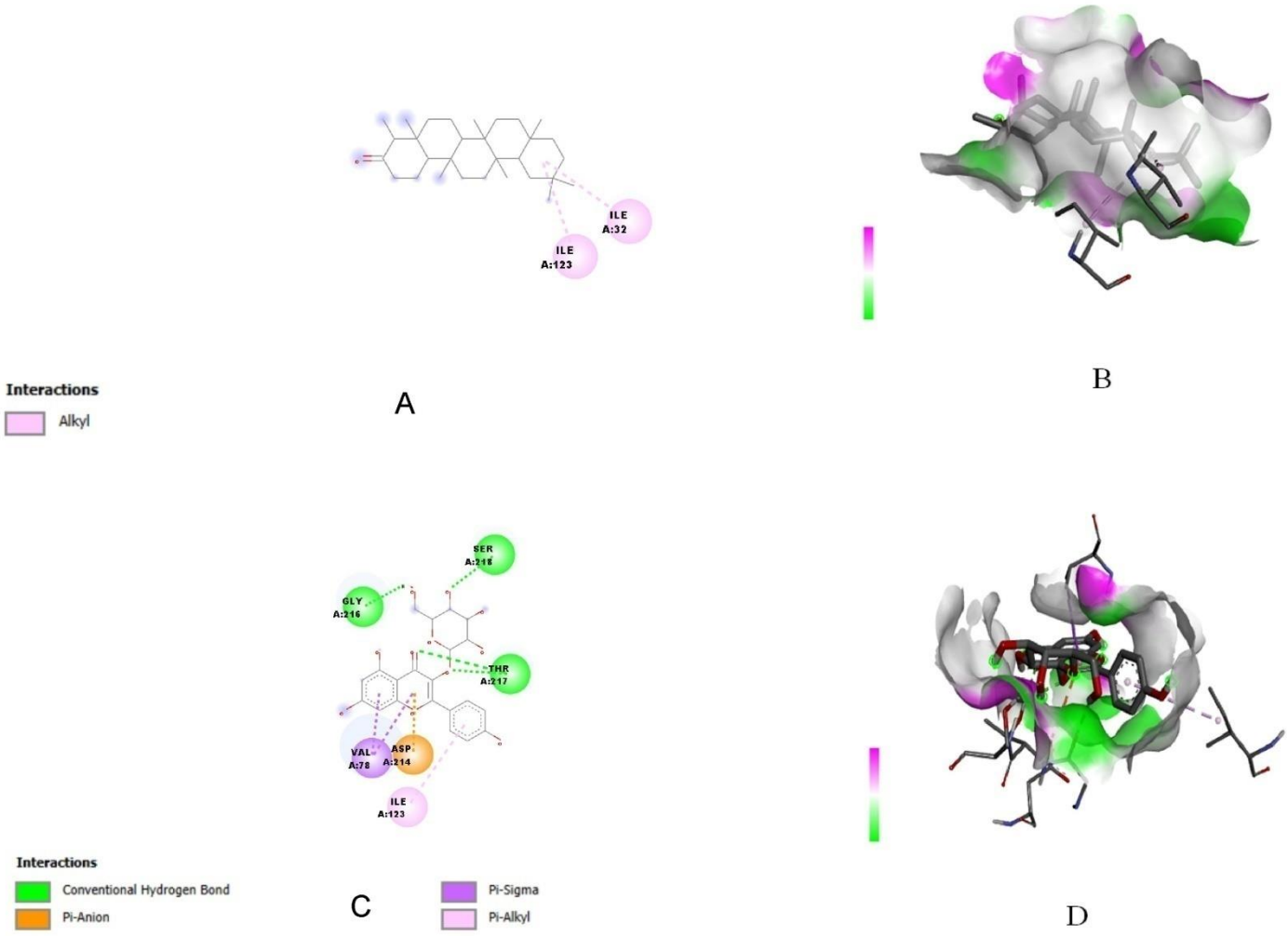

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 5 and 6 from *Alchornea cordifolia* (Table 3.2) with the amino-acid residues of Plasmepsin II receptor.

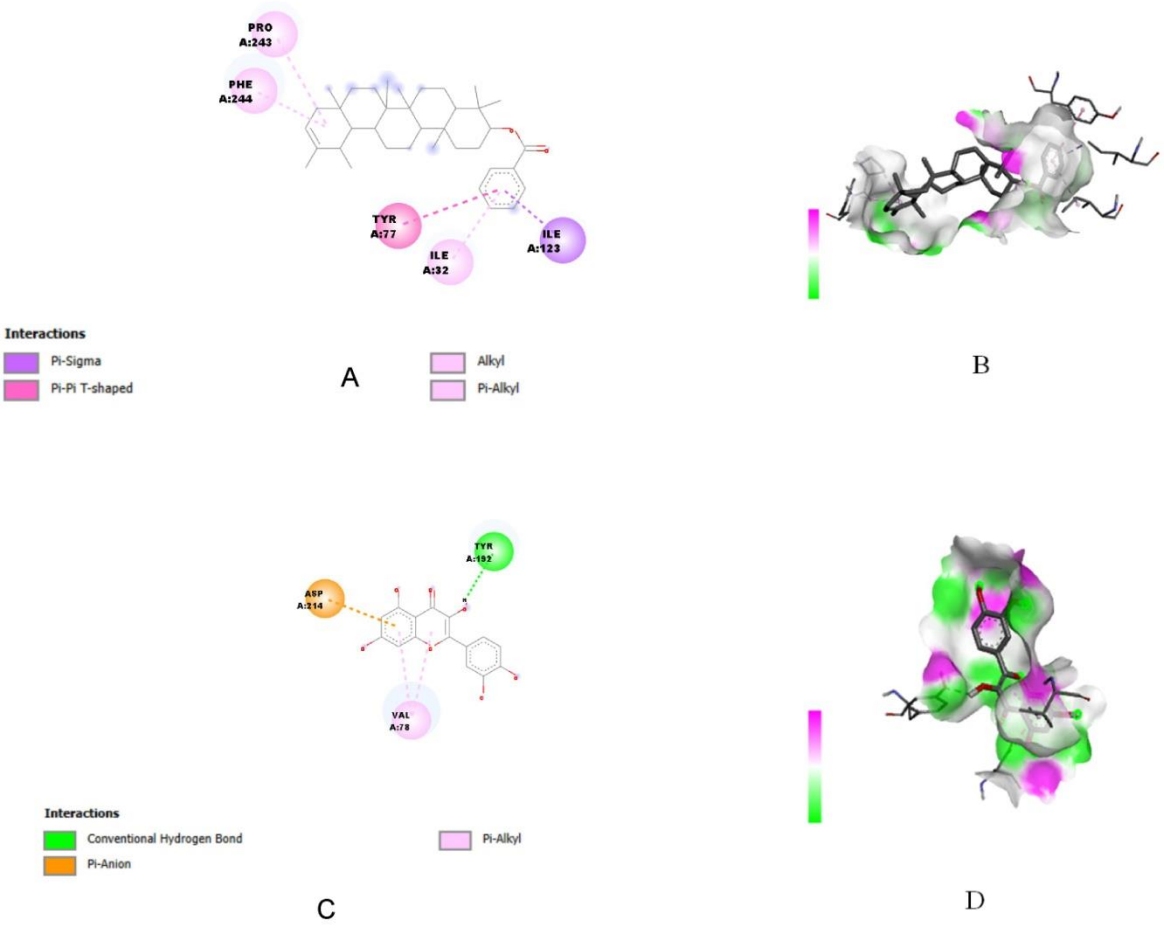

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 7 and 8 from *Alchornea cordifolia* (Table 3.2) with the amino-acid residues of Plasmepsin II receptor.

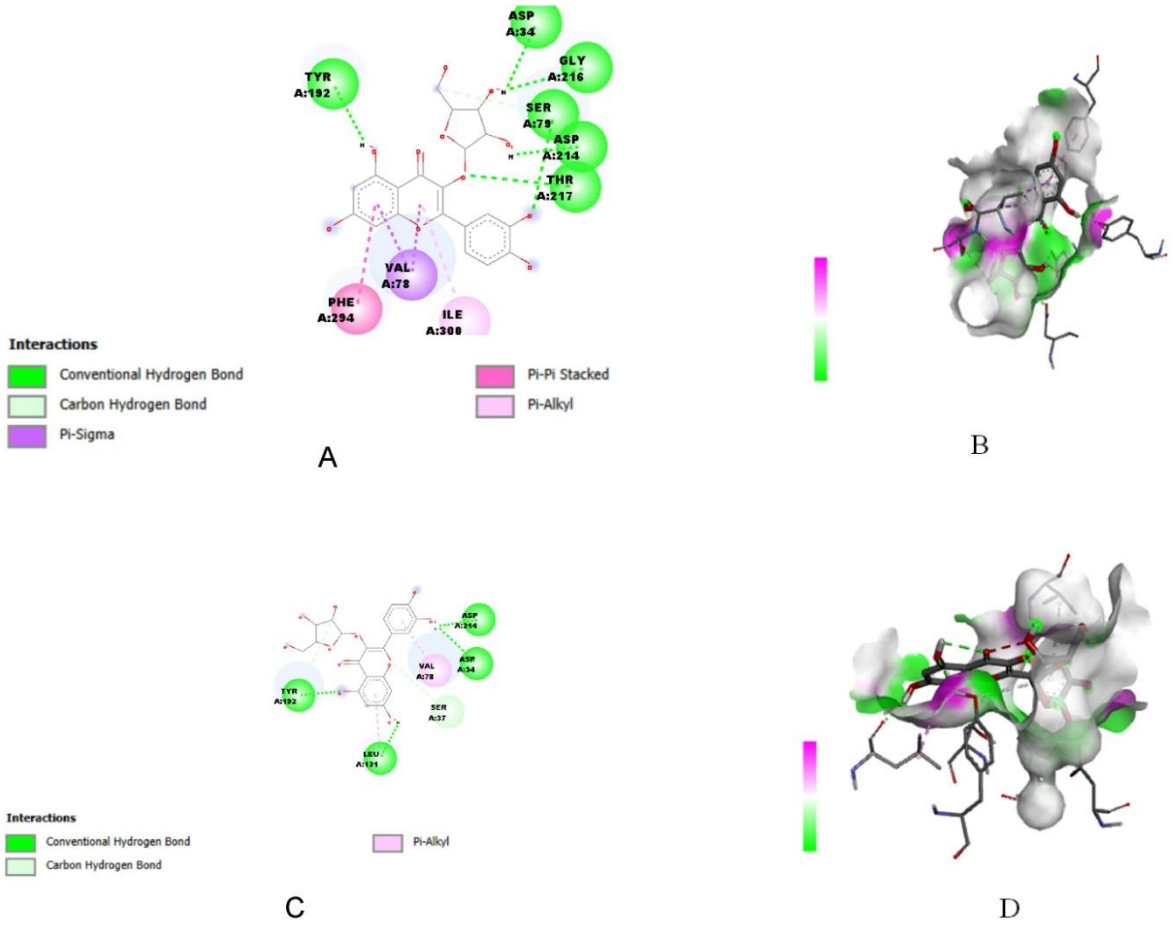

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 9 and 10 from *Alchornea cordifolia* (Table 3.2) with the amino-acid residues of Plasmepsin II receptor.

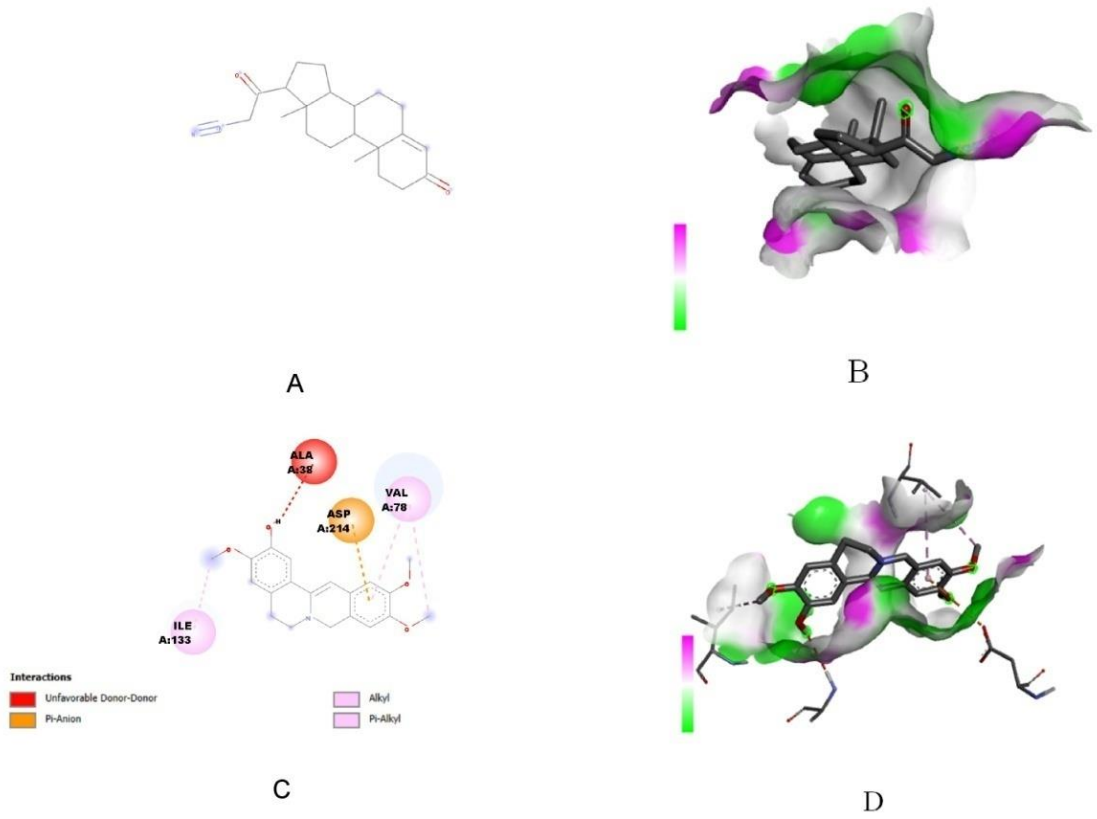

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 1 and 3 from *Enantia chlorantha* (Table 3.3) with the amino-acid residues of Plasmepsin II receptor.

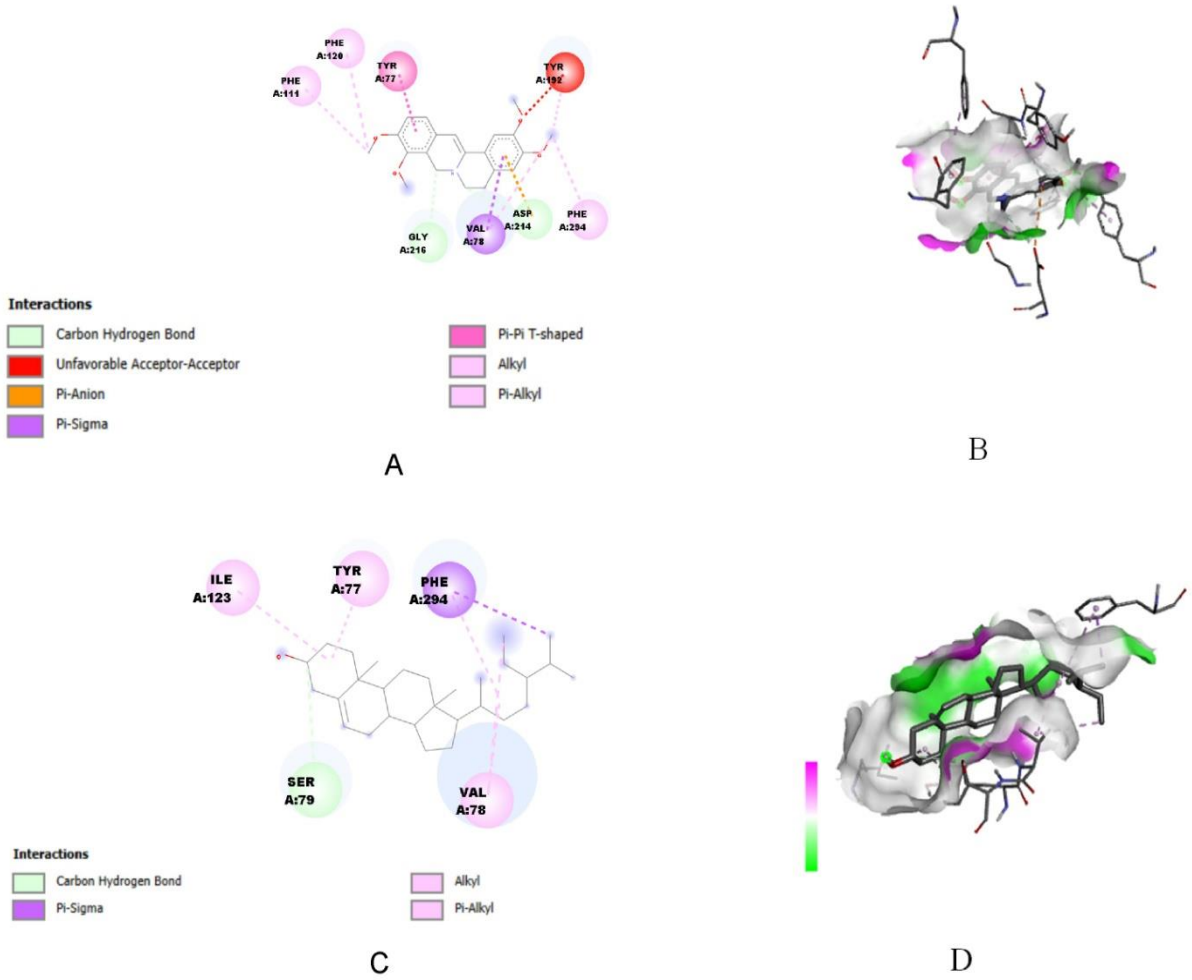

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 4 and 5 from *Enantia chlorantha* (Table 3.3)with the amino-acid residues of Plasmepsin II receptor.

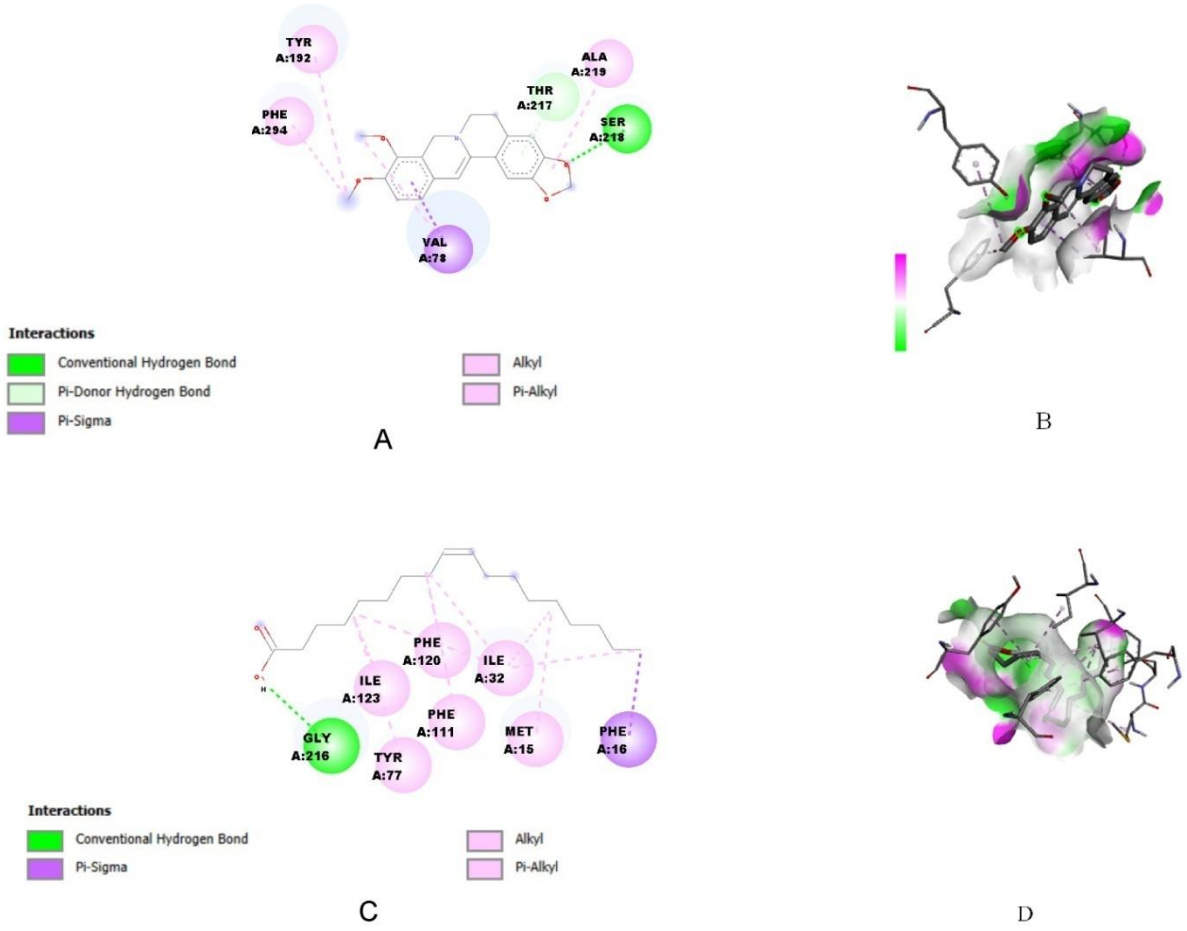

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 6 and 7 from *Enantia chlorantha* (Table 3.3) with the amino-acid residues of Plasmepsin II receptor.

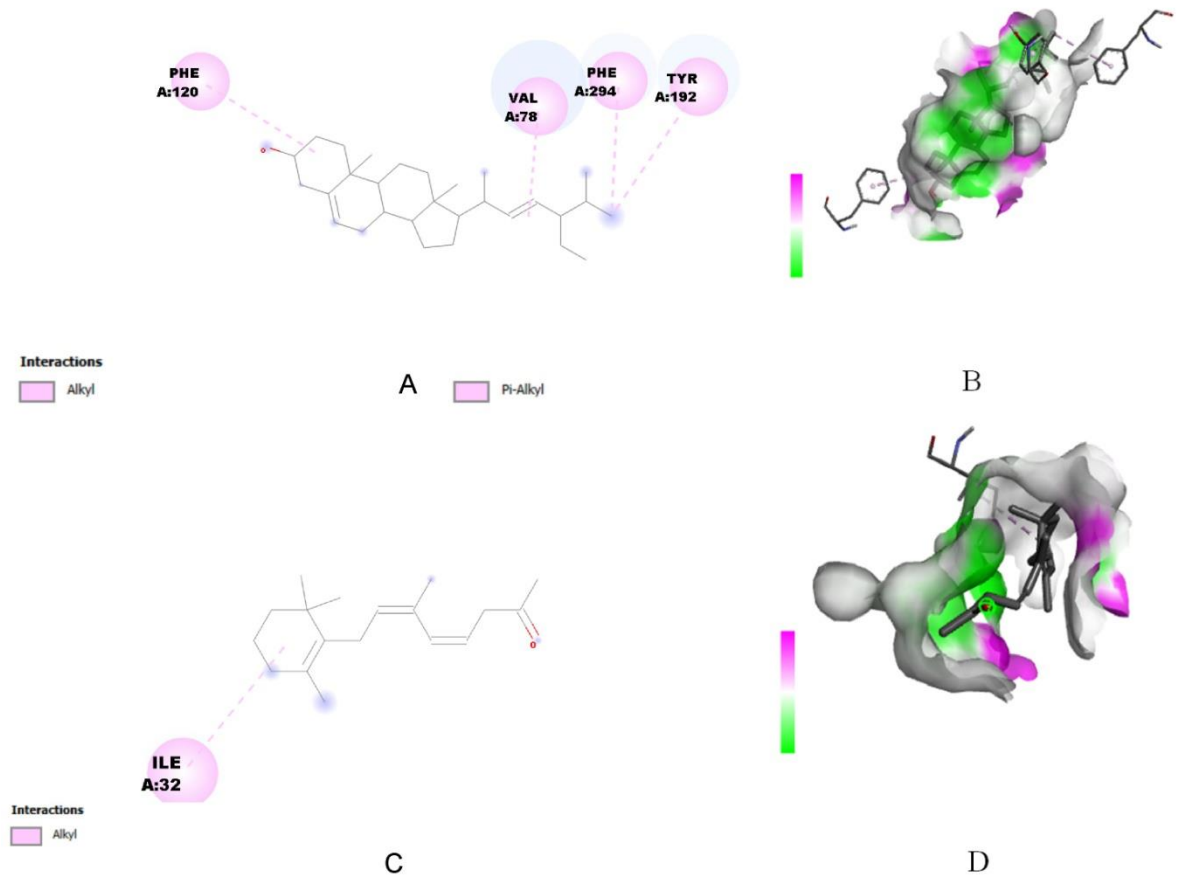

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 8 and 10 from *Enantia chlorantha* (Table 3.3) with the amino-acid residues of Plasmepsin II receptor

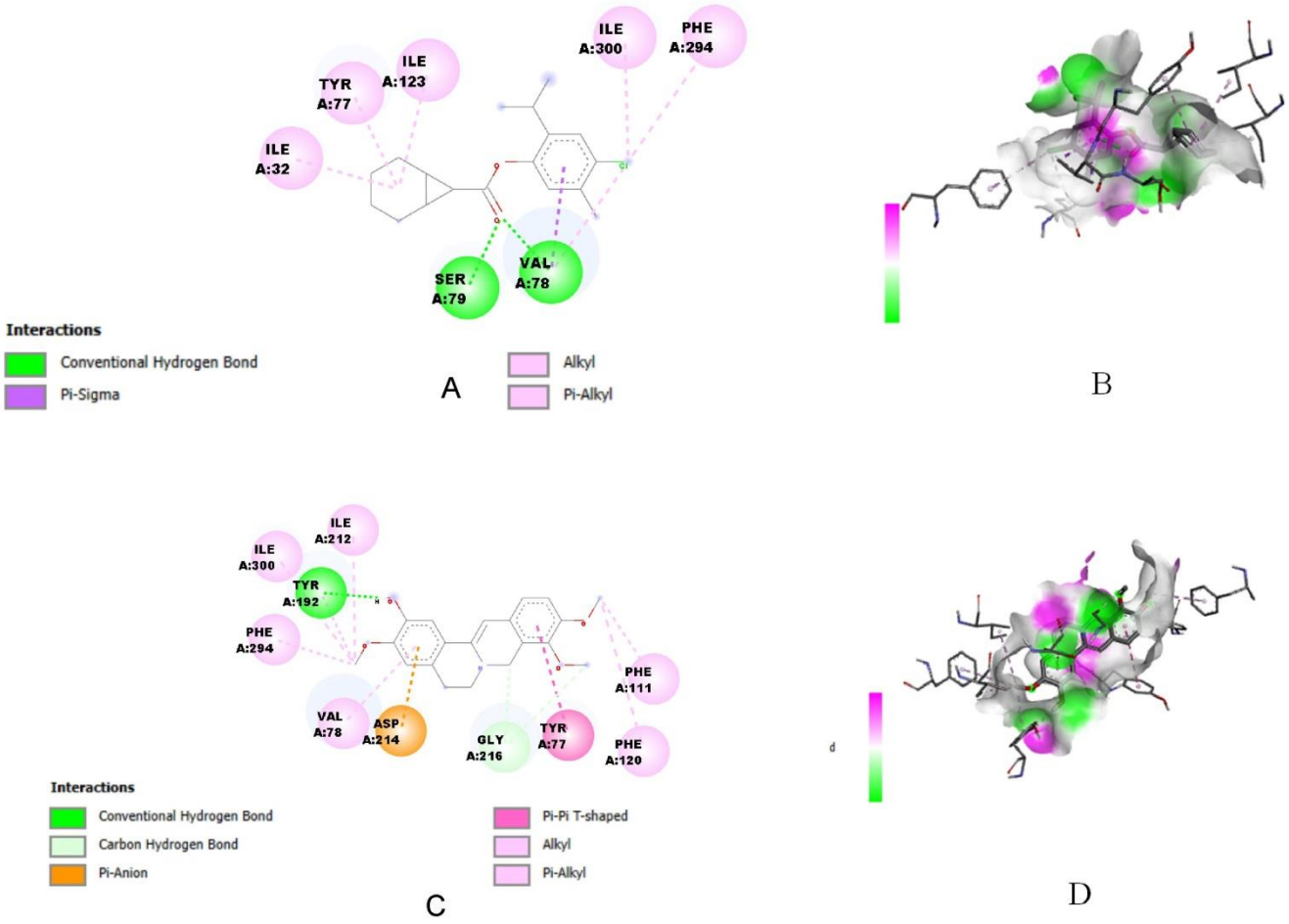

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 11 and 12from *Enantia chlorantha* (Table 3.3) with the amino-acid residues of Plasmepsin II receptor.

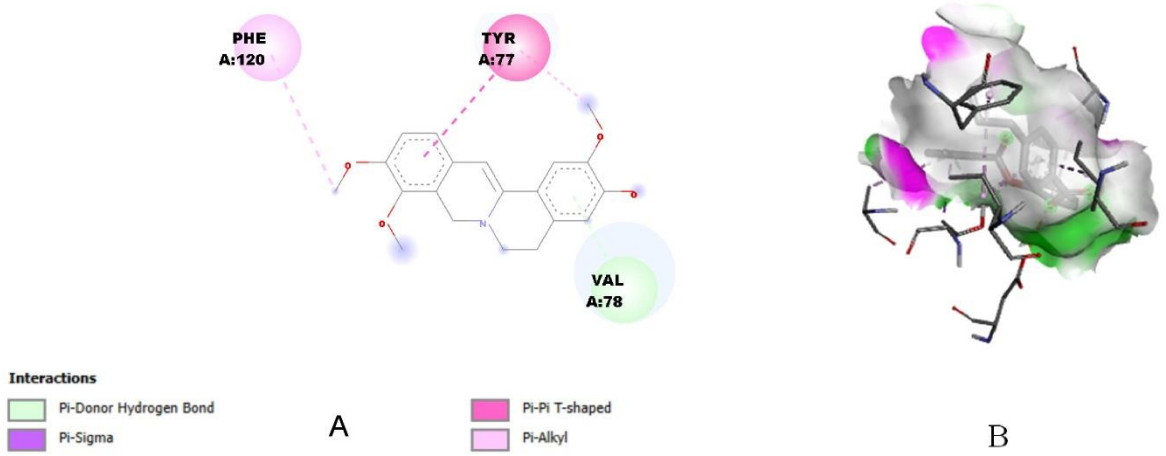

Figure 3.3: Showing the 2D (A & C) and 3D (B & D) molecular interactions of compounds 13 from *Enantia chlorantha* (Table 3.3) with the amino-acid residues of Plasmepsin II receptor.

3.3 Post-Docking Analysis

The amino acids that the ligands from both plants interacted with via conventional H-bonds or other bonds on the Plasmepsin II receptor are presented in Tables 3.4 and 3.5.

Table 3.4: Showing the various types of interaction between the ligands (*Alchornea cordifolia* compounds) and amino acids on the target protein (Plasmepsin II).

S/N	Compound	Conventional Hydrogen bond	Carbo Hydrogen Bond	Others
1	Acetyl aleuritolic acid	GLN 194		Unfavourable donor-donor (HIS 189)
2	Beta- Sitosterol		SER 79	Pi-sigma (PHE 294), Alkyl, Pi-

				alkyl (ILE 123, TYR 77)
3	Daucosterol	ASP 34, THR 217	SER 79	Pi-sigma (PHE 294), alkyl, Pi-alkyl (ILE 123, TYR 77, VAL 78)
4	Ellagic acid	ASP 34, THR 217		Pi-anion (ASP 214), Pi-sigma (VAL 78), Pi-alkyl (ILE 300)
5	Friedelin			Alkyl (ILE 32, ILE 123)
6	Kaempferol-3-O-galactoside	GLY 216, SER 218, THR 217		Pi-anion (ASP 214), Pi-sigma (VAL 78), Pi-alkyl (ILE 123)
7	Lup-20(29)-en-3c-ol (Lupenol)			Pi-sigma (ILE 123), Pi-pi T-shaped (TYR 77) Alkyl, Pi-alkyl (PRO 243, PHE 244)

8	Quercetin	TYR 192		Pi-anion (ASP 214), Pi-alkyl (VAL 78)
9	Quercetin-3-O-alpha-D-Arainoturanosiole	ASP 214, GLY 216, THR 217, SER 79, TYR 192, ASP 34	VAL 78	Pi-sigma (VAL 78), Pi-pi stacked (PHE 294), Pi-alkyl (ILE 300)
10	Quercetin-3-O-Arabinosiole	THR 192, LEU 131, ASP 34, ASP 214	SER 37	Pi-alkyl (VAL 78)
11	*4-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide	ASN 76, GLY 36, ASP 214, GLY 216, THR 217	TYR 77	Pi-sigma (ILE 123), Pi-sulfur (MET 76), Pi-pi T-shaped, Pi-donor Hydrogen bond, Pi-alkyl (TYR 192, LEU 131)
12	**Dihydroartemisinin	SER 79		Pi-sigma (TYR 77), Alkyl (VAL 78)

Table 3.5: Showing the various types of interaction between the ligands (*Alchornea cordifolia* compounds) and amino acids on the target protein (Plasmepsin II).

S/N	Compound	Conventional Hydrogen bond	Carbo Hydrogen Bond	Others
1	Diazoprogesterone			
2	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one			
3	Pseudocolumbamine			Pi-anion (ASP 214), Alkyl Pi-alkyl (ILE 133, VAL 78)
4	Palmatine		ASP 214, GLY 216	Pi-sigma (VAL 78), pi-anion (ASP 214), Pi-pi T-shaped (TYR 77), alkyl, pi-alkyl (PHE 111, PHE 120, PHE 294)
5	B-sitosterol		SER 79	Pi-sigma (PHE 294), alkyl, pi-alkyl (ILE 123,

			TYR 77, VAL 78)
6	Berberine	SER 218	Pi-donor hydrogen bond (THR 217), pi-sigma (VAL 78), alkyl, pi-alkyl (TYR 192, ALA 219, PHE 294)
7	7,8-Dihydro-8-hydroxypalmatine	GLY 216	Pi-sigma (PHE 16), alkyl, pi-alkyl (ILE 123, TYR 77, PHE 120, PHE 111, ILE 32, MET 15)
8	Stigmasterol		Alkyl, pi-alkyl (PHE 120, VAL 78, PHE 294, TYR 192)
9	Isohyobunone	THR 217	Alkyl, pi-alkyl (ILE 212, TYR 192, ILE 300)

10	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one			Alkyl (ILE 32)
11	4-Chloro-2-isopropyl-5-methylphenyl bicyclo[4.1.0]heptane-7-carboxylate	SER 79, VAL 78		Alkyl, pi-alkyl (ILE 32, TYR 77, ILE 123, ILE 300, PHE 294), pi-sigma (VAL 78)
12	Columbamine	TYR 192	GLY 216	Pi-anion (ASP 214), pi-pi T-shaped (TYR 77) alkyl, pi-alkyl (ILE 212, ILW 300, PHE 294, VAL 78, PHE 111, PHE 120)
13	Jatrorrhizine			Pi-dono hydrogen bond (BVAL 78), pi-pi T-shaped (TYR 77), pi-sigma (VAL

				78), pi-alkyl (PHE 120)
14	Magnoplorine	GLY 216	GLY 36	Attractive charge, pi-anion (ASP 34, ASP 214), pi-donor hydrogen bond (GLY 36), alkyl, pi-alkyl (VAL 78, ILE 123, ILE 32, ILE 290)
15	1,2-Benzenediol, o-(4-butylbenzoyl)-o'-(2-methylbenzoyl)	THR217		Pi-donor hydrogen bond (VAL 78), pi-anion (ASP 214), pi-sigma, alkyl pi-alkyl (PHE 120, ILE 32, ILE 123, VAL 78, ALA 219)

16	*4-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide	ASN 76, GLY 36, TYR 77 ASP 214, GLY 216, THR 217	Pi-sigma (ILE 123), Pi-sulfur (MET 76), Pi-pi T-shaped, Pi-donor Hydrogen bond, Pi-alkyl (TYR 192, LEU 131)
17	**Dihydroartemisinin	SER 79	Pi-sigma (TYR 77), Alkyl (VAL 78)

3.4 Results of the Physicochemical and Pharmacokinetic properties of *Alchornea cordifolia* and *Enantia chlorantha* ligands (phytoconstituents) are shown in Tables 3.6 – 3.7.

Table 3.6: Physiochemical Properties and Pharmacokinetic Parameters of the Phytoconstituents of *Alchornea cordifolia*

S/N	No of Rotatable bonds	No of H-bond acceptors	No of H-bond donors	TPSA	Consensus Log P	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski violations	Bioavailability Score	PAINS alerts
1	3	4	1	63.6	6.61	Low	No	No	No	Yes	No	No	-3.4	1	1	0
2	6	1	1	20.2	7.24	Low	No	No	No	No	No	No	-2.2	1	1	0
3	9	6	4	99.4	5.55	Low	No	No	No	No	No	No	-4.3	1	1	0
4	0	8	4	141	1	High	No	Yes	No	No	No	No	-7.4	0	1	1
5	0	1	0	17.1	7.44	Low	No	No	No	No	No	No	-1.9	1	1	0
6	4	11	7	190	-0.1	Low	No	No	No	No	No	No	-8.5	2	0	0
7	1	1	1	20.2	7.26	Low	No	No	No	No	No	No	-1.9	1	1	0
8	1	7	5	131	1.23	High	No	Yes	No	No	Yes	Yes	-7.1	0	1	1
9	4	11	7	190	0.13	Low	No	No	No	No	No	No	-8.3	2	0	1
10	3	11	7	190	-0	Low	No	No	No	No	No	No	-8.6	2	0	1

Table 3.7: Physiochemical Properties and Pharmacokinetic Parameters of the Phytoconstituents of *Enantia chlorantha*

S/N	No of Rotatable bonds	No of H-bond acceptors	No of H-bond donors	TPSA	Consensus Log P	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski violations	Bioavailability Score	PAINS alerts
1	2	4	0	72	3	High	Yes	No	No	Yes	No	No	-5.6	0	0.6	1
2	0	1	0	17	4	High	Yes	No	No	Yes	No	No	-3.6	0	0.6	0
3	3	4	1	52	2	High	Yes	Yes	No	No	Yes	Yes	-5.9	0	0.6	0
4	4	4	0	41	3	High	Yes	No	No	No	Yes	Yes	-5.8	0	0.6	0
5	6	1	1	20	7	Low	No	No	No	No	No	No	-2.2	1	0.6	0
6	2	4	0	41	3	High	Yes	Yes	No	No	Yes	Yes	-5.8	0	0.6	0
7	4	5	1	60	3	High	Yes	Yes	Yes	Yes	Yes	Yes	-6.3	0	0.6	0
8	5	1	1	20	7	Low	No	No	No	Yes	No	No	-2.7	1	0.6	0
9	2	1	0	17	4	High	Yes	No	Yes	Yes	No	No	-4.2	0	0.6	0
10	4	1	0	17	4	High	Yes	No	Yes	Yes	No	No	-4.2	0	0.6	0
11	4	2	0	26	5	High	Yes	Yes	Yes	Yes	No	No	-3.9	1	0.6	0
12	3	4	1	52	2	High	Yes	Yes	No	No	Yes	Yes	-5.9	0	0.6	0
13	3	4	1	52	2	High	Yes	Yes	No	No	Yes	Yes	-5.9	0	0.6	0
14	2	4	2	59	1	High	Yes	Yes	No	No	No	Yes	-6.4	0	0.6	0
15	9	4	0	53	6	High	No	No	Yes	No	No	No	-3.7	1	0.6	0

3.5 The results of ADME and Toxicity profiling of the ligands (phytoconstituents) of *Alchornea cordifolia* and *Enantia chlorantha* are shown in Tables 3.8 -3.9

Table 3.8: Toxicity Profile of the Phytoconstituents of *Alchornea cordifolia*

S/N	Ligands PubChem CID	Hepatotoxicity	Neurotoxicity	Nephro toxicity	Respiratory toxicity	Cardiotoxicity	Carcino genicity	Immuno toxicity	Mutagenicity	Cytotoxicity	LD50 (mg/kg)	Class
1	161616	-	-	-	+	+	-	+	-	-	2589	V
2	222284	-	-	+	-	+	-	+	-	-	890	IV
3	5742590	-	-	+	-	+	-	+	-	-	8000	VI
4	5281855	-	-	+	+	-	+	-	-	-	2991	IV
5	91472	-	+	-	-	-	-	+	-	-	500	IV
6	5282149	-	-	+	+	-	-	-	-	-	5000	V
7	236432	-	+	-	+	-	-	+	-	-	2000	IV
8	5280343	-	-	+	+	-	+	-	+	-	159	III
9	11968848	-	-	+	+	-	-	+	-	-	5000	V
10	12309865	-	-	+	+	-	-	+	-	-	5000	V

Where + = Toxic, - = Not toxic

Table 3.8: Toxicity Profile of the Phytoconstituents of *Enantia chlorantha*

S/N	Ligands PubChem CID	Hepatotoxicity	Neurotoxicity	Nephro toxicity	Respiratory toxicity	Cardiotoxicity	Carcino genicity	Immuno toxicity	Mutagenicity	Cytotoxicity	LD50 (mg/kg)	Class
1	104633	+	-	-	+	-	+	+	-	-	2300	V
2	13760785	-	+	-	-	-	-	-	-	-	5000	V
3	182406	-	+	-	+	-	-	+	+	-	200	III
4	19009	-	+	-	+	-	+	+	+	+	200	III
5	222284	-	+	-	+	-	-	+	-	-	890	IV
6	2353	-	+	-	+	-	+	+	+	+	200	III
7	493569	-	-	-	+	-	-	+	-	-	350	IV
8	5280794	-	+	-	+	-	-	+	-	-	890	IV
9	5318673	-	+	-	-	-	-	-	-	-	2500	V
10	5363697	-	+	-	-	-	-	-	-	-	10000	VI
11	579217	-	+	-	-	-	-	-	-	-	200	IV
12	72310	-	+	-	+	-	-	+	+	-	200	III
13	72323	-	+	-	+	-	-	+	+	-	200	III

14	73337	-	-	-	+	-	-	+	-	-	401	IV
15	91710667	+	-	+	-	-	-	-	-	-	2900	V

Where + = Toxic, - = Not toxic

CHAPTER FOUR

DISCUSSION

The unremitting malaria burden, combined with a growing number of cases of drug resistance, requires a new approach to the issue. This work took up this challenge by pursuing a two-fold exploration, aided by the abundant ethnobotanical history of West Africa and the predictive capabilities of contemporary computational biology/bioinformatics. We have concentrated on two plants, *Alchornea cordifolia and Enantia chlorantha,* whose combined use in traditional medicine is a monument to generations of observational experience. We wanted to go beyond anecdotal evidence and give them a rigorous, scientific validation of their potential. Using *in silico* methods to understand their molecular interactions with the central drug target, Plasmepsin II (1LEE), and supplementing this with *in vivo* safety studies, we have built a strong body of evidence. The discussion interprets our main findings by integrating the strands of binding affinity, molecular interactions, drug-likeness, and safety to provide a unified account of the promise of these plants as a source of novel antimalarial chemotypes.

This paper applied the phytoconstituents of *Alchornea cordifolia* and *Enantia chlorantha* to determine the anti-malarial drugs. Isolated compounds of *Alchornea cordifolia* twenty-six (26) and *Enantia chlorantha* twenty (20) were obtained by an extensive literature search. The results of GC-MS analysis of *Enantia chlorantha* provided forty-five (45) probable compounds. These compounds were docked against 1LEE, of which the compounds whose binding affinity is less or equal to -6.5 were presented in Figure 3.2 and Figure 3.3, respectively.

Dihydroartemisinin is an artemisin analog that has been reported to be used in the treatment of uncomplicated malaria; it was used in this experiment as a positive control to show the active amino acids that combine with the analog. It was used in this experiment as a positive control to show the active amino acids that combine with potential ligands.

The identification of the active amino acids at the binding site became possible with the help of4-amino-n-[4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl)-benzamide (R36) that was co-crystallized with the target protein, plasmepsin II (1LEE).

Plasmepsin II is an aspartic protease of the human intraerythrocytic parasite *Plasmodium falciparum*. It's used in the degradation of the host cell haemoglobin within the acidic food vacuole of the parasite and is a possible target. It is involved in the degradation of the host cell haemoglobin within the acidic food vacuole of the parasite and is a possible target for the development of antimalarial drugs.

4.1 Binding Affinity

Compounds (ligands) that are molecular docked against a known target generate a score called the docking score that can be used to predict the binding affinity and consequently activity of the ligand towards the protein target (Prakash, 2010; Wang and Zhu, 2016 and Dar and Mir, 2017).

Our *in-silico* investigation was based on the molecular docking of phytoconstituents of the two plants with *P. falciparum* Plasmepsin II (PDB ID: 1LEE). The outcomes were very impressive. Some of the compounds also exhibited binding affinities that not only competed but, in most cases, greatly exceeded that of the positive control, dihydroartemisinin (-7.1 kcal/mol).

From *Alchornea cordifolia*, Lup-20(29)-en-3β -ol (Lupenol) emerged as a standout candidate with an exceptional binding affinity of -9.1 kcal/mol. This was closely followed by Friedelin (-8.3 kcal/mol) and β -Sitosterol (-8.1 kcal/mol).

Likewise, *EnantiaChloranta* presented potent inhibitors, with Diazoprogesterone (-8.5 kcal/mol), Stigmasterol (-8.4 kcal/mol), and β-Sitosterol (-8.2 kcal/mol) taking the frontline. It also exhibited strong binding to berberine, a well-characterized alkaloid (-7.9 kcal/mol).

The implication here, which is profound, is that these natural compounds are intrinsically able to squirm into the active site within Plasmepsin II. Their higher binding efficiency than that of a frontline antimalarial implies that they may effectively outcompete the native substrate (haemoglobin), thereby disrupting the nutrient uptake pathway on which the parasite relies and causing its starvation. This computer-based argument provides a strong molecular explanation of the reported anti-plasmodial activity of these plants in a living organism, and the story shifts to a 'here is how it works' argument based on a molecular basis.

4.2 Molecular Interaction

Without a favourable binding mode, there is no meaning in the strong binding affinity. The analysis of post-docking interaction showed that complex molecular conversations mediate these high-affinity bonds. The common partners in these interactions were the catalytic dyad of Plasmepsin II —ASP34 and ASP214 —and other prominent residues, such as TYR77, SER79, and ILE123.

We have found that the most effective ligands interact with the enzyme via a complex combination of binding actions. As an example, Asp214, GLY216, THR217, SER79, and TYR192 were engaged in a large network of conventional hydrogen bonds with Quercetin-3-O-alpha-D-arabinofuranoside of *A. cordifolia*. This is a multi-point attachment characteristic with high-specificity inhibitors. Likewise, the native ligand R36 and several alkaloids from *E. chlorantha*, such as Palmatine and Columbamine, engaged in critical π-π T-shaped and π-anion interactions with TYR77 and ASP214, respectively. These interactions are crucial for stabilizing the ligand within the hydrophobic and acidic environment of the binding pocket.

Moreover, alkyl and π-alkyl interactions with residues such as ILE123, VAL78, and PHE294 also indicate the importance of van der Waals interactions and shape complementation. The binding of a compound such as Friedelin, which does not form hydrogen bonds, is specifically

due to extensive hydrophobic contacts, with the compound essentially burying itself in the active site. This is a major strength, as it offers diversity in interaction mechanisms. It postulates that a mixture of these phytochemicals may therefore have a multi-vector assault on Plasmepsin II, complicating the parasite's ability to develop resistance through single-point mutations.

4.3 ADMET Profiling and Drug-Likeness

A drug is not a potent inhibitor until it is safely delivered and it can reach its target in the body. Our ADMET and drug-likeness profiling (SwissADME and Protox-III) served as an important filter, distinguishing between active compounds that have the potential to be developed into viable drug leads and those that do not.

In this case, there was an interesting difference between these two plants. This was especially favourable about the alkaloid-rich profile of *Enantia chlorantha*. Berberine, Palmatine, Jatrorrhizine, and Magnoflorine passed the Lipinski rule of five with no violations. There are five criteria that the Lipinski Rule of 5 requires an orally active drug to satisfy: hydrogen bond donor ≤ 5; hydrogen bond acceptor ≤ 10; molecular weight ≤ 500 Daltons; octanol-water partition coefficient ≤ 5. They were highly absorbed by the gastrointestinal tract, indicative of good oral bioavailability. Surprisingly, they were also predicted to be BBB-permeant. Although this must be done with great caution regarding neurotoxicity, it may also have some benefits in combating cerebral malaria. Their principal demerits were forecasts of CYP enzyme inhibition and likely mutagenicity, which are important flags for further medicinal chemistry optimization.

On the contrary, numerous terpenoids and glycosylated flavonoids of *Alchornea cordifolia*, including Lupenol, Friedelin, and Daucasterol, were found to have low GI absorption, which is not in accordance with the Lipinski rule, because of high molecular weight and log P values. Although Lupenol displayed the most significant binding affinity, compared to other drugs, its

drug-likeness score was poor and therefore, might better serve as a synergistic drug rather than as a lead drug, which might hinder its use as a systemic oral medication, but could also be as a lead drug in other systems to validate Plasmepsin II as a target.

This contrast is not a fault but a manifestation of nature's chemical diversity. It posits that, in the polyherbal formulation, the *E. chlorantha* components can be bioavailable and systemically active agents, and the *A. cordifolia* Components may act through other pathways, including the enhancement of host immunity or higher-order synergistic activities at that site.

4.4 *In Vivo* Toxicity Assessment

Our computational predictions of safety required empirical validation. This was necessary because the acute oral toxicity test in Swiss mice was performed according to the established Lorke *et al*. method. The findings were unambiguous: the *A. cordifolia and E. chlorantha* complex extract demonstrated an extremely high safety margin.

There was no death or other major behavioural indications of poisoning even at the test dose of 5000mg/kg limit. This is what allows us to categorize the mixed extract as Category 5 (or Unclassified) under the Globally Harmonized System (GHS), which is, in effect, equivalent to it being practically non-toxic. The resulting LD50 is thus more than 5000 mg/kg, which is a very significant observation.

This *in vivo* safety profile provides strong support for the traditional use of these plants and is consistent with the predictions of ProTox-III, which, in most cases, reported low acute toxicity (Class IV and V) for these compounds. It gives a solid basis in stating that the therapeutic effect of this combination at adequate doses is associated with a small risk of acute adverse effects. This is an important step towards de-risking these herbal medicines to enable continued preclinical and clinical development.

for concern, although the *in vivo* results are clean, perhaps because of limited systemic exposure.

CHAPTER FIVE

CONCLUSION

The experiment in our study has brought the traditional applications of Alchornea cordifolia and Enantia chlorantha out of folklore into the realm of evidence-based science. Not only have we verified that the combined extract is safe, but we have also shed light on the molecular actors and mechanisms that may underlie its effectiveness.

The interplay between computational studies and laboratory experiments is the crux of our contribution. *In silico* analyses identify Lupenol, Berberine, and Palmatine as the top candidates targeting Plasmepsin II; however, ADMET profiling indicates that Berberine and Palmatine possess more favorable drug-like properties than Lupenol. In the meantime, the *in-vivo* study proves the *in-vivo* safety of the complex mixture of these compounds.

This integrated approach is a clear path to follow:

Lead Optimization: *E. chlorantha* alkaloids (e.g., Berberine derivatives) are the most promising lead that should be targeted to create a single-entity drug. Semi-synthetic alteration would help address anticipated CYP inhibition and mutagenicity, while also enhancing potency and selectivity.

Synergy Studies: Determine whether less bioavailable and highly potent compounds of the *A. cordifolia* (e.g., Lupenol) could work synergistically with the *E. chlorantha* alkaloids *in vitro* and *in vivo,* which would permit reduced and even safer doses of the two-wise extract.

Mechanistic Validation: Transition from prediction to confirmation using enzymatic assays to verify the inhibition of PMII and *in vitro* parasite cultures to show true antiplasmodial activity.

To sum up, this work can be regarded as evidence of the strength of integrating classic knowledge with state-of-the-art science. We have presented a strong, multi-layered case to support further study of the subjects *Alchornea cordifolia* and *Enantiachlorantha*. They are not merely the relics of the past but the future, safe, mechanically foresighted, and candidates in the current pressing war against drug-resistant malaria.